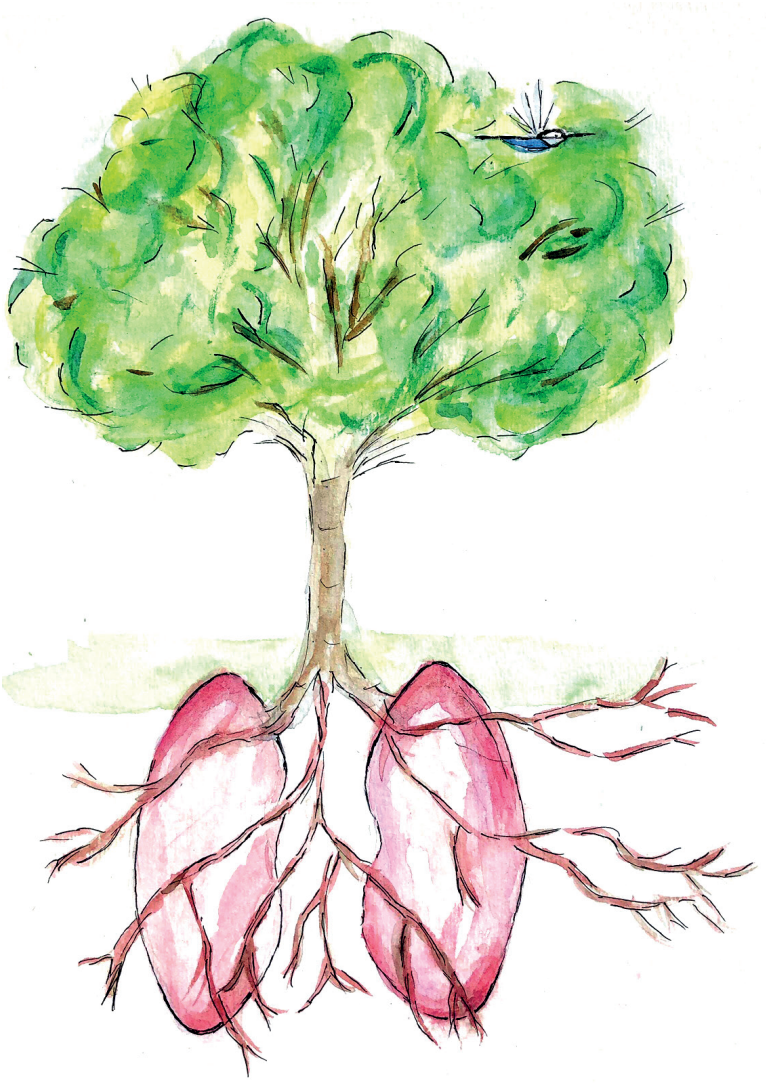
A Poorest-First Approach to RSV Vaccine Development



Natalie Isabelle Mazur

A POOREST-FIRST APPROACH TO RSV VACCINE DEVELOPMENT

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A POOREST-FIRST APPROACH TO RSV VACCINE DEVELOPMENT

RSV VACCIN ONTWIKKELING GERICHT OP ONTWIKKELINGSLANDEN

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

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Natalie Isabelle Mazur

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te Malden, Verenigde Staten

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Dedicated to my loving parents, Angela B. Romijn and Eric Mazur, my grandparents Daan and Annie (Opa en Oma); Hélène and Peter (Tita en Annapapa), and Mordechai, Rose and Nellie

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Part 1

INTRODUCTION

1

RESPIRATORY SYNCYTIAL VIRUS

Keep your face always towards the sunshine and the shadows will fall behind you.
Walt Whitman (1819-1892)

Chapter 1 — Respiratory Syncytial Virus

Virology

Respiratory syncytial virus (RSV) is a negative-sense single-stranded RNA virus encoding for 11 proteins. RSV mainly infects the ciliated airway epithelial cells of the respiratory tract causing damage and inflammation to the bronchioles. Two surface proteins attachment (G) and fusion (F), play a role in RSV binding and fusion respectively. The F and G protein are viral epitopes that play a role in virus neutralization and are therefore important targets in vaccine development [this thesis]. The RSV F protein is a highly conserved class I viral fusion protein[1]. The discovery and stabilization of the prefusion (pre-F) conformation of the RSV surface F glycoprotein has provided a new target for vaccines and monoclonal antibodies (mAbs), as the pre-F epitope is more immunogenic than the postfusion (post-F) viral epitope[2].

Other RSV viral proteins are less frequently employed in vaccine design. The viral envelope protein, small hydrophobic (SH), has a transmembrane and extracellular domain and likely plays a role in viral replication and inflammasome activation[3]. The matrix protein (M), forms the inner envelope. Four proteins make up the nucleocapsid inside the viral envelope: nucleoprotein (N) which binds RNA; phosphoprotein (P) which is a polymerase cofactor, polymerase (L); and M2-1 which is a transcription factor, M2-2 likely has a regulatory role in RNA replication. NS1 and NS2 are non-structural proteins that downregulate RNA synthesis and inhibit type I interferon[4].

Epidemiology

The hospitalization burden of bronchiolitis is well recognized as the number one cause of hospitalization for infants and young children globally. Epidemics due to RSV have a seasonal pattern: occurring in winter months in temperate climates and the rainy season in tropical climates[5]. In children under 5 years it is estimated that 33.1 million episodes of acute lower respiratory illness (ALRI) and 118,200 deaths are attributable to RSV globally[6]. RSV is the second leading cause of death in the infant period following malaria when attributing mortality burden by pathogen[7]. More than 99% of this mortality burden is in developing countries[6]. Monitoring of RSV mortality beyond hospital admissions is needed, especially in areas where healthcare access is limited and mortality risk is elevated. Minimally-invasive tissue sampling and cause-of-death panels have been validated and set up in low-resource settings to be able to ascertain cause of death due to RSV[8]. Furthermore, the World Health Organization (WHO) has piggybacked on global influenza surveillance to set up RSV surveillance in 14 countries[9]. Recent global burden estimates are high: 4.1 million RSV-associated ALRI hospitalizations under 5 years of age annually[10].

The burden of RSV is not limited to young infants; RSV infection in adults is substantial and the disease burden is comparable to that of influenza. Older adults with comorbidities such as underlying heart or lung disease are at elevated risk of severe RSV disease. 4-10% of high-risk adults will develop acute RSV infection annually[11].

Overall, RSV vaccine candidates aim to protect three target populations at risk for severe disease: (1) young infants (0-6 months of age), (2) older infants and young children (>6 months of age), and (3) older adults (65 years or older).

Pathogenesis & Host Response

RSV mainly infects the ciliated airway epithelial cells (AEC's) of the respiratory tract and causes both damage and inflammation of the bronchioles[12]. Direct viral damage and cytopathology do not play a major role in the pathology of RSV infection but instead the host response plays an essential role in airway damage[12]. Neutrophils are the predominant cells responsive in RSV pathogenesis and are implicated in mucin production[13]. Natural immunity to RSV is incomplete, and reinfection occurs throughout life[14].

In the 1960's concerns of enhanced respiratory disease (ERD) following vaccination with formalin-inactivated RSV (FI-RSV) severely hindered inactivated virus and subunit vaccine development. However, research on ERD has also guided vaccine development towards a desired immune response to RSV. ERD is associated with induction of poorly neutralizing antibodies in vaccine recipients[15] and animal models of ERD suggest a Th-2 biased T cell response[16]. For this reason, an RSV vaccine ideally elicits potent neutralizing antibodies without a Th2 bias. While a definitive correlate of protection against RSV infection remains elusive, cell-mediated immunity[17], mucosal IgA[18], and neutralizing antibodies[19–22] have been associated with protection from RSV infection. Ultimately, the outcome of large phase III trials will help to define a correlate of protection from clinical RSV disease.

Clinical Presentation

Bronchiolitis is a clinical diagnosis which occurs primarily in children under 6 months of age. Physicians should use a history and physical exam to recognize the diagnosis based on symptoms and signs of rhinorrhea, cough, wheeze, tachypnea, increased respiratory effort, grunting, nasal flaring and retractions. Severe disease is defined clinically by increased respiratory effort, hypoxemia and eventually respiratory failure[23]. Symptoms of difficulty breathing and worsening disease include retractions, nasal flaring, cyanosis, and increased respiratory rate. When considering hospitalization it is important to evaluate the impact of respiratory symptoms on feeding ability, hydration, and mental status[23]. A randomized controlled trial in which pulse oximetry readings were artificially elevated resulted in significantly decreased hospital admissions; researchers urge clinicians to not value oxygen saturation too highly as a single marker for disease severity. Prematurity, age below 3 months, bronchopulmonary dysplasia, congenital heart disease, Down syndrome and immunodeficiency are well-established risk factors for severe disease [24–27]. For bronchiolitis a chest radiograph has little diagnostic added value and findings are often non-specific with hyperinflation and patchy atelectasis[28]. Although bronchiolitis is a clinical diagnosis, the gold standard for viral diagnosis can be performed through PCR on a nasopharyngeal swab. More recently molecular point-of-care clinical tests have been developed and implemented with high sensitivity and specificity[29]. The added value of viral diagnosis is largely in promoting RSV awareness and potentially decreased likelihood of antibiotic treatment[30].

Current Management

There is currently no treatment or vaccine against RSV. There is a prophylaxis, palivizumab (a mAb against the F protein of RSV) which is given to high-risk babies in developed countries to prevent severe RSV disease. An evidence-based approach to RSV clinical management can be summed up in three words: less is more. Most often, bronchiolitis is self-limiting and resolves within 21 days after symptom onset[31] . In the case of severe disease, supportive care is the cornerstone of clinical management: hydration via the nasogastric or intravenous route and supplemental oxygen in the inpatient setting. Most therapeutic interventions (inhaled bronchodilators, systemic corticosteroids, ribavirin, antibiotics, chest physiotherapy, epinephrine, antitussives) are not recommended in global management guidelines [this thesis]. In fact, reduction of the use of non-evidence-based therapies for bronchiolitis is essential in the inpatient setting. For RSV bronchiolitis, mechanical ventilation in the intensive-care unit can be life-saving, yet the role of non-invasive respiratory support strategies (i.e. high flow nasal canula) remains unclear[32] . The lack of access to mechanical ventilation may explain higher RSV mortality in lower and lower-middle income countries (LMICs): 100% versus 24% of RSV deaths had access to the intensive care unit in high-income countries and LMICs respectively [this thesis].

RSV Transmission & Prevention

RSV is spread by close contact with direct inoculation of large-particle aerosols or by self-inoculation after touching contaminated surfaces. Small-particle aerosols do not seem to be a major mode of transmission[33]. RSV survives differentially on fomites. RSV can survive longer on non-porous surfaces such as countertops, plastic or glass for six to 12 hours. RSV may be transferred from these surfaces to hands with subsequent recovery of infectious virus from the skin and inoculation[34]. In a controlled human infection model with adult volunteers it was found that a minimum dose of 3.2 LogTCID50 of virus is needed for infection[35]. Many measures have been introduced to prevent nosocomial infection of RSV including hand hygiene regimens, gloves, gowns, masks, eye protection, and cohorting. In a review of different precautionary measures, nursing with gowns and gloves was the only effective measure to prevent RSV infection[36].

Ethics

After the tragedy of the 1960's vaccine-enhanced disease in a clinical trial on orphan children in the United States, little research was performed on RSV vaccines despite an ethical imperative[37]. International organizations such as the WHO failed to recognize the burden and importance of RSV as global health threat until 2015. Since then, RSV vaccine development has accelerated but no vaccine for LMICs is on the horizon [this thesis]. Many vaccine trials conducted lack data in LMIC settings. Large phase III vaccine trials conducted in vulnerable populations (South African, Alaskan-natives[38,39]) failed to meet the ethical principles of post-trial access. These ethical principles have been developed to protect vulnerable research populations ensure access to successful drugs after the end of a study.

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Part 2

GLOBAL LIFE-THREATENING LOWER RESPIRATORY TRACT INFECTIONS

2

GLOBAL RESPIRATORY SYNCYTIAL VIRUS–RELATED INFANT COMMUNITY DEATHS

Clinical Infectious Diseases, 2021

We must look a long time before we can see.
Henry David Thoreau (1817-1862)

Clinical Infectious Diseases

RSV COMMUNITY MORTALITY SUPPLEMENT

Global Respiratory Syncytial Virus–Related Infant Community Deaths

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Background. Respiratory syncytial virus (RSV) is a leading cause of pediatric death, with >99% of mortality occurring in low- and lower middle-income countries. At least half of RSV-related deaths are estimated to occur in the community, but clinical characteristics of this group of children remain poorly characterized.

Methods. The RSV Global Online Mortality Database (RSV GOLD), a global registry of under-5 children who have died with RSV-related illness, describes clinical characteristics of children dying of RSV through global data sharing. RSV GOLD acts as a collaborative platform for global deaths, including community mortality studies described in this supplement. We aimed to compare the age distribution of infant deaths <6 months occurring in the community with in-hospital.

Results. We studied 829 RSV-related deaths <1 year of age from 38 developing countries, including 166 community deaths from 12 countries. There were 629 deaths that occurred <6 months, of which 156 (25%) occurred in the community. Among infants who died before 6 months of age, median age at death in the community (1.5 months; IQR: 0.8–3.3) was lower than in-hospital (2.4 months; IQR: 1.5–4.0; $P < .0001$). The proportion of neonatal deaths was higher in the community (29%, 46/156) than in-hospital (12%, 57/473, $P < 0.0001$).

Conclusions. We observed that children in the community die at a younger age. We expect that maternal vaccination or immunoprophylaxis against RSV will have a larger impact on RSV-related mortality in the community than in-hospital. This case series of RSV-related community deaths, made possible through global data sharing, allowed us to assess the potential impact of future RSV vaccines.

Keywords: community death; lower respiratory tract infection; respiratory syncytial virus.

As part of the global agenda for 2030 set by the United Nations, Sustainable Development Goal (SDG) 3 urgently calls for ending preventable deaths of children under 5 years of age. Globally, respiratory syncytial virus (RSV) is a leading cause of death after malaria for infants [1]. More than 99% of these RSV pediatric deaths occur in the developing world [2]. Current global mortality estimates are almost exclusively based on in-hospital RSV mortality. However, it is likely that a significant proportion of these deaths occur outside the hospital, especially in low-income settings [3]. A recent meta-analysis estimated that out-of-hospital mortality was 2-fold higher than in-hospital mortality in 3 low-income and lower-middle-income countries (L(M)ICs) [3]. Thus, the burden of out-of-hospital RSV deaths appears to be at least as high as in-hospital deaths. Despite the magnitude of the problem, understanding

[a]RSV GOLD collaborators are listed in the Notes section.

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the clinical characteristics of pediatric RSV-related mortality in the community remains a key knowledge gap.

Addressing the knowledge gap on community deaths can give key insights to inform policy for a future RSV vaccine. More than 50 vaccine candidates are in clinical development for RSV [4]. Different approaches to RSV prevention confer varying degrees and duration of protection. Currently, 2 major approaches are in development for infants: (1) maternal vaccination and (2) passive antibody prophylaxis. Recent late-phase trial data show the potential degree and duration of protection for these different approaches, with infant monoclonals giving a higher degree and duration of protection than a maternal vaccine. The recently published phase III results of a post-fusion F protein maternal RSV vaccine show an antibody half-life of 49.1 days with 44.4% efficacy (95% confidence interval [CI]: 19.6-61.5%) against severe RSV lower respiratory tract infection (LRTI) through the first 3 months of life [5]. Prophylaxis with an extended half-life monoclonal antibody shows a longer duration of protection with 70.1% efficacy (95% CI: 52.3–81.2%) against RSV LRTI through the first 5 months of life [6]. To estimate the potential impact of RSV-preventive interventions against mortality in the developing world, it is essential to characterize children dying of RSV in the community.

Studying community deaths is difficult given the challenges associated with virological studies in deaths that occur in the community. To date, the largest case series of community RSV-related deaths includes 11 deaths at home in a single, urban setting in Argentina [7]. Although in-hospital deaths are challenging to capture in L(M)ICs given the lack of diagnostic capacity, capturing community deaths is even more challenging due to difficulty in ascertaining cause-of-death based on the low specificity of verbal autopsy data and difficulty obtaining postmortem patient samples. However, in the past years, several studies supported by the Bill & Melinda Gates Foundation (BMGF) aimed to measure RSV mortality in the community: Z-PRIME (the Zambia Pertussis RSV Infant Mortality Estimation study); community-based studies in Argentina, India, and Pakistan; as well as the Child Health and Mortality Prevention Surveillance (CHAMPS) in South Africa, Bangladesh, Kenya, Mali, Mozambique, Ethiopia, and Sierra Leone [8]. The RSV Global Online Mortality Database (RSV GOLD) provides the unique opportunity to pool data from all of these studies and compare global individual-level patient data of children dying in the community to children dying in-hospital.

Respiratory syncytial virus–preventive interventions aim to prevent infant death in accordance with the SDGs. Estimated impact of a maternal vaccine or infant monoclonal on pediatric deaths will guide policy decisions and accelerate access to life-saving interventions. The primary aim of this article is to describe global community pediatric deaths under 6 months and compare this group with in-hospital deaths in upper-middle-income countries (UMICs) and L(M)ICs.

METHODS

Study Site, Design, and Population

RSV GOLD is a global online registry for children under the age of 5 years who died with laboratory-confirmed RSV infection after 1 January 1995 [9]. Individual patient-level data are collected using an online questionnaire. Variables collected in the RSV GOLD database have been published previously [9]. Data are collected through active outreach to researchers and physicians worldwide. Investigators of BMGF-funded community mortality studies were specifically asked to share data collected through 2 March 2021. The data from these community studies have been published in this supplement issue. Two community studies (Z-PRIME and Pakistan Community Mortality studies) included children younger than 6 months of age; other studies recruited children through at least 12 months of age (Supplementary Table 1). Data from studies submitted to the RSV GOLD registry were collected both prospectively and retrospectively.

In this analysis RSV-related deaths above 1 year of age, nosocomial deaths, and deaths in high-income countries were excluded (Figure 1). Based on the expected duration of protection for infant RSV-preventive interventions, the primary aim of this study was to compare the age distribution of RSV-related infant deaths under 6 months occurring in the community with those in-hospital. The secondary aim was to describe age at death for children dying of RSV in the first year of life in the community. In order to achieve our secondary aim, to describe the age distribution under 1 year, we analyze the population ("12m cohort"), in which we excluded 2 community studies that only enrolled children up to 6 months of age.

Data Collection and Case Definition

Case definitions of a community death varied between different BMGF-funded community mortality studies (Supplementary Table 1). For community deaths submitted to RSV GOLD that did not originate from these studies, a community death was defined as a child who did not die in the hospital or a child who was not hospitalized and location of death was unknown (n = 2). As in our previous publications, we included any death with laboratory-confirmed RSV infection and did not require RSV to be the primary cause of death (Supplementary Table 5) [9]. Neonates were defined as children through 1 month of age.

Upon submission to the database, data-quality checks were performed by the RSV GOLD team to ensure the completeness and accuracy of the data. To this end, case data were verified for missingness, plausibility, and accuracy through direct communication with collaborators as soon as possible after case submission. Minimum essential data for inclusion were the key variables age at death, year of death, and laboratory-confirmed RSV infection.

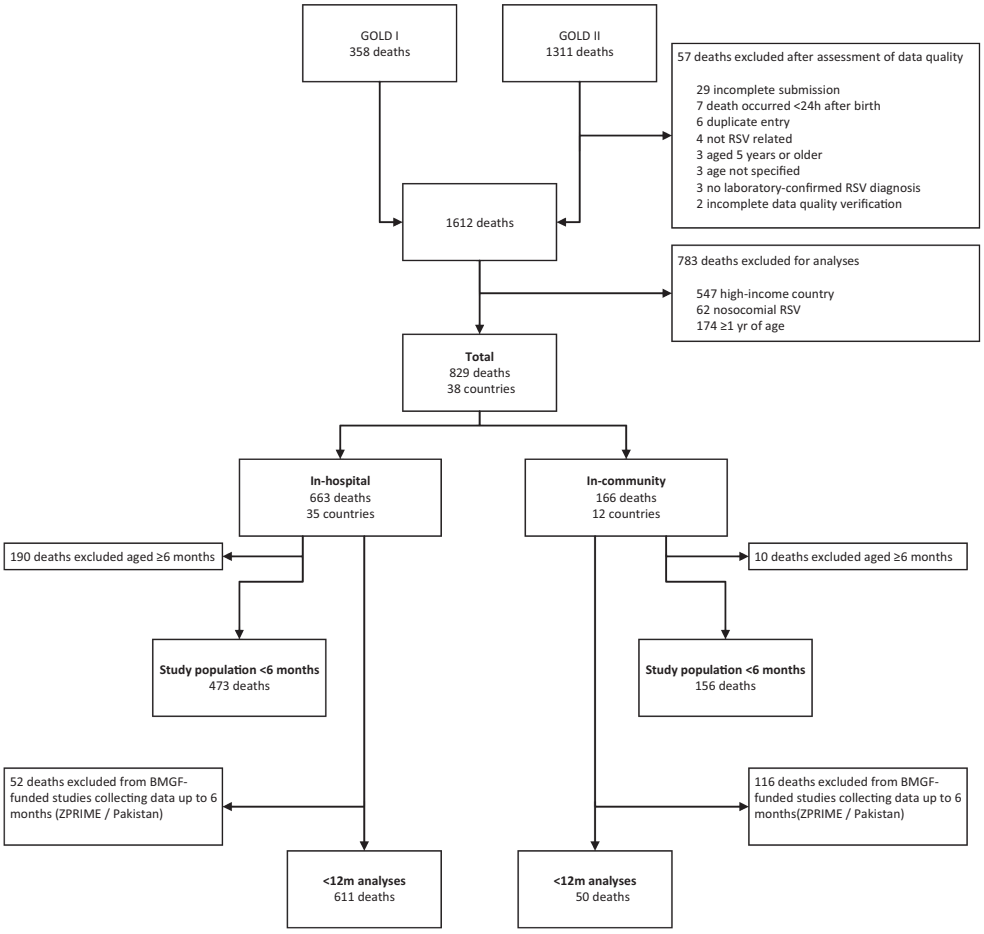

Figure 1. Flowchart of children included in this study. Flowchart shows children excluded via both data quality and per definition of study population. For the primary analysis we analyzed 629 children dying under age 6 months (473 in-hospital deaths and 156 community deaths). For the secondary analysis we analyzed 661 children dying under age 12 months (611 in-hospital deaths and 50 community deaths). GOLD I: Pediatric deaths published as a retrospective case series from 1 November 2014 to 31 October 2015 [9]. GOLD II includes pediatric deaths collected after this publication. Abbreviations: m, months; BMGF, Bill & Melinda Gates Foundation; GOLD, Global Online Mortality Database; RSV, respiratory syncytial virus; ZPRIME, Zambia Pertussis RSV Infant Mortality Estimation Study.

Statistical Analyses

For continuous variables, the means or medians were reported and differences between 2 groups were tested with a Mann-Whitney *U* test. Categorical variables were described with frequencies and percentages and compared between groups using Fisher's exact test. We did not perform imputation for missing data because data were not missing for essential variables and for other variables there was no clear correlation on which to build a multiple imputation model.

We considered $P < .05$ to be significant for all analyses. Despite multiple comparisons, we chose not to correct for an increased false-positive rate due to the exploratory nature of the study and small sample size. The statistical analysis was performed using R version 4.0.2 (R Core Team 2020, Vienna, Austria) with the following packages: ggplot2 [10], ggpubr [11], rnaturalearthdata [12], dplyr [13], and qwraps2 [14].

We performed 2 sensitivity analyses: (1) without the Z-PRIME data and (2) restricting the data to community

mortality studies. As the majority of the data for community deaths originated from the Z-PRIME study in Zambia, we performed a sensitivity analysis that excluded the Z-PRIME cases to ensure that this overrepresentation of Zambia deaths did not lead to different results. Furthermore, we tested the assumption that community deaths from the Z-PRIME data are representative for community deaths from other L(M)ICs by testing the observed characteristics for significant differences.

Ethical Considerations

The institutional research board of the University Medical Center Utrecht waived the requirement for parental informed consent in 2014 since the study concerns only anonymized secondary data. Collaborators sharing data were encouraged to adhere to local standards for ethics approval in accordance with the RSV GOLD Ethics Guideline [15].

RESULTS

Study Population

The overall study population included 829 pediatric deaths under 1 year of age from 38 countries classified as UMIC or L(M)IC according to the World Bank income group classification (Figure 1, Supplementary Table 2). Of these, 166 deaths occurred in the community. The world maps in Figure 2A and 2B show the global distribution of community and in-hospital deaths, respectively. The study population of infants under 6 months consisted of 629 deaths, of which 156 (25%) deaths from 12 different countries occurred in the community (Supplementary Table 4). Most community deaths were from Zambia (72%, 112/156). Community deaths were submitted from 2009 onwards, while data for in-hospital deaths were shared from 1995 onwards. The 12m cohort comprises 661 children, of whom 8% (50/661) died in the community and 92% (611/661) died in-hospital.

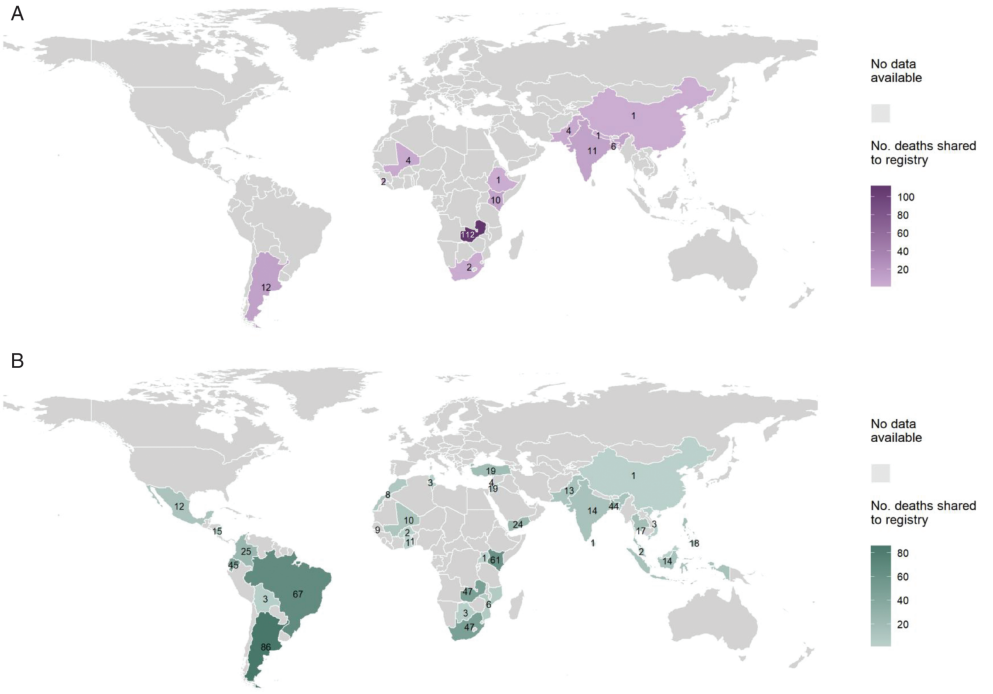

Figure 2. *A*, World map showing L(M)ICs and UMICs that shared RSV-confirmed community deaths under 12 months of age and number of RSV-confirmed community deaths shared to the registry. The color gradient of purple indicates number of deaths shared, with darker purple representing increased number of deaths shared. Numbers of deaths are visible on the map. *B*, World map showing L(M)ICs and UMICs that shared RSV-confirmed in-hospital deaths under 12 months of age and number of deaths of RSV-confirmed in-hospital deaths shared to the registry. The color gradient of green indicates number of deaths shared, with darker green representing increased number of deaths shared. Numbers of deaths are visible on the map. Abbreviations: L(M)ICs, lower-income-lower-middle-income country; RSV, respiratory syncytial virus; UMIC, upper-middle-income country.

Age at Death of Community Versus In-Hospital Deaths

Median age at death was significantly lower for community deaths (1.5 months; IQR: 0.8–3.3) than in-hospital deaths (2.4 months; IQR: 1.5–4.0; $P < .0001$) (Table 1, Figure 3A). Deaths in the community included a higher proportion of neonates (29%, 46/156) than deaths occurring in-hospital (12%, 57/473; $P < .0001$). Similar results were found for the 12m cohort (Table 2, Figure 3A). In the 12m cohort, median age at death was lower in the community (2.1 months; IQR: 1.3–5.0) compared with in-hospital (4.0 months; IQR: 2.0–6.1; $P = .02$) (Table 2, Figure 3B). Similarly, for the 12m cohort, a higher proportion of deaths occurred in the neonatal period in the community (14%, 7/50) than in-hospital (7%, 40/611), although this difference was not statistically significant ($P = .08$).

Clinical Characteristics of Community vs In-Hospital Deaths

For infants who died under 6 months, clinical characteristics of community and in-hospital deaths were largely comparable. For children under 6 months with comorbidity data, 31% (11/35) of infants dying in the community had a comorbidity compared with 44% (162/368) who died in-hospital. However, data on comorbidities were missing for 78% of community deaths, limiting the power to analyze this characteristic (comorbidities are specified in Supplementary Table 8). The proportion of premature infants did not differ significantly between community (25%, 11/44) and in-hospital (32%, 81/253; not significant) deaths. We note that prematurity data were missing for a substantial proportion of community (72% 112/156) and in-hospital (47%, 220/473) deaths for infants under 6 months. The reported mean gestational age was lower for deaths in-hospital compared with those occurring in the community (36.2 vs 38.5 weeks; $P = .005$).

The secondary analysis of the 12m cohort was remarkably similar to the primary analysis of children dying before age 6 months. Among infants dying in the community, 28% (10/36) had a comorbidity compared with 46% (240/525, $P = .04$) of infants dying in-hospital. In the 12m cohort, the proportion of premature infants did not differ significantly between community versus in-hospital deaths (Table 2), although reported gestational age was significantly lower for infants dying in-hospital than in the community (36.3 vs 38.4 weeks; $P = .006$).

Sensitivity Analyses

We performed a sensitivity analysis excluding the majority of the community deaths, which originated from a single Zambian study site (71%, 112/156). The age at death in the community did not differ significantly for children who died in Zambia (n = 112; data shown in Gill et al in this supplement issue) compared with children from other countries (n = 44; 2.0 months; IQR: 1.3–3.3 months). The proportion of children with prematurity was similar for community deaths in Zambia and community deaths elsewhere (Forman et al, data published elsewhere in this supplement issue). After excluding the Zambia data, we found that age at death remained lower for children who died in the community (2.0 months; IQR: 1.3–3.3) compared with children who died in-hospital (2.5 months; IQR: 1.8–4.0), although this difference was not statistically significant ($P = .07$) (Supplementary Table 6). The proportion of neonates was similar in the in-hospital and community deaths (Supplementary Table 6).

We performed a sensitivity analysis restricted to data obtained from the community mortality study sites (144 community deaths and 68 in-hospital deaths) to rule out bias due to differences in methodology of data collection, because data in this subset were collected systematically in the community and in the hospital setting (Supplementary Table 7). In this analysis, we observed a lower median age at death in the community compared with in-hospital, although differences were smaller than in the main analysis and not statistically significant (1.5 vs 2.0 months; $P = .26$).

Table 1. Clinical Characteristics of Children Under 6 Months Who Died with Respiratory Syncytial Virus In-Hospital Versus in the Community in Lower-income Middle-Income Countries and Upper-Middle-Income Countries

Clinical Characteristics	All Deaths (n = 629)	Community (n = 156)	In-Hospital (n = 473)	*P*
Sex, male, % (n/N)	54 (330/615)	55 (78/142)	53 (252/473)	NS
Age at death, months, median (IQR)	2.0 (1.1-4.0)	1.5 (0.8-3.3)	2.4 (1.5-4.0)	<.0001
Neonatal deaths, % (n/N)	16 (103/629)	29 (46/156)	12 (57/473)	<.0001
Comorbidity, % (n/N)	43 (173/403)	31 (11/35)	44 (162/368)	NS
Prematurity, % (n/N)	31 (92/297)	25 (11/44)	32 (81/253)	NS
Gestational age, weeks, mean (SD, n)	36.6 (3.5, 145)	38.5 (2.4, 23)	36.2 (3.6, 122)	.005
Birth weight, kg, median (IQR, n)	2.8 (2.2-3.2, 156)	3.0 (2.4-3.3, 30)	2.8 (2.2-3.2, 126)	NS
Month and year of death, minimum–maximum	July 1995–February 2021	February 2009–July 2020	July 1995–February 2021	…
Not immunized, % (n/N)	30 (71/235)	36 (15/42)	29 (56/193)	NS
Other children in household, % (n/N)	75 (118/158)	82 (18/22)	74 (100/136)	NS
Mother uneducated, % (n/N)	10 (23/231)	6 (6/103)	13 (17/128)	NS
Father uneducated, % (n/N)	6 (9/161)	1 (1/86)	11 (8/75)	.01

P values are provided for the comparison between community and in-hospital deaths. Abbreviations: IQR, interquartile range; NS, not significant; SD, standard deviation.

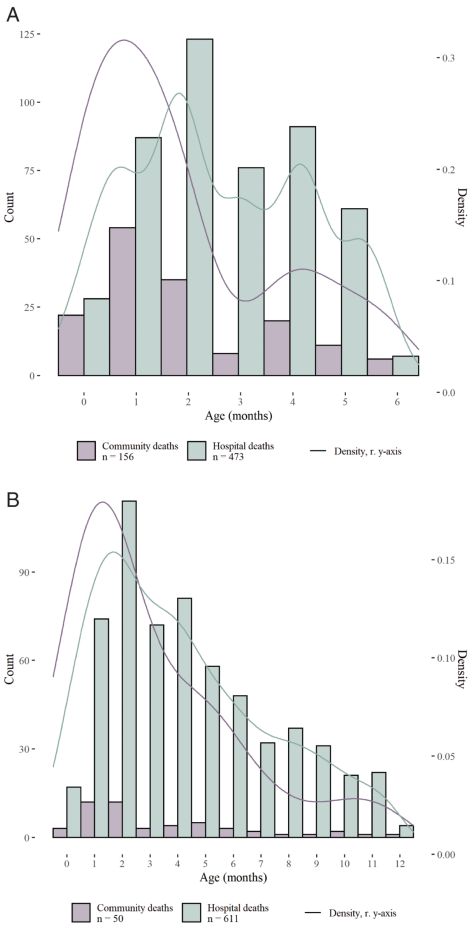

Figure 3. *A*, Histogram and density plot of age at death for children under 6 months who died with RSV in the community compared with in-hospital in L(M)ICs and UMICs. The histogram shows number of deaths (count, left *y*-axis) shared to the registry by age at death in months (rounded to the nearest integer) from age 0 up to 6 months for all infants under 6 months of age. Lines show the kernel density estimate of age at death in months (density, right *y*-axis). Deaths that occurred in the community are shown in purple, while deaths that occurred in the hospital are shown in green. *B*, Histogram and density plot of age at RSV-related death for children under 12 months who died in the community compared with in-hospital in L(M)ICs and UMICs. The histogram shows number of deaths shared (count, left *y*-axis) to the registry by age at death from age 0 up to 12 months for the 12m cohort. Lines show the kernel density estimate of age at death in months (density, right *y*-axis). Deaths that occurred in the community are shown in purple, while deaths that occurred in the hospital are shown in green. Abbreviations: L(M)ICs, lower income and lower middle income country; RSV, respiratory syncytial virus; UMIC, upper-middle-income country.

Moreover, in this sensitivity analysis, the proportion of neonates was similar in the in-hospital and community deaths (Supplementary Table 7).

DISCUSSION

As a result of global data sharing by collaborators, this study is the first global case series to compare RSV-related mortality in the community with in-hospital deaths in L(M)ICs and UMICs. The aim of this study was to understand differences between infants dying in the community and infants dying in-hospital in order to inform RSV vaccine–development strategies for low-resource settings. We found that children dying in the community were generally younger than children dying in-hospital. A larger proportion of deaths in the community involved neonates in the primary analyses but not the sensitivity analyses, possibly due to a larger proportion of deaths originating from L(M)ICs in the community. The younger age at death in the community may be explained by difficulty of caregivers in recognizing respiratory danger signs at a younger age, resulting in delayed or no access to care for younger children with RSV LRTI. We conclude that RSV-prevention strategies targeting infants in the first months of life will likely have a larger impact on mortality occurring in the community than in-hospital. Thus, we expect a high impact of infant RSV immunization strategies via maternal vaccination or infant immunoprophylaxis.

The RSV GOLD database serves as a platform that can bundle data from study sites around the world to allow for a high-level analysis of RSV-related mortality around the globe. Previous publications on community deaths did not describe age distribution for RSV-related illness but instead described risk factors for community deaths in Argentina [7], leading causes of deaths determined by minimally invasive autopsies in the CHAMPS sites [8], and estimates of the proportion of out-of-hospital deaths in South Africa [16]. Previously we published a case series of in-hospital deaths in which we found the median age at death to be 5 months in L(M)ICs and 4 months in UMICs in children under 5 years of age. In this analysis, we found the median age at RSV-related death in infants from UMICs and L(M)ICs who died before 1 year of age to be similar (4.0 months; IQR: 2.0–6.1).

There were several limitations of this study. First, the community mortality studies contributing to the RSV GOLD registry were not designed identically and used different definitions for community deaths (Supplementary Table 1). A second limitation concerns the reporting of age at death by collaborators. There are 2 ways in which collaborators may have rounded age at death to age in months, which could introduce bias in our analysis. Due to general conceptualization of age, collaborators may have rounded age in months down. This rounding method may have introduced systematic bias for the group of children who died in-hospital because age at death was most frequently shared in months for in-hospital deaths and in days for community deaths. Second, collaborators may round age to the nearest integer, which would mean that the cutoffs applied for our analyses exclude children whose age was rounded to 1 (neonates), 6 (primary analysis), or 12 (secondary analysis) months.

Table 2. Clinical Characteristics of Children Under 12 Months Who Died with Respiratory Syncytial Virus In-Hospital Versus in the Community in Lower-Middle-Income Countries and Upper-Middle-Income Countries, Excluding Deaths From Studies Recruiting Only Those Under 6 Months

Clinical Characteristics	All Deaths (n = 661)	Community (n = 50)	In-Hospital (n = 611)	*P*
Sex, male, % (n/N)	56 (369/661)	56 (28/50)	56 (341/611)	NS
Age at death, months, median (IQR)	4.0 (2.0-6.0)	2.1 (1.3-5.0)	4.0 (2.0-6.1)	.02
Neonatal deaths, % (n/N)	7 (47/661)	14 (7/50)	7 (40/611)	NS
Deaths <6 months, % (n/N)	70 (461/661)	80 (40/50)	69 (421/611)	NS
Comorbidity, % (n/N)	45 (250/561)	28 (10/36)	46 (240/525)	.04
Prematurity, % (n/N)	28 (101/356)	24 (9/37)	29 (92/319)	NS
Gestational age, weeks, mean (SD, n)	36.6 (3.5, 195)	38.4 (2.5, 27)	36.3 (3.5, 168)	.01
Birth weight, kg, median (IQR, n)	2.8 (2.2-3.2, 208)	3.0 (2.5-3.3, 30)	2.8 (2.2-3.2, 178)	NS
Month and year of death, minimum–maximum	July 1995–February 2021	February 2009–February 2020	July 1995–February 2021	…
Not immunized, % (n/N)	13 (33/258)	19 (5/27)	12 (28/231)	NS
Other children in household, % (n/N)	73 (160/220)	90 (19/21)	71 (141/199)	NS
Mother uneducated, % (n/N)	12 (19/155)	8 (2/25)	13 (17/130)	NS
Father uneducated, % (n/N)	7 (6/81)	5 (1/21)	8 (5/60)	NS

P values are provided for the comparison between community and in-hospital deaths. Abbreviations: IQR, interquartile range; NS, not significant; SD, standard deviation.

For example, the observed difference in proportion of neonatal deaths could be influenced by misclassification bias. To this end, age reported in months may have been rounded to age 1 month for deaths in the first month of life and subsequently these children would not be classified as neonatal deaths more often for the in-hospital group. In summary, for both rounding methods, age at death may have been underestimated for children dying in the hospital, which would mean that the difference in age between community and in-hospital deaths may have been underestimated. A third limitation of the study is the quality and completeness of the data. An inherent weakness in global data sharing is that primary data cannot be verified and data-collection systems differ in quality. With extensive data-quality checks and direct verification with collaborators, we attempted to limit the impact of this methodological weakness. For some variables (comorbidity, prematurity), a high proportion of data were missing.

More than 50% of community death data originate from a single study site (Zambia). Our conclusions regarding age at death did not change when excluding these deaths from the analysis. An important limitation is the difficulty of measuring mortality in the community, which may have resulted in missed deaths. Data from the community were from a small number of countries while hospital deaths were shared from a larger number of countries over a longer period of time, which may account for differences in the data. Data from most studies were obtained from systematic postmortem sampling, which may not be comparable to the way in which data were obtained for the in-hospital group and which could explain the different findings in the sensitivity analyses. For this reason, the age difference could also be explained by limitations in study methodology as children with RSV may present with nonspecific symptoms to the hospital at a younger age and not be tested in L(M)ICs. However, in a sensitivity analyses limited to comparable groups in-hospital and in the community, we observed the same trend of lower age at death in the community. Despite data-quality verification processes, there are major limitations of the study methodology as published previously [9].

Future steps should consist of analysis of a larger case series including more community deaths and a larger global distribution, which will allow for more robust conclusions regarding vaccine impact on infant mortality. Prospective, real-time data sharing of RSV-related death in L(M)ICs will contribute to increased data quality and completeness of data, including more detailed information on age at death, allowing for a better comparison between community and in-hospital RSV-related deaths. A uniform definition of RSV-related deaths in the community will allow for better collection and understanding of global community mortality. Future studies would be strengthened by enhanced systems for data collection of key clinical characteristics such as immunization status, prematurity, and comorbidity for community deaths, as this information was frequently missing for this population.

Conclusions

Community deaths are thought to represent more than half of all RSV-related deaths globally [17]. Characterizing these deaths is essential to estimate the impact of future preventive interventions. Due to global data sharing and efforts of BMGF-funded community mortality studies and other collaborators, the RSV GOLD database has served as a platform to aggregate robust data for analysis of RSV-related pediatric mortality on a global level. We show that infants under 6 months of age die at a younger age in the community than in-hospital. Modeling studies will have to translate these findings into expected impact of upcoming maternal vaccines and next-generation monoclonal antibodies against RSV. For the first time, we show evidence that maternal vaccination or infant monoclonal prophylaxis may have a greater impact on RSV-related community

mortality than in-hospital mortality. Ultimately, clinical trials and postmarketing surveillance studies will provide further evidence to evaluate the impact of these interventions on pediatric RSV mortality in the community versus in-hospital.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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DESCRIBING GLOBAL PEDIATRIC RSV DISEASE AT INTENSIVE CARE UNITS IN GAVI-ELIGIBLE COUNTRIES USING MOLECULAR POINT-OF-CARE DIAGNOSTICS: THE RSV GOLD-III STUDY PROTOCOL

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He who has a why to live for can bear almost any how.
Friedrich Nietzsche (1844-1900)

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Describing global pediatric RSV disease at intensive care units in GAVI-eligible countries using molecular point-of-care diagnostics: the RSV GOLD-III study protocol

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Abstract

Background: Respiratory syncytial virus (RSV) infection is an important cause of hospitalization and death in young children. The majority of deaths (99%) occur in low- and lower-middle-income countries (LMICs). Vaccines against RSV infection are underway. To obtain access to RSV interventions, LMICs depend on support from Gavi, the Vaccine Alliance. To identify future vaccine target populations, information on children with severe RSV infection is required. However, there is a lack of individual patient-level clinical data on instances of life-threatening RSV infection in LMICs. The RSV GOLD III—ICU Network study aims to describe clinical, demographic and socioeconomic characteristics of children with life-threatening RSV infection in Gavi-eligible countries.

Methods: The RSV GOLD-III—ICU Network study is an international, prospective, observational multicenter study and will be conducted in 10 Gavi-eligible countries at pediatric intensive care units and high-dependency units (PICUs/HDUs) during local viral respiratory seasons for 2 years. Children younger than 2 years of age with respiratory symptoms fulfilling the World Health Organization (WHO) "extended severe acute respiratory infection (SARI)" case definition will be tested for RSV using a molecular point-of-care (POC) diagnostic device. Patient characteristics will be collected through a questionnaire. Mortality rates of children admitted to the PICU and/or HDU will be calculated.

Discussion: This multicenter descriptive study will provide a better understanding of the characteristics and mortality rates of children younger than 2 years with RSV infection admitted to the PICU/HDU in LMICs. These results will contribute to knowledge on global disease burden and awareness of RSV and will directly guide decision makers in their efforts to implement future RSV prevention strategies.

Trial registration number: NL9519, May 27, 2021

Keywords: Respiratory syncytial virus, Children, Pediatric intensive care unit, Study design, Lower-middle-income countries, Burden, Awareness

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Background

Respiratory syncytial virus (RSV) infection is an important cause of hospitalization and mortality due to lower respiratory tract infection (LRTI) in children under 5 years of age worldwide [1]. Annual RSV-related hospital admissions and in-hospital deaths in this age group have been estimated to be 3.2 million and 59,600, respectively, while overall annual RSV-related mortality including community deaths could be as high as 118,200 [1]. The majority of deaths (99%) occur in low- and lower-middle-income countries (LMICs) due to lack of access to healthcare and poor quality of care in health facilities [1], and children under 2 years of age are disproportionally affected [2, 3]. As the Haemophilus influenza type b and pneumococcal conjugate vaccines are introduced and scaled up in LMICs, the global burden of child pneumonia attributable to bacterial causes has decreased and the proportional contribution of viral pathogens has increased. RSV now remains one of the major pathogens that needs to be tackled in order to achieve sustainable development goal 3.2—end preventable deaths of newborns and children under 5 years of age by 2030.

Currently there is no immunization available against RSV, although several vaccine and monoclonal antibody candidates are under clinical development [4]. The most advanced maternal vaccine candidate has completed a phase 3 trial but did not meet the primary endpoint [5]. A new extended half-life monoclonal antibody developed by SanofiPasteur / MedImmune, nirsevimab (previously MEDI8897), has met the primary endpoint of reducing RSV LRTI in healthy infants in a recent phase III trial [6].

Gavi, the Vaccine Alliance (previously: Global Alliance for Vaccines and Immunizations), is an international organization that invests in vaccines to protect children's lives and health in LMICs. Every 5 years, Gavi develops a new vaccine investment strategy (VIS) to prioritize new and under-used vaccines and to make these available to LMICs through the Gavi vaccine support programme. RSV interventions, including both maternal vaccine and monoclonal antibodies, were considered as one of the six prioritized vaccine programmes as part of Gavi VIS for the 2021–2025 funding period [7]. It is anticipated that future RSV vaccines or monoclonal antibodies will be most efficacious in targeting severe disease leading to poor outcome (e.g. oxygen supplementation, ICU admission, and death).

The majority of Gavi-eligible countries have sparse or no individual patient-level data to make decisions on target populations for RSV interventions when these become available in the next 5–10 years. These data will be important for cost-effectiveness analyses of potential RSV interventions to assist policy makers in making decisions related to resource allocation for RSV interventions [8]. Patient-data will also contribute to local disease awareness. Defining burden in terms of RSV incidence and case-fatality ratios in Gavi-eligible countries has been challenging due to insufficient diagnostic capabilities for RSV surveillance [9]. Interviews with stakeholders revealed that RSV prevention received low priority at national and government level due to lack of information about disease and disease burden, and some respondents suggested that RSV diagnostics would help to improve value proposition [10].

This study aims to obtain individual patient-level data from children who have been admitted with severe RSV infection at the (pediatric) intensive care unit (ICU) or high-dependency unit (HDU) in Gavi-eligible countries through implementation of RSV point-of-care testing to pave the way for future vaccine introduction.

Methods/design

Study design and study site selection

The RSV GOLD III study is a prospective, observational, multi-centre study and will be conducted at 11 sites in 10 Gavi-eligible countries. The study was initiated at the first study sites in April, 2021. The total duration of the study is 2 years for each participating study site. To select study sites, we sent out an open invitation for collaboration to researchers and physicians from various LMICs from the existing RSV GOLD network. Before the start of the study, we collected information about potential study sites through email correspondence and teleconferences. The minimum collected information included but was not limited to the location and catchment area of the hospital, the hospital staff to be in charge of performing the study, logistics of the hospital (languages spoken, freezer availability, respiratory seasonality), pediatric ward, pediatric or neonatal ICU, and (pediatric) HDU availability and number of beds, the annual number of respiratory illness-related admissions, and mortality rates. If admission data were not available, estimations were made by the study team based on number of beds and information from local collaborators. We selected study sites based on a high expected number of RSV inclusions, quality of the communication and engagement of local collaborators.

Study sites

The study will be conducted in the following LMICs: Afghanistan, Cameroon, Ghana, Haiti, Mozambique, Nepal (2 hospitals), Nigeria, Sudan, Tanzania, and The Gambia. The study will also be conducted at 2 sites in the Netherlands to allow for a comparison of patient characteristics with patients from a high-income country (HIC). Table 1 provides the characteristics of the participating LMIC study sites.

Table 1 Characteristics of the participating RSV GOLD III - ICU Network study sites in low- and lower-middle-income countries

City, Country	Hospital	Number of PICU Beds	Number of HDU Beds	Number of NICU beds	Estimated annual number of children < 2 years admitted to PICU / HDU / NICU with severe acute respiratory infection	Respiratory season
Zaria, Nigeria	Ahmadu Bello University Teaching Hospital	NA	32*	8	155	April–November
Mazar-e-Sharif, Afghanistan	Balkh regional hospital	35	NA	8	650	October–March
Banjul, The Gambia	Edward Francis Small Teaching Hospital	NA	20	37	100	October-May
Khartoum, Sudan	Jafar Ibn Auf Specialized Hospital for Children	8	9	16	100	December-May
Kathmandu, Nepal	Kanti Children's Hospital	12	8	16	130	July-March
Kathmandu, Nepal	Tribhuvan University Teaching Hospital	4	6	8	32	July-March
Douala, Cameroon	Laquintinie Hospital Douala	20	NA	NA	180	September-January April-June
Accra, Ghana	Korle Bu Teaching Hospital	6	18	50**	70	June-November
Maputo, Mozambique	Maputo Central Hospital	21	NA	70**	85	March-August
Dar es Salaam, Tanzania	Muhimbili National Hospital	13	NA	19**	270	December-May
Port-au-Prince, Haiti	Saint-Damien Hospital	10	NA	16**	40	August-January

PICU pediatric intensive care unit, *HDU* high dependency unit, *NICU* neonatal intensive care unit, *NA* not available

*Emergency unit serves as HDU

**No recruitment

Study objectives

Primary objective

To describe the clinical, demographic, and socioeconomic characteristics of RSV-positive children under 2 years of age who have been admitted with suspected RSV infection at ICUs or HDUs in Gavi-eligible countries.

Secondary objectives

1. To determine proportional RSV-related mortality in children under 2 years of age at participating ICUs or HDUs.
2. To compare clinical, demographic, and socioeconomic characteristics between children with fatal and non-fatal RSV infection.
3. To compare clinical, demographic, and socioeconomic characteristics between children with RSV infection from LMICs and HICs.
4. To describe RSV seasonality in the study locations.
5. To compare clinical, demographic, and socioeconomic characteristics between children with (fatal) RSV infection and children with (fatal) influenza infection.
6. To confirm the point-of-care (POC) RSV test using conventional or real-time RSV PCR.
7. To estimate the sensitivity of the World Health Organization (WHO) "extended severe acute respiratory infection (SARI)" case definition for hospital-based surveillance for severe RSV infection. [11]
8. To compare the burden of RSV infection and mortality rates between children who do and do not meet the WHO "extended SARI" case definition.

Study participants

For this study, 2 different groups of children (A and B) are distinguished. For each group, a subject must meet all the eligibility criteria in order to participate (Table 2). Children from group A will be tested for RSV at all study sites and for influenza at 3 study sites (Ghana, Mozambique, Nepal) (Fig. 1):

Group A. Children with suspected RSV disease (all study sites)

1. Children < 2 years of age at time of sampling;
2. Who are admitted to an ICU and/or HDU and meet the WHO "extended SARI" case definition;

Table 2 Eligibility criteria for RSV GOLD III—ICU Network Study

Inclusion criteria	
Group A	**Group B**
Age < 2 years at time of sampling; Admitted to PICU / HDU / NICU; Meeting WHO "Extended SARI" case definition: Severe (defined as requiring hospitalization); and Acute (defined as onset within the last 10 days); and Respiratory infection (defined as having cough or shortness of breath) In infants less than 6 months, additionally include those who present with: Apnea (temporary cessation of breathing from any cause); and/or Sepsis, defined as: Fever (37.5 °C or above) or hypothermia (less than 35.5 °C); and Shock (defined as lethargy, fast breathing, cold skin, prolonged capillary refill or fast weak pulse); and Seriously ill with no apparent cause Signed and dated written informed (deferred) consent obtained from the parent(s)/legal representative(s) of the subject, or in accordance with local regulations	Age < 2 years at time of sampling; Admitted to PICU / HDU / NICU; Signed and dated written informed (deferred) consent obtained from the parent(s)/legal representative(s) of the subject, or in accordance with local regulations

PICU pediatric intensive care unit, *HDU* high dependency unit, *NICU* neonatal intensive care unit, *WHO* world health organization, *SARI* severe acute respiratory infection

Children who do not meet the WHO "extended SARI" case definition will be tested for RSV at 3 study sites (group B):

Group B. Children who are not suspected to have RSV disease (Ghana, Mozambique, Nepal)

1. Children < 2 years of age at time of sampling;
2. Who are admitted to an ICU and/or HDU and do not meet the WHO "extended SARI" case definition;

WHO "extended SARI" RSV surveillance case definition

In this study, the global case definition developed by the WHO for hospital-based RSV surveillance is used to identify children hospitalized with suspected RSV infection. The RSV surveillance case definition was recently modified based on results from the WHO RSV surveillance pilot which showed that the use of an *extended* SARI case definition, not requiring fever to identify a suspect case, substantially increased the number of RSV infections detected [3].

In group A, subjects will be tested for RSV when they are admitted to the ICU meeting the WHO "extended SARI" case definition for hospital-based surveillance for severe RSV infection:

- Severe (defined as requiring hospitalization); and
- Acute (defined as onset within the last 10 days); and
- Respiratory infection (defined as having cough or shortness of breath)

In infants less than 6 months, additionally include those who present with:

- Apnea (temporary cessation of breathing from any cause); and/or
- Sepsis, defined as:
- Fever (37.5 °C or above) or hypothermia (less than 35.5 °C); and
- Shock (defined as lethargy, fast breathing, cold skin, prolonged capillary refill or fast weak pulse); and
- Seriously ill with no apparent cause

In group B subjects not fulfilling the WHO "extended SARI" case definition will also be tested for RSV.

Exclusion criteria

Neonates younger than 4 days old will not be tested for RSV or influenza due to the high incidence of respiratory symptoms related to intrapartum-related complications or prematurity in this group.

Sample collection

A nasopharyngeal swab (flocked swab, COPAN, 3 ml universal transport medium (UTM)) will be obtained as soon as possible but no later than 72 h after admission to the PICU and/or HDU. Samples will be taken by trained healthcare staff.

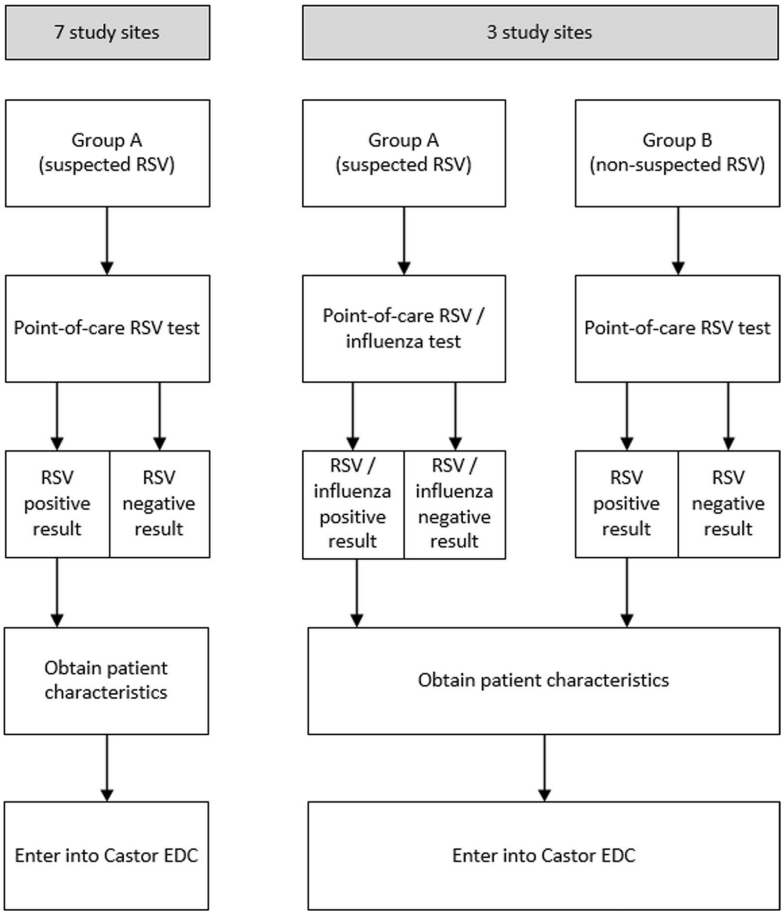

Fig. 1 Schematic diagram of the study workflow. Group A: children < 2 years admitted to the PICU and/or HDU meeting the WHO "extended SARI" case definition (10 study sites). Group B: children < 2 years admitted to the PICU and/or HDU not meeting the WHO "extended SARI" case definition (3 study sites: Ghana, Mozambique, Nepal)

Point-of-care testing

The samples will be tested for RSV and influenza using the highly sensitive and specific point-of-care (POC) ID NOW test [12–14]. On-site training for local study staff on performing the POC test will be provided by the RSV GOLD team. If site visits are not possible, the training will be given online. Refresher training will be provided before the start of each new respiratory season or if required by the study sites.

Point-of-care test confirmation

Although the POC ID NOW RSV test has shown high sensitivity and specificity of 100% and 97%, respectively, in previous studies [13], polymerase chain reaction (PCR) remains the gold standard for confirmation of a positive or negative test. POC RSV test confirmation is an optional part of the protocol. Samples will preferably be shipped to the University Medical Centre Utrecht (UMCU) laboratory for conventional or real-time PCR testing in order to confirm the POC RSV test and ensure

quality of the data. Researchers may decide to perform viral testing and/or sequencing at a local, national or academic reference laboratory accredited to international quality standards with validated conventional or real-time RSV PCR tests when the capacity is available or when shipment of samples to UMCU is not possible.

Per study site, the following samples will be confirmed:

- All RSV-positive samples.
- A matched (by age and month of admission) number of RSV-negative samples.

Sample storage

Nasopharyngeal samples will be stored in freezers before shipment. For budget restrictions, influenza-positive samples will not be confirmed through PCR and will not be stored.

RSV sequencing

RSV sequencing is an optional part of the protocol. Samples sent for confirmation will be stored at the UMCU or study site to allow for sequencing if the sample is RSV-positive. Investigating the molecular heterogeneity of RSV isolates can be important to determine susceptibility or resistance to future RSV monoclonal antibodies or vaccines.

Data collection and data management

From each study site baseline data will be obtained using questionnaires to evaluate local clinical treatment and management availability and standards. Included patients will be followed up until death or discharge. Participant data will be collected through a case report form and parental questionnaire (Additional file 1: Table S1). These data will be entered by hospital study staff into Castor Electronic Data Capture (EDC) system [15]. Data validation, data analysis and interpretation of the data will be performed by the Utrecht-based study team in collaboration with the site investigators.

Sample size

RSV

The aim is to include all children < 2 years of age meeting the case definition admitted to the PICU and/or HDU at each study site each year during the respiratory season. There is no maximum number of patients each study site needs to recruit. We estimated the following number of inclusions for all study sites (Table 3):

Table 3 Estimated number of RSV-positive patients and RSV-related deaths

	RSV-positive patients per study site (N, range)	Total RSV-positive patients (N)	Total RSV-related deaths (N)
Group A	30–120	840	84
Group B	10–30	60	6
Total	NA	900	90

Group A

Based on an estimated number of admissions, we expect to recruit 2800 patients across 10 study sites, 100–400 patients at each study site. Assuming 30% of patients who fulfill the WHO case definition "extended SARI" will have a positive RSV test, we expect to capture 840 RSV-positive children, 30–120 children at each study site. Assuming a mortality rate of 10% we expect approximately 3–12 RSV-related deaths at each study site, in total N = 84 deaths. We consider this number sufficient for descriptive purposes.

If 2800 patients will be recruited and 30% RSV-positives are observed (N = 840), this produces a two-sided 95% Clopper-Pearson confidence interval with a width equal to 0.034, ranging from 28 to 32%. In the smallest estimate, 100 patients will be recruited per site. With an assumed RSV-positive sample proportion of 30%, a sample size of 100 patients produces a two-sided 95% confidence interval with a width equal to 0.187, with a lower limit of 21% and an upper limit of 40%.

In 840 RSV-positive patients, an observed proportion of mortality of 10% will produce a two-sided 95% confidence interval with a width equal to 0.042, producing a lower limit of 8.1% and an upper limit of 12.2%. In the smallest estimate, 100 patients will be recruited per site. With an observed mortality proportion of 10%, a sample size of 100 patients produces a two-sided 95% confidence interval with a width equal to 0.127, corresponding to a lower limit of 4.9% and an upper limit of 17.6%. We consider those estimates sufficiently precise.

Group B

We expect to recruit 1200 patients in 3 study sites, 200–600 patients at each study site. Assuming 5% of patients will have a positive RSV test, we expect to capture 60 RSV-positive children, 10–30 children at each study site. Assuming a mortality rate of 10% in children who tested positive for RSV, we expect an additional 1–3 RSV-related deaths at each study site, in total N = 6 deaths.

When the sample proportion is 5%, a sample size of 1200 patients produces a two-sided 95% confidence interval with a width equal to 0.026, corresponding to a confidence interval from 3.8 to 6.4%.

In 60 RSV-positive patients, an observed proportion of mortality of 10% will produce a two-sided 95%

confidence interval with a width equal to 0.167, with a lower limit of 3.8% and an upper limit of 20.5%.

Sensitivity WHO case definition

The sensitivity of the WHO "extended SARI" case definition will be calculated using the total population from the 3 sites that included group A and B for RSV testing. In total, we expect to recruit 800 patients from group A and 1200 patients from group B at 3 study sites, in total 2000 patients. We will not adapt the sample size for this purpose as this is a secondary endpoint of the study.

Influenza (secondary objective)

Per site we expect to test for influenza in 100–400 (group A) children. Assuming a 5% positivity rate we expect to capture 42 influenza-positive children, 5–20 children at each study site. Assuming a mortality rate of 10%, we expect approximately 1–2 influenza-related deaths at each study site for group A, in total N = 4 deaths.

Statistical analysis

We will describe characteristics of RSV-positive children. Chi-square tests and nonparametric tests will be used to compare clinical and demographic characteristics between children where appropriate. RSV-positive children from group A and group B will be presented as proportions with 95% confidence intervals. The estimated case fatality ratio in RSV positive children will be provided with 95% confidence intervals. We will also report the mortality rate with 95% confidence intervals, and total RSV-related mortality in group A and B. Subgroup analysis per site will also be performed. We will calculate the sensitivity of the WHO case definition "extended SARI". We will divide the number of RSV-positive children meeting the case definition (group A) by the total number of RSV-positives regardless of whether the case definition was met and express it as a percentage. No formal statistical analysis plan was written before the start of this descriptive study.

Burden of disease

The estimated number of RSV-related PICU and/or HDU admissions and deaths in children with respiratory infection in the specific country of participating study sites will be quantified. A numerator (the number of POC-confirmed RSV-related admissions and deaths at the PICU and/or HDU) and denominator (population in the hospital catchment area) will be defined. In case the catchment population is not readily available because the facility is not the only one providing in-patient care to the population, it will be estimated based on reviewing hospital administrative datasets and using a hospital admission survey [16]. In the case of limited administrative data or limited resources to perform a hospital admission survey, burden of RSV disease may be described in terms of the proportion of RSV-related PICU and/or HDU admissions (or deaths) among all PICU and/or HDU admissions with LRTI. Other markers of disease burden will also be reported, such as length of stay, duration of oxygen supplementation, etc.

Ethical considerations

The study will be conducted according to the principles of the Declaration of Helsinki (version 2013), and local law and regulations. Risks and burdens for study subjects are considered minimal. No other safety issues are expected due to the set-up and nature of the study. No Data Safety Monitoring Board will be appointed and no (Severe) Adverse Events will be reported. Written informed consent will be obtained from each patient-participant by research staff or in accordance with local regulations prior to enrolment in the study.

The intended benefits resulting from this study can be divided into 1) direct benefits and 2) indirect benefits. The primary direct benefit for study participants is timely and proper diagnosis of RSV infection which may result in the prevention of unnecessary or inappropriate use of antibiotics. Secondary direct (patient) benefits consist of:

a) The ability to determine that RSV is not the cause of disease and that an alternative diagnosis should be considered in case of a negative test;
b) The ability to provide parents of RSV-positive children who have been admitted to PICU and/or HDU or who died with information about the cause of or contribution to death.

Indirect (societal) benefits consist of:

a) The ability to provide information on disease burden and target populations to policy makers when a vaccine becomes available;
b) Giving medical staff insights into the incidence of RSV/influenza-related admissions and mortality at their hospital;
c) Identifying RSV and influenza as important causes of PICU and/or HDU admission and death;
d) Capacity building by supplying a reliable POC test to confirm or rule out RSV and influenza as a cause of respiratory infection;
e) Capacity building through involving local site investigators in conducting clinical research;
f) Increasing overall RSV awareness of hospital staff and parents of young children.

For children from HICs, no direct benefits apply, as RSV testing is part of routine care.

Dual ethical review was performed to ensure that the ethical standards in this study are no less stringent than those applicable in the country of the sponsoring organization. This protocol was therefore submitted for ethics review in The Netherlands as well as to all local (and/or national) research ethics committees.

Discussion

Although global estimates show a high RSV burden in children from LMICs, individual patient-level data are lacking due to limited availability of RSV testing in these countries. The critical lack of diagnostic capacity hampers the ability to distinguish RSV from other causes of severe respiratory infection in children to justify the need for vaccine introduction in LMICs. Disease awareness is essential to introduce RSV interventions, which will become available in the near future. RSV GOLD is the first global online registry for children younger than 5 years who have died with RSV infection. The first results of the RSV GOLD study have been previously published [2, 17]. The registry was extended after publication (RSV GOLD II) and data collection is still ongoing. In order to increase real-time data collection from LMICs, funding was obtained to establish a network of PICUs in 10 different Gavi-eligible countries (RSV GOLD III).

The RSV GOLD III—ICU Network study is a novel collaboration between RSV GOLD and 10 study teams from 3 different continents, aiming at collecting individual patient-level data of young children with severe RSV infection through POC RSV testing. Data analyses will provide insights into potential risk factors for fatal RSV infection at the PICU and/or HDU and differences between patients from various income settings.

Some challenges remain for this type of study. First, this study will take place mainly in tertiary level facilities in urban areas where access to healthcare is likely better than in rural areas. Results may therefore not be representative of the whole country. Second, the definition of an ICU and HDU may differ from country to country and even within a single healthcare system. For example, in some countries, the capacity to mechanically ventilate differentiates a PICU bed from a HDU bed, while in other countries, a PICU bed may be defined as a bed within a hospital area with a higher patient: nurse ratio. In 2017, the task force of The World Federation of Societies of Intensive and Critical Care Medicine proposed a global definition and stratified ICUs based on the intensity of care provided [18]. For this study, we included both PICUs and HDUs according to the definition of the participating study sites, where the most severely ill children are usually admitted. To characterize differences between participating PICUs and HDUs, we will collect information on the capacity of care, such as the number of available ventilators and attending healthcare staff. Third, due to budgetary constraints, we are unable to extend RSV testing to the regular wards. We will therefore likely miss a proportion of potential study participants in case PICU and/or HDU beds are occupied and children with severe respiratory infection are admitted to the regular wards instead. We will make an effort in collecting information on the number of refusals to estimate the potential impact of this study limitation. Also, influenza testing is limited to 3 study sites. However, this study will provide insights into the characteristics of hospitalized children with severe RSV infection in LMICs including complete granular age distribution data which can be used for modelling studies on the impact of upcoming maternal vaccines and monoclonal antibodies against RSV. Fourth, for some study sites it may be difficult to calculate a catchment population due to the presence of other PICUs in the area. We will collect information on the number of other available PICUs and will adjust for this in our calculations. Based on preliminary data we estimate that 2–4 study sites will not have sufficient data to estimate the catchment population.

Finally, the SARS-CoV2 pandemic may affect the number of respiratory admissions, thus study results may not be representative of regular respiratory seasons in participating countries. Since the duration of the study is 2 years and most of the recruitment will take place in 2022, we do not expect this to be a major limitation.

In summary, this global prospective multicenter study will provide a better understanding of the characteristics and mortality rates of children younger than 2 years who are admitted to the PICU and/or HDU with severe RSV infection in LMICs. These results will not only contribute to knowledge on global disease burden and awareness of RSV, but will also provide valuable information to healthcare policy makers on the impact of future RSV prevention strategies.

Abbreviations

RSV: Respiratory Syncytial Virus; LRTI: Lower respiratory tract infection; LMIC: Low- and lower-middle income country; VIS: Vaccine investment strategy; ICU: Intensive care unit; HDU: High-dependency unit; NICU: Neonatal intensive care unit; HIC: High-income country; WHO: World Health Organization; SARI: Severe acute respiratory infection; UTM: Universal transport medium; POC: Point-of-care; PCR: Polymerase chain reaction; UMCU: University Medical Centre Utrecht; EDC: Electronic data capture.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-021-06544-3.

Additional file 1. Table S1. Patient variables collected.

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Authors' contributions
LB and YL designed and co-authored the protocol. All site investigators from the RSV GOLD III—ICU Network study group reviewed the protocol and provided feedback. YL and LB drafted the initial manuscript. All authors read, commented on and approved the final manuscript version. All authors read and approved the final manuscript.

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Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
The study protocol (version 1.0, date 01-08-2020) was reviewed by the Research Ethics Committee of University Medical Centre Utrecht (METC Utrecht), the Netherlands (20-536). A waiver was provided for ethics review of participating Dutch sites (date 29-09-2020). For dual review purposes, the METC Utrecht reviewed the protocol with a focus on ethical conduct of research in low resource settings, using the Declaration of Helsinki and CIOMS guidelines. The study protocol was subsequently reviewed by all site investigators and comments were incorporated into the new version (version 1.2, date 15-11-2020). Ethics approval was obtained by the Institutional Review Boards of participating study sites:Afghanistan: Institutional Review Board, Ministry of Public Health: A.0121.0263;Cameroon: Cameroon Bioethics Initiative Ethics Review and Consultancy Committee: 1156;Ghana: Korle Bu Teaching Hospital Scientific and Technical Committee: KBTH-STC 00056/2021;Haiti: Comité National de Bioéthique c/o Association Médicale Haitienne: Ref 2021-19;Mozambique: Comité Nacional de Bioética para Saúde: IRB00002657; Ref:344/CNBS/21;Nepal: Ethical Review Board (ERB), Nepal Health Research Council (NHRC): 849/2020; Institutional Review Committee (IRC), Institute of Medicine, Tribhuvan University: 391/077/078; Institutional Review Committee (IRC), Kanti Children's Hospital: 50/2020-021;Nigeria: Health Research Ethics Committee, Ahmadu Bello University Teaching Hospital Shika, Zaria, Nigeria: ABUTHHZ/HREC/H22/2021;Sudan: National Health Research Ethics Review Committee: 7-1-21;Tanzania: Muhimbili National Hospital Institutional Ethics Review Board: MNH/IRB/I/2021/39;The Gambia: Edward Francis Small Teaching Hospital Research & Ethics Committee: 27th July, 2021.The study has been registered in the Netherlands Trial Register (NL9519) on May 27, 2021.Informed consent will be obtained from the parent(s) or legal representative(s) by research staff prior to enrolment in the study.

Consent for publication
Not applicable.

Competing interests
LJB has regular interaction with pharmaceutical and other industrial partners. He has not received personal fees or other personal benefits. UMCU has received major funding (>€100,000 per industrial partner) for investigator-initiated studies from AbbVie, MedImmune, Janssen, the Bill and Melinda Gates Foundation, Nutricia (Danone) and MeMed Diagnostics. UMCU has received major cash or in kind funding as part of the public private partnership IMI-funded RESCEU project from GSK, Novavax, Janssen, AstraZeneca, Pfizer and Sanofi. UMCU has received major funding by Julius Clinical for participating in the INFORM study sponsored by MedImmune. UMCU has received minor funding for participation in trials by Regeneron and Janssen from 2015 to 2017 (total annual estimate less than €20,000). UMCU received minor funding for consultation and invited lectures by AbbVie, MedImmune, Ablynx, Bavaria Nordic, MabXience, Novavax, Pfizer, Janssen (total annual estimate less than €20,000). LJB is the founding chairman of the ReSViNET Foundation. NIM has regular interaction with pharmaceutical and other industrial partners. She has not received personal fees or other personal benefits. HN reports grants from Innovative Medicines Initiative and National Institute of Health Research; personal fees and grants from WHO and Sanofi; and personal fees from the Bill & Melinda Gates Foundation, Janssen, Abbvie, and Reviral.GvT is involved in public private partnerships (EU Innovative Medicines Initiative consortia). She has not received personal fees or other personal benefits.

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Publisher's Note

4

SEVERITY OF RESPIRATORY SYNCYTIAL VIRUS LOWER RESPIRATORY TRACT INFECTION WITH VIRAL COINFECTION IN HIV-UNINFECTED CHILDREN

Clinical Infectious Diseases, 2016

The question is not what you look at, but what you see.
Henry David Thoreau (1817-1862)

Clinical Infectious Diseases

MAJOR ARTICLE

Severity of Respiratory Syncytial Virus Lower Respiratory Tract Infection With Viral Coinfection in HIV-Uninfected Children

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Background. Molecular diagnostics enable sensitive detection of respiratory viruses, but their clinical significance remains unclear in pediatric lower respiratory tract infection (LRTI). We aimed to determine whether viral coinfections increased life-threatening disease in a large cohort.

Methods. Molecular testing was performed for respiratory viruses in nasopharyngeal aspirates collected from children aged <5 years within 24 hours of hospital admission during sentinel surveillance for severe acute respiratory illness (SARI) hospitalization conducted in South Africa during February 2009–December 2013. The primary outcome was life-threatening disease, defined as mechanical ventilation, intensive care unit admission, or death.

Results. Of 2322 HIV-uninfected children with respiratory syncytial virus (RSV)–associated LRTI, 1330 (57.3%) had RSV monoinfection, 38 (1.6%) had life-threatening disease, 575 (24.8%) had rhinovirus, 347 (14.9%) had adenovirus (ADV), and 30 (1.3%) had influenza virus. RSV and any other viral coinfection was not associated with severe disease (odds ratio [OR], 1.4; 95% confidence interval [CI], OR, 0.74; 95% CI, .39–1.4), ADV coinfection had increased odds of life-threatening disease (adjusted OR, 3.4; 95% CI, 1.6–7.2; $P = .001$), and influenza coinfection had increased odds of life-threatening disease and prolonged length of stay (adjusted OR, 2.1; 95% CI, 1.0–4.5; $P = .05$) compared with RSV monoinfection.

Conclusions. RSV coinfection with any respiratory virus is not associated with more severe disease when compared to RSV alone in this study. However, increased life-threatening disease in RSV-ADV and RSV-influenza coinfection warrants further study.

Keywords. respiratory syncytial virus; viral coinfection; lower respiratory tract infection disease severity.

Respiratory syncytial virus (RSV) is a global health problem, causing an estimated 66 000–199 000 deaths per year globally in children <5 years of age [1]. The clinical manifestations of RSV infection range widely from a mild, self-limiting upper respiratory tract infection (URTI) to severe lower respiratory tract infection (LRTI), which may lead to death. Risk factors for severe disease include premature birth, low birth weight, immunocompromised status, chronic lung disease, congenital heart disease, human immunodeficiency virus (HIV) infection, and Down syndrome [2–8]; however, the majority of infants hospitalized for RSV LRTI are previously healthy children [9].

Currently, management options for RSV-associated disease are limited, with supportive treatment as the cornerstone of clinical care [10]. Therefore, it is essential to gain insight into factors contributing to disease severity to effectively direct future preventive and therapeutic interventions.

The development of sensitive molecular diagnostics for the detection of respiratory viruses has given insight into the viral respiratory dynamics during severe respiratory infection [11]. There are conflicting data on whether viral coinfection results in more severe RSV-associated LRTI. Whereas some studies report an association for RSV–human metapneumovirus (HMPV) coinfection and less severe disease [12–14], others

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report more severe disease associated with RSV-HMPV, RSV-rhinovirus (RV), RSV-adenovirus (ADV), and any coinfection compared to identification of RSV alone [15–21]. Furthermore, no association with disease severity for RSV-RV, RSV-HMPV, and any viral coinfection has been reported by others [13, 22–25]. The majority of these studies are limited by assessment over a single season [15, 18, 26], lack of adjustment for confounders [13,15,20,22–25], and small sample size (38–666 RSV cases), all of which could bias the interpretation of the results.

The aim of this study was to evaluate the effect of respiratory viral coinfection on disease severity among children hospitalized with RSV-associated LRTI.

METHODS

Study Site, Design, and Population

Children <5 years of age hospitalized with severe acute respiratory illness (SARI) were enrolled in a prospective, hospital-based, sentinel surveillance study conducted at 6 sites in 4 provinces in South Africa from February 2009 through December 2013 as described elsewhere [27]. Four rural, periurban, and urban hospital sites enrolled children in 3 provinces (Gauteng, Mpumalanga, KwaZulu-Natal), and 2 sites were added in a fourth province (North West) in June 2010. There were a total of 24 pediatric intensive care unit (ICU) beds available across all sites.

Data Collection and Case Definition

SARI was defined among hospitalized children as follows: physician-diagnosed sepsis or LRTI in children aged 2 days to 3 months; or physician-diagnosed LRTI in children aged 3 months to 5 years, presenting within 7 days of symptom onset. Exclusion criteria were transfer from another hospital, neonates who were never discharged after delivery, and children residing outside of the hospital catchment area. A nasopharyngeal aspirate (NPA) in 4 mL of normal saline and a blood sample were collected from the child, ideally within 24 hours of admission but up to 7 days after onset of symptoms. Specimens were transported within 72 hours of collection to the National Institute for Communicable Diseases in Johannesburg for viral and bacterial analysis. Demographic and hospitalization data were collected by interview and record review, and children were followed up to hospital discharge.

Laboratory Testing

RSV infection was confirmed via multiplex real-time reverse-transcription polymerase chain reaction (PCR) assay performed on collected NPAs. NPAs were also tested for 9 other viruses: ADV, parainfluenza viruses 1, 2, and 3 (PIV1–3), influenza A and B viruses, HMPV, RV, and enterovirus (EV) with the same molecular testing technique [28]. RV clades A, B, and C were detected in the primer set utilized [29]. ADV testing was not done from August to October 2009 due to limited availability of reagents [28]. PCR data were semiquantitative and specimens with a cycle threshold (Ct) value <37 were considered positive. To detect pneumococcal infection, both blood culture for *Streptococcus pneumoniae* and whole blood *lytA* PCR were performed on blood specimens, although blood cultures were not systematically performed on all patients [30]. HIV testing was performed on a whole blood specimen or dried blood spot using an HIV PCR assay for children <18 months of age and HIV enzyme-linked immunosorbent assay for children ≥18 months of age. Quality Control for Molecular Diagnostics external quality assessments for all viruses in the panel were performed as well as annual World Health Organization panels for influenza alongside live and post hoc data quality checks.

Outcomes

The primary outcome of this study, life-threatening disease, was defined as a composite outcome of mechanical ventilation, ICU admission, or death. The secondary outcome was life-threatening disease or prolonged length of hospital stay ≥5 days.

Statistical Analyses

Continuous variables were described using mean (standard deviation) or median (interquartile range [IQR]). Differences in mean/median of continuous variables were tested with the 2-sided *t* test or a nonparametric Mann-Whitney test when appropriate. Categorical variables were described with frequencies and percentages and compared between groups using χ^2 test or Fisher exact test if there were <5 observations in one group.

Logistic regression was used to assess the association between any viral coinfection (at least one of the following viruses detected: HMPV, RV, ADV, EV, influenza, PIV1, PIV2, PIV3) and virus-specific coinfections on the study outcomes as described above among RSV-positive children. In addition, we compared ADV-RSV and influenza-RSV coinfections to ADV and influenza monoinfection, as coinfection with these pathogens among RSV-positive children was found to be associated with increased risk of life-threatening disease. This analysis was implemented to assess whether ADV and influenza monoinfection were the driver of severe disease. Results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Multivariate logistic regression was performed by use of the manual forward stepwise procedure including variables with a P value < .2 in univariate analyses. The analysis was adjusted for age using the subgroups <6 months and ≥6 months. The primary analysis was conducted on the HIV-uninfected population; subsequently, a separate analysis was performed for the HIV-infected population due to significantly elevated mortality rate and altered immune status of this subgroup.

We considered $P < .05$ to be significant for all analyses. Statistical analysis was performed using STATA/SE software, version 13.1 (StataCorp, College Station, Texas).

Ethical Considerations

The study protocol was approved by the University of the Witwatersrand Ethics Committee and the KwaZulu-Natal Human Biomedical Research Ethics Committee (protocol M081042 and BF157/08). Details of consenting, which included written informed consent from the parent or primary caregiver of the child, have been described [30]. This surveillance was deemed nonresearch by the US Centers for Disease Control and Prevention (NRD 2012 6197).

RESULTS

Study Population

During February 2009 to December 2013, 10 128 children <5 years of age were enrolled, including 2404 (23.7%) with RSV-associated LRTI. Our total HIV-uninfected population with RSV-associated LRTI was 2322 children. We performed a sensitivity analysis to validate HIV status and found that the untested and HIV-negative population did not differ in baseline characteristics or underlying conditions, and both had a similar mean RSV Ct value of 25.1 (SD, 5.1) and 25.7 (SD, 4.6) (P = .004), respectively (Supplementary Table 1).

Prevalence of Viral Coinfection

Table 1 details the prevalence of respiratory virus coinfections among children hospitalized for RSV-associated LRTI, including stratification by age groups <6 months and ≥6 months of age. The prevalence of any respiratory viral coinfection was more common among children aged ≥6 months (529 [51.1%]) compared with those aged <6 months (463 [36.0%]) (P < .001). The prevalence of RSV-PIV1, RSV-PIV2, and RSV-PIV3 coinfection was <1% in both groups. Rhinovirus was the most prevalent coinfecting virus, found among 23.5% of children aged <6 months and 26.4% of children aged ≥6 months; followed by RSV-ADV coinfection (8.3% and 23.2% in children aged <6 months and ≥6 months, respectively; P < .001) and RSV-EV coinfection (5.6% and 11.5% in children aged <6 months and ≥6 months, respectively; P < .001). The different permutations of viral coinfections in the RSV-infected population are elucidated in a coinfection matrix (Supplementary Table 2).

We compared the prevalence of viruses in the presence (n = 2404) or absence of RSV (n = 7447) and found that the presence of RSV was associated with a lower prevalence of all other respiratory viruses during RSV season (Supplementary Figure 1). In the RSV-negative population, 19.3% (1436/7447) of children hospitalized for LRTI had 2 or more viruses detected in the respiratory tract, with the most prevalent viruses being RV and ADV, respectively.

Demographic and Clinical Characteristics

We examined the prevalence of demographic and clinical characteristics among RSV monoinfection cases and those with any respiratory virus coinfection, stratified by age <6 months and ≥6 months. A total of 1287 children were aged <6 months and 1035 were aged ≥6 months. The median age for RSV monoinfection was 4.2 months (IQR, 1.9–9.6 months), and 6.6 months (IQR, 3.0–14.7 months) for RSV with any viral coinfection. Age was associated with RSV and any viral coinfection in both children <6 months of age (P < .001) and aged ≥6 months (P = .05; Table 2).

We described the demographics and underlying conditions of RSV monoinfection and coinfections in Table 2. Underlying conditions were not more prevalent in viral coinfection than in RSV monoinfection (P = .29 for children aged <6 months; P = .38 for children aged ≥6 months).

Respiratory Viral Coinfections and Disease Severity

Within the RSV-positive population <5 years old, 26 children (1.1%) were admitted to the ICU, 21 children (0.90%) needed

Table 1. Respiratory Viral Coinfections, Stratified by Age Group, in HIV-Uninfected Children Aged <5 Years With Respiratory Syncytial Virus–Associated Lower Respiratory Tract Infection at 6 Sentinel Sites in South Africa, 2009–2013

	All Ages (N = 2322)		<6 mo (n = 1287)		≥6 mo (n = 1035)		
Infection	Frequency	(%)	Frequency	(%)	Frequency	(%)	*P* Value[a]
RSV monoinfection	1330	(57.3)	824	(64.0)	506	(48.9)	<.001
RSV + any coinfection[b]	992	(42.7)	463	(36.0)	529	(51.1)	<.001
RSV-HMPV	26	(1.1)	16	(1.2)	10	(0.97)	.53
RSV-RV	575	(24.8)	302	(23.5)	273	(26.4)	.11
RSV-ADV	347	(14.9)	107	(8.3)	240	(23.2)	<.001
RSV-EV	191	(8.2)	72	(5.6)	119	(11.5)	<.001
RSV-Influenza	30	(1.3)	11	(0.85)	19	(1.8)	.04
RSV-PIV1	12	(0.52)	2	(0.16)	10	(0.97)	.01
RSV-PIV2	12	(0.52)	4	(0.31)	8	(0.77)	.12
RSV-PIV3	20	(0.86)	12	(0.93)	8	(0.77)	.70

Abbreviations: ADV, adenovirus; EV, enterovirus; HIV, human immunodeficiency virus; HMPV, human metapneumovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus; RV, rhinovirus.

[a]The *P* value is given for a comparison of the prevalence of a certain coinfection in the age group <6 mo and ≥6 mo.

[b]Any viral respiratory coinfection with HMPV, RV, ADV, EV, influenza, PIV1, PIV2, or PIV3.

Table 2. Demographic and Clinical Characteristics, Stratified by Age Group, for Any Coinfection or Respiratory Syncytial Virus Monoinfection in HIV-Uninfected Children <5 Years of Age at 6 Sentinel Sites in South Africa, 2009–2013

Characteristic	<6 mo			≥6 mo		
	RSV Monoinfection (n = 824)	Any Coinfection[a] (n = 463)	*P* Value	RSV Monoinfection (n = 506)	Any Coinfection[a] (n = 529)	*P* Value
Demographics						
Age, mo, median (IQR)	2.4 (1.3–3.8)	2.8 (1.9–4.0)	<.001	12.8 (8.1–21.0)	14.0 (9.0–22.7)	.05
Female sex	354/824 (43.0)	199/463 (43.0)	.99	223/506 (44.1)	227/529 (42.9)	.71
Race, black	810/823 (98.4)	454/463 (98.1)	.63	493/504 (97.8)	519/528 (98.3)	.58
Duration of symptoms, d, median (IQR)	2 (1–3)	2 (1–3)	.44	2 (1–3)	2 (1–3)	.16
Premature birth[b]	16/822 (2.0)	12/463 (2.6)	.45	4/503 (0.80)	5/528 (0.95)	.99
DOB within 10 wk of start of RSV season	524/824 (63.6)	295/463 (63.7)	.97	182/506 (36.0)	210/529 (39.7)	.22
RSV Ct value, mean (SD)	24.8 (4.5)	25.6 (4.6)	.002	25.8 (4.6)	26.3 (5.3)	.15
Crowding (≥5 people in the household)	82/813 (10.1)	57/455 (12.5)	.18	31/499 (6.2)	56/525 (10.7)	.01
Underlying conditions[c]						
Underlying illness	20/823 (2.4)	16/463 (3.5)	.29	15/505 (3.0)	21/528 (4.0)	.38
Whole blood PCR + *Streptococcus pneumoniae*	29/453 (6.4)	6/254 (2.4)	.02	13/297 (4.4)	18/277 (6.5)	.26
Outcome						
Primary outcome	17/810 (2.1)	12/460 (2.6)	.56	2/496 (0.4)	7/511 (1.4)	.18
Secondary outcome	363/811 (44.8)	189/458 (41.3)	.23	115/499 (23.1)	100/523 (19.1)	.12

Data are presented as no./No. (%) unless otherwise indicated.

Abbreviations: Ct, cycle threshold; DOB, date of birth; IQR, interquartile range; PCR, polymerase chain reaction; RSV, respiratory syncytial virus; SD, standard deviation.

[a]Any viral respiratory coinfection with HMPV, RV, ADV, EV, influenza, PIV1, PIV2, or PIV3.

[b]Born at <37 weeks gestation age.

[c]Underlying conditions included asthma, chronic renal failure, splenectomy/asplenia, autoimmune disease, seizure disorders, malignancy, chronic lung disease, heart failure, organ transplant, diabetes, kwashiorkor/marasmus, prematurity, valvular heart disease, immunosupressive therapy, burns, nephrotic syndrome, obesity, cirrhosis/liver failure, coronary artery disease, sickle cell, immunoglobulin deficiency, spinal cord injuries, chronic obstructive pulmonary disease/emphysema, or other as specified by parent(s).

mechanical ventilation, and 8 children died (0.34%). Seventeen of the 21 children (81%) who received mechanical ventilation were admitted to the ICU. Sixty-seven percent of children were hospitalized for <5 days. When comparing RSV with any respiratory viral coinfection to RSV monoinfection, we found no overall association between any viral infection and life-threatening disease (OR, 0.74; 95% CI, .39–1.4; *P* = .36; Table 3). We found the same to be true for our secondary outcome, including extended length of stay (adjusted OR [aOR], 0.83; 95% CI, .69–1.0; *P* = .05) Table 4. After adjusting for confounders, RSV-ADV coinfection had a 3.4 increased odds of life-threatening disease compared with RSV monoinfection (95% CI, 1.6–7.2; *P* = .001; Table 3). RSV-ADV coinfection was not associated with the secondary outcome (aOR, 1.0; 95% CI, 0.77–1.3; *P* = .77, Table 4). When we compared RSV-ADV coinfection to ADV monoinfection, we found no relation to life-threatening disease (aOR, 0.78; 95% CI, .37–1.6; *P* = .51) and decreased life-threatening disease and extended length of stay (aOR, 0.51; 95% CI, .38–.70; *P* < .001). The median ADV Ct value was significantly lower in ADV monoinfection (29.7; IQR, 20.8–34.3) compared with RSV-ADV infection (33.2; IQR, 30.1–35.5) (*P* < .001). Finally, RSV-influenza showed increased odds for our secondary outcome including prolonged length of stay (aOR, 2.1; 95% CI 1.0–4.5; *P* = .05) Table 4. We identified an increased odds of our secondary outcome for RSV-influenza when compared to influenza alone (aOR, 2.1; 95% CI, 1.0–4.4; *P* = .04). No other viral coinfections showed increased odds of severe disease compared with RSV monoinfection.

In the HIV-infected population, 6.3% (n = 5) of children had life-threatening disease. Mean RSV Ct value was significantly lower in the HIV-infected population than the HIV-uninfected population (RSV Ct value 27.1 [SD, 5.0] vs 25.5 [SD, 4.8]; *P* = .003). Similarly, in this population, we found no association between any viral coinfection and more severe disease compared with RSV monoinfection [Supplementary Table 4].

RSV Viral Load and Disease Severity

We found a mean Ct value of 25.1 (SD, 4.6) for children aged <6 months and 26.0 (SD, 5.0) for children aged ≥6 months (*P* < .001). RSV viral load was not associated with life-threatening disease in children with RSV monoinfection or children with RSV with any coinfection. When included in our multivariate model, RSV Ct values were not found to be associated with life-threatening disease (aOR, 1.0; 95% CI, .94–1.1) or the secondary outcome including increased length of stay (aOR, 1.0; 95% CI, .98–1.0).

Table 3. Primary Outcome in Univariate and Multivariate Analyses of Respiratory Syncytial Virus Viral Coinfection and Life-threatening Disease in HIV-Uninfected Children <5 Years of Age at 6 Sentinel Sites in South Africa, 2009–2013

Coinfection	Life-threatening Disease	MV, ICU, Death, no./No. (%)	OR (95% CI)	*P* Value	aOR (95% CI)	*P* Value
Any[a]	No	19/1306 (1.5)	0.74	.36		
	Yes	19/971 (2.0)	(.39–1.4)			
HMPV	No	38/2251 (1.7)	…	…		
	Yes	0/26 (0.0)				
RV	No	31/1715 (1.8)	0.69	.37		
	Yes	7/562 (1.3)	(.30–1.6)			
ADV	No	27/1937 (1.4)	2.4	.02	3.4	.001
	Yes	11/340 (3.2)	(1.2–4.8)		(1.6–7.2)	
EV	No	35/2091 (1.7)	0.96	.95		
	Yes	3/186 (1.6)	(.29–3.2)			
Influenza	No	38/2248 (1.7)	…	…		
	Yes	0/29 (0.0)				
PIV1	No	37/2266 (1.6)	6.0	.09		
	Yes	1/11 (9.1)	(.75–48.3)			
PIV2	No	38/2265 (1.7)	…	…		
	Yes	0/12 (0.0)				
PIV3	No	37/2257 (1.6)	3.2	.27		
	Yes	1/20 (5.0)	(.41–24.2)			

Univariate analysis: All factors with $P < .20$ were entered into the multivariate model. Multivariate analysis: Only factors with $P < .05$ are shown. Multivariate analysis was adjusted for the covariates prematurity and age, which were found to be significant in univariate analysis and subsequently in multivariate analysis using the manual forward stepwise procedure. Primary outcome data were missing for 45 RSV–infected children.

Abbreviations: ADV, adenovirus; aOR, adjusted odds ratio; CI, confidence interval; EV, enterovirus; HMPV, human metapneumovirus; ICU, intensive care unit; MV, mechanical ventilation, OR, odds ratio; PIV, parainfluenza virus; RV, rhinovirus, RSV: respiratory syncitial virus.

[a]Any viral respiratory coinfection with HMPV, RV, ADV, EV, influenza, PIV1, PIV2, or PIV3.

DISCUSSION

In general, our study did not corroborate the findings from previous smaller studies, that children hospitalized with LRTI characterized by RSV coinfection with respiratory viruses had more severe disease compared with children with RSV monoinfection [15, 31, 32]. We did, however, identify an association between RSV-ADV coinfection and life-threatening disease, which may be indicative of synergistic pathogenesis leading to respiratory failure or that severe disease in these children was largely driven by coinfection with ADV. The association of RSV-ADV coinfection with severe disease was, however, not evident when we assessed prolonged hospitalization. Our data are supported by findings of another study in which RSV-ADV coinfection showed statistically significant increases in hospital length of stay, days with supplemental oxygen use, ICU admission, and mechanical ventilation compared with RSV monoinfection in children hospitalized for LRTI, although no comparison was made with ADV monoinfection [33]. In a study of mixed RSV-ADV infection, RSV-ADV coinfection was not found to be more severe than ADV alone when examining duration of fever, oxygen requirement, and length of hospital stay [34]. Another study of 9 RSV-confirmed infants found that 75% of children with RSV-ADV coinfection died despite mechanical ventilation [35]. Even though ADV alone may be responsible for more severe disease, clinical features such as hospital stay were not found to differ between RSV and ADV hospitalized LRTI [36]. However, increased pathogenicity may be explained by distinctly different immunological responses produced by RSV and ADV. ADV induces interferon-γ production activating the classical antiviral defense mechanism and heightened mononuclear cell activation compared with RSV, possibly leading to more severe disease with coinfection [37].

The increased odds of severe disease for RSV-ADV coinfection may warrant further exploration on a host and pathogen level. Virus–virus interactions can be classified into 3 categories [1]: viral genes or gene products interacting directly [2], host environment changes that result in indirect interaction, or [3] immunological interactions [38]. It is plausible that similar mechanisms that enhance bacterial superinfection may also enhance viral superinfection, namely, depletion of host defenses due to initial viral infection [39].

We found that coinfection of RSV and any other virus was not related to disease severity. This is in line with a retrospective study which found that clinical severity did not differ between RSV monoinfection and viral coinfection with 17 different respiratory viruses [40]. A recent meta-analysis of clinical disease severity and viral coinfection vs monoinfection found no clinical difference in severity between these 2 groups even when constrained to more pathogenic respiratory viruses (influenza, RSV, HMPV, PIV) [19]. Another meta-analysis of single and multiple virus respiratory infections (influenza, RV, ADV, HMPV, coronavirus, bocavirus, PIV1–3) and severity of disease concluded that the influence of coinfection on disease severity remains unclear due to the heterogeneity of results [41].

Table 4. Secondary Outcome in Univariate and Multivariate Analyses of Respiratory Syncytial Virus Viral Coinfection and Life-threatening Disease or Length of Stay of ≥5 Days in HIV-Uninfected Children <5 Years of Age at 6 Sentinel Sites in South Africa, 2009–2013

Coinfection	Secondary Outcome	MV, ICU, Death, or LOS ≥5 d, no./No. (%)	OR (95% CI)	*P* Value	aOR (95% CI)	*P* Value
Any[a]	No	478/1310 (36.5)	1.3	<.001		
	Yes	289/981 (29.5)	(1.2–1.6)			
HMPV	No	758/2265 (33.5)	1.1	.90		
	Yes	9/26 (34.6)	(.47–2.4)			
RV	No	599/1724 (34.7)	0.79	.03		
	Yes	168/567 (29.6)	(.64–.97)			
ADV	No	671/1946 (34.5)	0.73	.02		
	Yes	96/345 (27.8)	(.57–.94)			
EV	No	721/2101 (34.3)	0.61	.005		
	Yes	46/190 (24.2)	(.43–.86)			
Influenza	No	753/2261 (33.3)	1.8	.13	2.1	.05
	Yes	14/30 (46.7)	(.85–3.6)		(1.0–4.5)	
PIV1	No	765/2280 (33.6)	0.44	.30		
	Yes	2/11 (18.2)	(.09–2.0)			
PIV2	No	765/2279 (33.6)	0.40	.23		
	Yes	2/12 (16.7)	(.09–1.8)			
PIV3	No	761/2271 (33.5)	0.85	.74		
	Yes	6/20 (30.0)	(.33–2.2)			

Univariate analysis: All factors with $P < .20$ were entered into the multivariate model. Multivariate analysis: Only factors with $P < .05$ are shown. Multivariate analysis was adjusted for the covariates prematurity and age, which were found to be significant in univariate analysis and subsequently in multivariate analysis using the manual forward stepwise procedure. Secondary outcome was missing for 31 RSV–infected children.

Abbreviations: ADV, adenovirus; aOR, adjusted odds ratio; CI, confidence interval; EV, enterovirus; HMPV, human metapneumovirus; ICU, intensive care unit; LOS, hospital length of stay; MV, mechanical ventilation, OR, odds ratio; PIV, parainfluenza virus; RV, rhinovirus, RSV: respiratory syncitial virus.

[a]Any viral respiratory coinfection with HMPV, RV, ADV, EV, influenza, PIV1, PIV2, or PIV3.

In our study, the highest prevalence of viral coinfection was detected in HIV-uninfected children aged ≥6 months hospitalized for LRTI. This is in accordance with findings from a number of studies that found multiple viral respiratory infection to be associated with older age [21, 33, 42, 43] when compared to RSV monoinfection. Increased rates of virus infection with increasing age have been described previously in this surveillance population [30]. The increased prevalence of respiratory viral coinfections among children aged ≥6 months may be explained by increased exposure to respiratory viruses, an increased immune response during primary infection that discourages viral coinfection, or increased susceptibility due to waning maternal antibodies [44].

In the presence of RSV, our data show lower prevalence of non-RSV viruses in children hospitalized for viral respiratory illnesses during the RSV season (Supplementary Figure 1). This could be indicative of viral interference in which the presence of RSV in the community inhibits infection by or circulation of other viruses. Evidence of viral interference has been found in studies of children who received influenza vaccine [45, 46] and among children receiving immunoprophylaxis for RSV [47]. In both groups, the prevalence of nonpreventively targeted viruses was higher than among comparison groups that did not receive vaccination or immunoprophylaxis. However, these speculations and the clinical relevance of some of these identified viruses need further exploration with a more suitable study design.

The strength of this study lies in the large sample size, which allowed us to look at different permutations of coinfection within the RSV population and compare them to RSV monoinfection only and to draw conclusions about an infrequent, yet important, outcome. Furthermore, we were able to control for important confounders of disease severity including age and prematurity. Finally, we did not limit our assessment of respiratory viral coinfection to a single season.

There were some limitations to our study. Given that viral data were collected at one time point after disease onset, it is difficult to link viral detection to etiology of LRTI. Some respiratory viruses are frequently detected in asymptomatic children and infants. RSV, HMPV, influenza, and ADV are significantly more prevalent in symptomatic children, whereas RV is commonly found in asymptomatic individuals [48]. Another study of infants up to 12 months of age found that detection of RSV, RV, influenza, ADV, and HMPV are highly associated with symptoms, with an OR >4 for presence of symptoms, whereas for EV, detection is not significantly associated with symptoms [49]. In South Africa, ADV was only moderately associated with severe disease as it was commonly identified in controls; the attributable fraction of ADV detection was 10.1% [50]. Viral detection may also be an artefact of prolonged viral shedding: ADV, for example, is known to exhibit longer low-level shedding [51]. In the RSV-ADV coinfection population, we found more frequent low-level virus than in the population with ADV monoinfection, which may be indicative of prolonged viral shedding and acute infection, respectively (Figure 1). The multiplex PCR used was limited in its ability

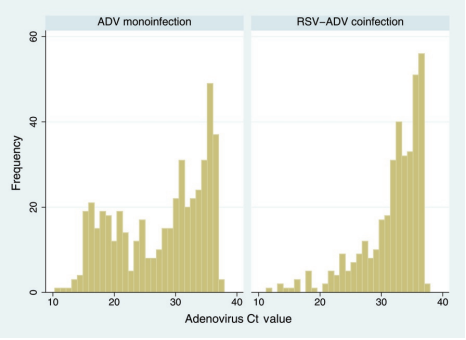

Figure 1. Histogram of adenovirus cycle threshold (Ct) values in human immunodeficiency virus-uninfected children <5 years of age with adenovirus (ADV) monoinfection and those with respiratory syncytial virus (RSV)–ADV coinfection at 6 sentinel sites in South Africa, 2009–2013.

to discriminate between RV and EV due to cross-reactivity; therefore, these coinfections are not optimally characterized within this population. Furthermore, the definition of any viral coinfection is limited by the respiratory viruses we did not test for, although the clinical significance of many of those (eg, human coronavirus and human bocavirus) as LRTI etiological agents also remain to be fully elucidated. Another limitation was that we only had semiquantitative data for viral load.

CONCLUSIONS

The present study contributes to a better understanding of the role of viral coinfection in children hospitalized for RSV-associated LRTI. Molecular diagnostics for respiratory viruses may serve as an important diagnostic tool in pediatric LRTI, but the possible synergy of multiple viruses in the respiratory tract is an area with no clear consensus. In our study, we found that RSV and any respiratory viral coinfection was not associated with more severe disease. The association between RSV-ADV coinfection and life-threatening disease in hospitalized children <5 years of age warrants further exploration and may be explained by enhanced ADV disease alone.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention or the National Institute for Communicable Diseases, South Africa.

Potential conflicts of interest. Authors certify no potential conflicts of interest. The authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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5

ESTIMATED IMPACT OF MATERNAL VACCINATION ON GLOBAL PEDIATRIC INFLUENZA-RELATED IN-HOSPITAL MORTALITY

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I make myself rich by making my wants few.
Henry David Thoreau (1817-1862)

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Research paper

Estimated impact of maternal vaccination on global paediatric influenza-related in-hospital mortality: A retrospective case series

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ABSTRACT

Background: Influenza virus infection is an important cause of under-five mortality. Maternal vaccination protects children younger than 3 months of age from influenza infection. However, it is unknown to what extent paediatric influenza-related mortality may be prevented by a maternal vaccine since global age-stratified mortality data are lacking.

Methods: We invited clinicians and researchers to share clinical and demographic characteristics from children younger than 5 years who died with laboratory-confirmed influenza infection between January 1, 1995 and March 31, 2020. We evaluated the potential impact of maternal vaccination by estimating the number of children younger than 3 months with in-hospital influenza-related death using published global mortality estimates.

Findings: We included 314 children from 31 countries. Comorbidities were present in 166 (53%) children and 41 (13%) children were born prematurely. Median age at death was 8·6 (IQR 4·5–16·6), 11·5 (IQR 4·3–24·0), and 15·5 (IQR 7·4–27·0) months for children from low- and lower-middle-income countries (LMICs), upper-middle-income countries (UMICs), and high-income countries (HICs), respectively. The proportion of children younger than 3 months at time of death was 17% in LMICs, 12% in UMICs, and 7% in HICs. We estimated that 3339 annual influenza-related in-hospital deaths occur in the first 3 months of life globally.

Interpretation: In our study, less than 20% of children is younger than 3 months at time of influenza-related death. Although maternal influenza vaccination may impact maternal and infant influenza disease burden, additional immunisation strategies are needed to prevent global influenza-related childhood mortality. The missing data, global coverage, and data quality in this study should be taken into consideration for further interpretation of the results.

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1. Introduction

Influenza virus is an important cause of mortality due to acute lower respiratory infection (ALRI) in young children. In 2018, influenza was associated with an estimated 15,300 (uncertainty range [UR] 5800–43,800) in-hospital deaths in children younger than 5 years of age globally [1]. More than one third of in-hospital deaths occurred in children younger than 6 months and the majority (82%) occurred in low-income (LICs) and lower-middle-income countries (LMICs) [1]. Children younger than 6 months of age have higher rates of influenza-associated hospitalisation and influenza-related mortality when compared to older children in high-income countries (HICs) [2,3].

Paediatric influenza vaccines are licensed for children aged 6 months and older [4]. This leaves a gap in protection during the first 6 months of life. Maternal vaccination has the potential to protect infants early in life through transplacental transfer of maternal antibodies against influenza virus. The World Health Organization (WHO) announced in 2012 that pregnant women should have the

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Research in Context

Evidence before this study

Influenza infection was associated with an estimated 15,300 (uncertainty range [UR] 5800–43,800) in-hospital acute lower respiratory infection (ALRI) deaths in children younger than 5 years in 2018, of which 36% occurred in infants under 6 months. The potential impact of maternal vaccination on paediatric mortality is unclear as age-stratified data under 6 months at time of death are lacking and maternally-derived influenza antibodies provide protection up to the first 3 months of life. We searched for articles published in English from January 1, 2014 to February 2, 2020 using PubMed and search terms concerning influenza infection and childhood mortality. Publications originated from high-income countries (HICs) only and there were no multi-country case series.

Added value of this study

To our knowledge, this is the first global case series of children who died with laboratory-confirmed influenza infection reporting age-stratified data in young children. We provide clinical and demographic characteristics of 314 paediatric in-hospital deaths with laboratory-confirmed influenza infection from 31 countries. In our study, 91 children (29%) were from LMICs. Less than 20% of children dying in-hospital with influenza were younger than 3 months at time of death and children from LMICs were younger (8·6 months) than children from HICs (15·5 months) at time of death.

Implications of all the available evidence

Our data show that maternal vaccination may prevent a higher proportion of influenza-related in-hospital deaths in children in LMICs than in other World Bank income regions. However, even in LMICs, more than 80% of children are 3 months or older at time of influenza-related in-hospital death. This implies that a maternal vaccine could maximally prevent a small proportion of global paediatric influenza-related in-hospital mortality and other strategies are expected to have greater impact. By applying the proportions of children younger than 3 months at time of death to global mortality estimates, we calculated that maternal vaccination can potentially prevent up to 3339 (UR 1287–9886) influenza-related in-hospital deaths in children younger than 5 years annually. The missing data, global coverage, and data quality in this study should be taken into consideration for further interpretation of the results.

highest priority for seasonal influenza vaccination [5]. Since then, several countries have incorporated maternal influenza vaccination into routine immunisation programs, although implementation has been negligible in LMICs due to logistical challenges, vaccine acceptance, and costs [6]. In 2014, the Board of Gavi, the Vaccine Alliance, advocated for more evidence of vaccine impact on infants' health outcome to support maternal influenza vaccination programmes in countries eligible for their support [7]. The efficacy of maternal influenza vaccination has been demonstrated in 4 randomised controlled trials, reducing laboratory-confirmed influenza in infants by 30–63% in the first 6 months of life [8–11].

In the secondary analysis of a maternal influenza vaccine trial duration of protection by maternal vaccination was shown to be limited to the first 3 months of life due to the decline of maternally-derived antibodies [12]. However, it remains unknown to what extent maternal influenza vaccination could prevent influenza-related mortality in infants, as global granular data on age distribution under 6 months at time of death are lacking [13]. Studies examining vaccine efficacy against influenza-related mortality from countries where maternal vaccination has been implemented exclude children younger than 6 months of age or do not collect maternal vaccination data [2,13,14]. Thus, the potential impact of maternal influenza vaccination on paediatric influenza-related mortality remains unknown.

To gain insight into the age distribution and clinical characteristics of influenza-related mortality in young children worldwide, we initiated the FLU Global Online Mortality Database (FLU GOLD) study. Using retrospective data, we determined the characteristics and age distribution of children younger than 5 years who died in-hospital with laboratory-confirmed influenza infection worldwide. Applying the proportions of children younger than 3 months at time of death to global mortality estimates, we estimated the potential impact of maternal influenza vaccination on in-hospital paediatric-related death.

2. Methods

2.1. Study design and patients

The FLU GOLD study was initiated in October 2017. We invited our existing global respiratory syncytial virus (RSV) GOLD network [15], consisting of individual investigators, research groups, and clinicians, to share individual-level data of children aged 0–59 months who had died with laboratory-confirmed influenza infection between January 1, 1995 and March 31, 2020. YNL, NIM, FSV, and LJB had full access to all the data in the study. We excluded community deaths due to limited available data (n = 8) and children with influenza-related mortality after stem cell transplantation. Additionally, we searched the literature using PubMed for "influenza" combined with "death", "deaths", "died", "mortality", "fatality", or "case fatality ratio (CFR)" and "pediatric", "pediatrics", "child", or "children" and invited authors to share additional (unpublished) cases. Collaborators were invited to share data between October 13, 2017, and March 31, 2020 through a link to a questionnaire (**Supplementary Material**) in Research Online, an electronic data capture platform [16].

2.2. Definitions

We collected demographic and clinical characteristics and compared these between children from different income groups. Countries of origin were categorised as LMIC (LIC and LMIC combined), upper-middle-income countries (UMIC), and HIC according to the World Bank classifications for 2020 [17]. We compared age distribution at time of death for the 3 income groups and between the following 3 patient populations: children with comorbidities, healthy term children, and healthy preterm children (without comorbidities). A comorbidity was defined as at least one underlying disease, such as congenital heart disease, chronic lung disease or a genetic disorder. Prematurity was defined as gestational age less than 37 weeks. If data for comorbidities or prematurity were not reported, we assumed that the children were healthy term. We calculated weight-for-age z-scores as previously described [15]. We determined the proportion of children who died within the influenza virus epidemic season by comparing age at death and seasonality within the country of origin as estimated by a recent systematic analysis on global patterns of monthly influenza virus activity [18]. We compared the proportion of in-hospital deaths under 6 months of age in our study to the proportions from published studies used for the recent global influenza burden study from the Respiratory Virus Global Epidemiology Network [1].

2.3. Age at influenza infection

We calculated age at influenza infection by subtracting the number of days between onset of influenza-related symptoms and influenza-related death from age at influenza-related death. We then determined the proportion of children under 3 months of age at time of influenza infection.

2.4. Community-acquired and hospital-acquired influenza-related death

We differentiated between children with community-acquired and hospital-acquired influenza infection. In case the setting where influenza had been acquired was not provided, and if there were no strong indications of nosocomial infection based on timeframe and clinical disease course, we assumed the infection had been community-acquired. We assumed that deaths occurred within the hospital if data on location of death were missing.

2.5. Potential impact of maternal influenza vaccination on influenza-related in-hospital death

To evaluate the minimum expected impact of maternal vaccination on influenza-related deaths assuming 100% vaccine efficacy and complete vaccination coverage, we multiplied the proportion of children younger than 3 months at time of community-acquired in-

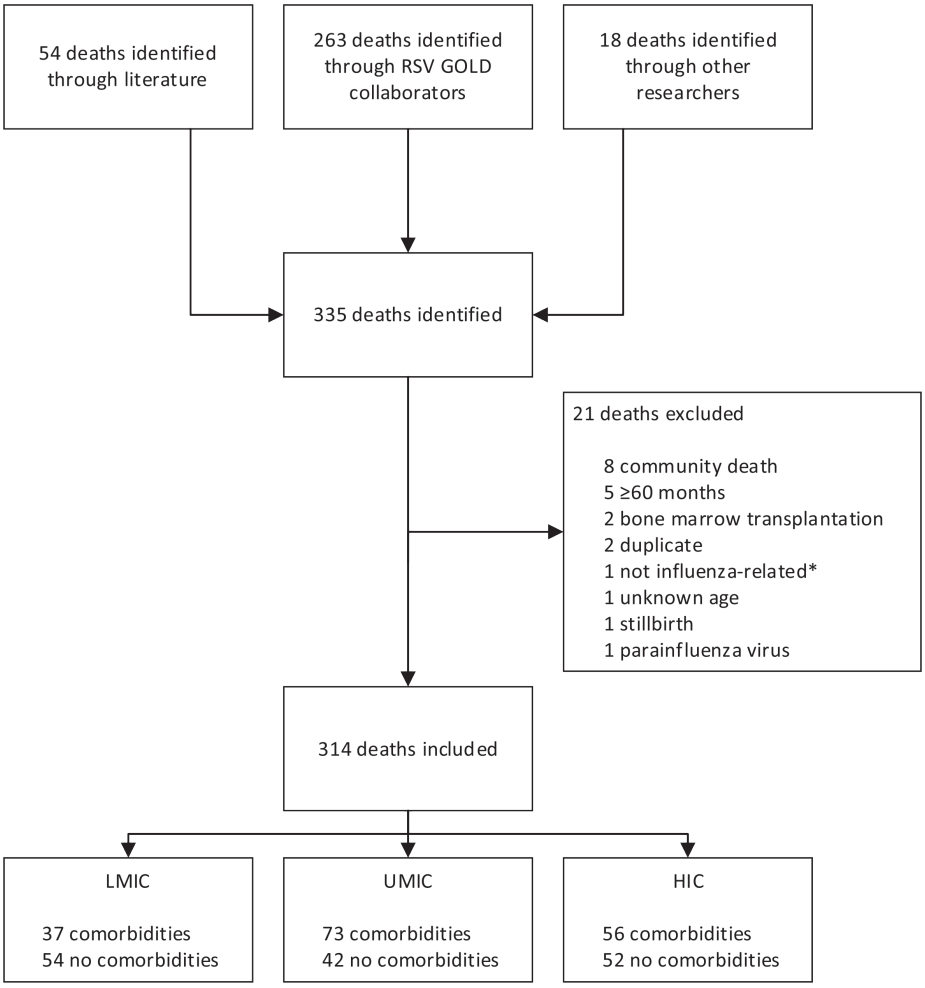

Fig. 1. Flowchart of included deaths.

RSV=respiratory syncytial virus. GOLD=global online database. LMIC=low-income and lower-middle-income countries. UMIC=upper-middle-income countries. HIC=high-income countries. *For 1 child the collaborator had indicated in the comments that the death was not influenza-related.

hospital death by the estimated total number of global influenza-associated ALRI in-hospital deaths under 5 years of age for each World Bank income group [1].

2.6. Sensitivity analyses

We compared demographic and clinical characteristics between different income groups, excluding cases with missing data for comorbidities or prematurity. We performed subgroup analyses and compared characteristics for children with hospital-acquired and community-acquired influenza-related death. Furthermore, we differentiated between seasonal and pandemic influenza-related deaths by excluding children with influenza A(H1N1)pdm09 who died within the timeframe of the WHO-declared pandemic (June 2009 - August 2010) from the analyses. Lastly, we analysed to what extent our results were sensitive to the contribution of a large number of cases from Ecuador, United Kingdom, Kenya, Turkey and South Africa (n = 145) by excluding these countries from our analyses.

2.7. Ethical approval

Since de-identified secondary patient data were used in the FLU GOLD study, parental informed consent was waived by the institutional research board of the University Medical Centre Utrecht. Ethical approval was obtained for individual collaborating institutes when required.

2.8. Statistical analysis

We report descriptive statistics for all variables. Continuous variables are presented as median with interquartile ranges (IQR). Categorical variables are presented as frequencies and proportions. We used the χ^2-test or the Fisher's exact test to determine statistical significance between groups for categorical parameters. We report conservative exact p values instead of asymptotic p values because of the small sample size. The Mann-Whitney U test was used for all continuous parameters. We applied the Bonferroni correction for multiple testing between World Bank income groups. All statistical analyses were performed with SPSS (version 21·0; IBM Corp, Armonk, NY).

2.9. Role of the funding source

The funder had no role in study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the paper for publication. YNL, NIM, FSV, and LJB had full access to all the data in the study and NIM had final responsibility for the decision to submit for publication.

3. Results

We obtained data from both published and unpublished cases and included 314 influenza-related deaths from 6 WHO regions and 31 countries (9 LMICs [29%], 9 UMICs [29%], and 13 HICs [42%]) across the world (Figs. 1 and 2, Supplemental Table 1). The majority of cases were shared through the RSV GOLD network and additional cases were identified through the literature search [19,20]. In total, 91 (29·0%) children were from LMICs, 115 (36·6%) were from UMICs and 108 (34·4%) were from HICs.

3.1. Clinical and demographic characteristics

Comorbidities were present in at least 166 (52·9%) children and 41 (13·1%) children were born prematurely (Table 1). Paediatric influenza vaccination had been administered to 11 (3·5%) children. Out of 31 children for which data on maternal immunisation were available, 2 children (Chile, n = 1 and US, n = 1) had mothers who had been vaccinated against influenza during pregnancy. Age at death for these children was 15 and 16 months, respectively. Type of diagnostic influenza tests used and additional characteristics for children from different income countries are included in Supplemental Tables 2 and 3, respectively. Co-pathogens were identified in respiratory samples of 114 children. RSV was the most common co-infection (Supplemental Table 4).

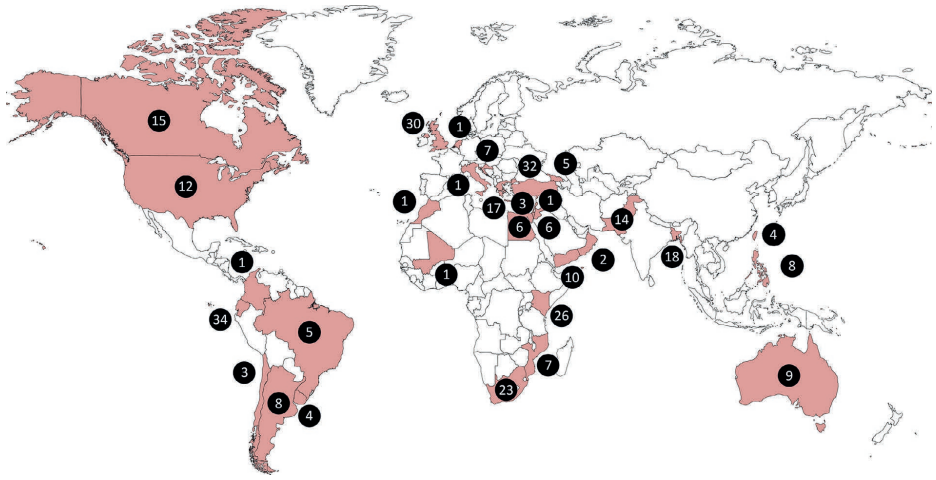

Fig. 2. Countries of children with influenza-related in-hospital death included in the analysis.
Number of included deaths are given for each country (in pink) from which collaborators shared data.

Table 1
Clinical and demographic characteristics of children younger than 5 years with influenza-related in-hospital death.

	LMICs (*n* = 91)	*p* value*	UMICs (*n* = 115)	*p* value†	HICs (*n* = 108)	*p* value‡	Total (*n* = 314)
Male sex	46 (50·5%)	0·58	53 (46·1%)	0·08	62/107 (57·9%)	0·32	161/313 (51·4%)
Age at death (months)	8·6 (4·5–16·6)	0·12	11·5 (4·3–24·0)	0·06	15·5 (7·4–27·0)	<0·001	12·0 (5·0–24·0)
<3 months at death	15 (16·5%)	0·42	14 (12·2%)	0·27	8 (7·4%)	0·07	37 (11·8%)
<6 months at death	30 (33·0%)	0·76	35 (30·4%)	0·07	21 (19·4%)	0·04	86 (27·4%)
Year of death	2015 (2011–2018); *n* = 91	0·32	2014 (2012–2017); *n* = 112	0·004	2012 (2010–2016); *n* = 107	0·001	2013 (2011–2017); *n* = 310
Age at infection (months)	7·6 (2·9–17·4); *n* = 64	0·07	11·3 (3·8–23·9); *n* = 107	0·08	15·9 (6·7–29·7); *n* = 79	0·001	11·5 (4·5–24·0); *n* = 250
<3 months at infection	19 (20·9%)	0·86	22 (19·1%)	0·09	11 (10·2%)	0·05	52 (16·6%)
Type of influenza							
A H1N1 pre-pandemic	3/81 (3·7%)	1·00	5/108 (4·6%)	0·45	2/102 (2·0%)	0·66	10/291 (3·4%)
A H1N1 pdm-09	14/81 (17·3%)	0·03	34/108 (31·5%)	0·09	44/102 (43·1%)	<0·001	92/291 (31·6%)
A H1N2	0/81	1·00	1/108 (0·9%)	1·00	0/102		1/291 (0·3%)
A H3N2	6/81 (7·4%)	0·17	16/108 (14·8%)	0·08	7/102 (6·9%)	1·00	29/291 (10·0%)
B Yamagata	0/81		0/108		0/102		0/291
B Victoria	0/81		0/108	0·49	1/102 (1·0%)	1·00	1/291 (0·3%)
A unsubtyped	32/81 (39·5%)	0·02	25/108 (23·1%)	1·00	23/102 (22·5%)	0·02	80/291 (27·5%)
B unsubtyped	26/81 (32·1%)	0·75	32/108 (29·6%)	0·65	27/102 (26·5%)	0·42	85/291 (29·2%)
Other^	2/81 (2·5%)	0·58	1/108 (0·9%)	1·00	0/102	0·20	3/291 (1·0%)
Comorbidity§	37 (40·7%)	0·001	73 (63·5%)	0·10	56 (51·9%)	0·12	166 (52·9%)
Prematurity§	8 (8·8%)	0·28	16 (13·9%)	0·71	17 (15·7%)	0·20	41 (13·1%)
Gestational age (weeks)	38·0 (34·0–39·0); *n* = 11	0·84	38·0 (35·0–39·0); *n* = 38	0·54	38·0 (34·3–40·0); *n* = 52	0·95	38·0 (35·0–39·0); *n* = 101
Length of stay in hospital (days)	6·5 (3·0–12·8); *n* = 84	0·005	9·0 (4·0–19·0); *n* = 109	0·95	11·5 (3·0–25·8); *n* = 88	0·02	8·0 (3·0–18·0); *n* = 281
Intensive care unit (ICU) admission	47/83 (56·6%)	0·10	75/109 (68·8%)	<0·001	86/89 (96·6%)	<0·001	208/281 (74·0%)
Length ICU admission (days)	4·5 (1·8–12·3); *n* = 38	0·004	10·5 (4·0–24·5); *n* = 52	0·17	8·0 (2·0–22·0); *n* = 83	0·10	8·0 (3·0–19·5); *n* = 173
ICU not available	13/83 (15·7%)	<0·001	0/109		0/89	<0·001	13/281 (4·6%)
Respiratory support	40/82 (48·8%)	<0·001	68/72 (94·4%)	0·17	88/89 (98·9%)	<0·001	196/243 (80·7%)
Mechanical ventilation	16/82 (19·5%)	<0·001	48/72 (66·7%)	<0·001	82/89 (92·1%)	<0·001	146/243 (60·1%)
Respiratory support not available	31/82 (37·8%)	<0·001	0/72		0/89	<0·001	31/243 (12·8%)
Time between onset of symptoms and hospital admission (days)	4·0 (2·0–7·0); *n* = 64	<0·001	2·0 (0·0–4·0); *n* = 110	0·96	2·0 (1·0–3·0); *n* = 80	<0·001	2·0 (1·0–4·3); *n* = 254
Time between onset of symptoms and death (days)	12·0 (7·0–19·8); *n* = 64	0·63	12·0 (7·0–24·0); *n* = 107	0·83	12·0 (5·0–27·0); *n* = 79	0·73	12·0 (6·0–22·3); *n* = 250
Death during influenza season	64 (70·3%)	0·08	65/112 (58·0%)	0·57	60/96 (62·5%)	0·28	189/299 (63·2%)

Data are n (%), n/N (%) or median (IQR); n. Statistical comparisons with χ^2 test using exact p values, Fisher's exact test or Mann-Whitney U test with p values of less than 0·0167 taken to be significant according to the Bonferroni correction for multiple testing. LMIC=low-income and lower-middle-income countries. UMIC=upper-middle-income countries. HIC=high-income countries. *Low-income or lower-middle-income country versus upper-middle-income country. †Upper-middle-income country versus high-income country. ‡Low-income or lower-middle-income country versus high-income country. ^These children were diagnosed with unsubtyped influenza C. §Considered absent when missing (children with missing data for comorbidity; *n* = 27, and prematurity; *n* = 150).

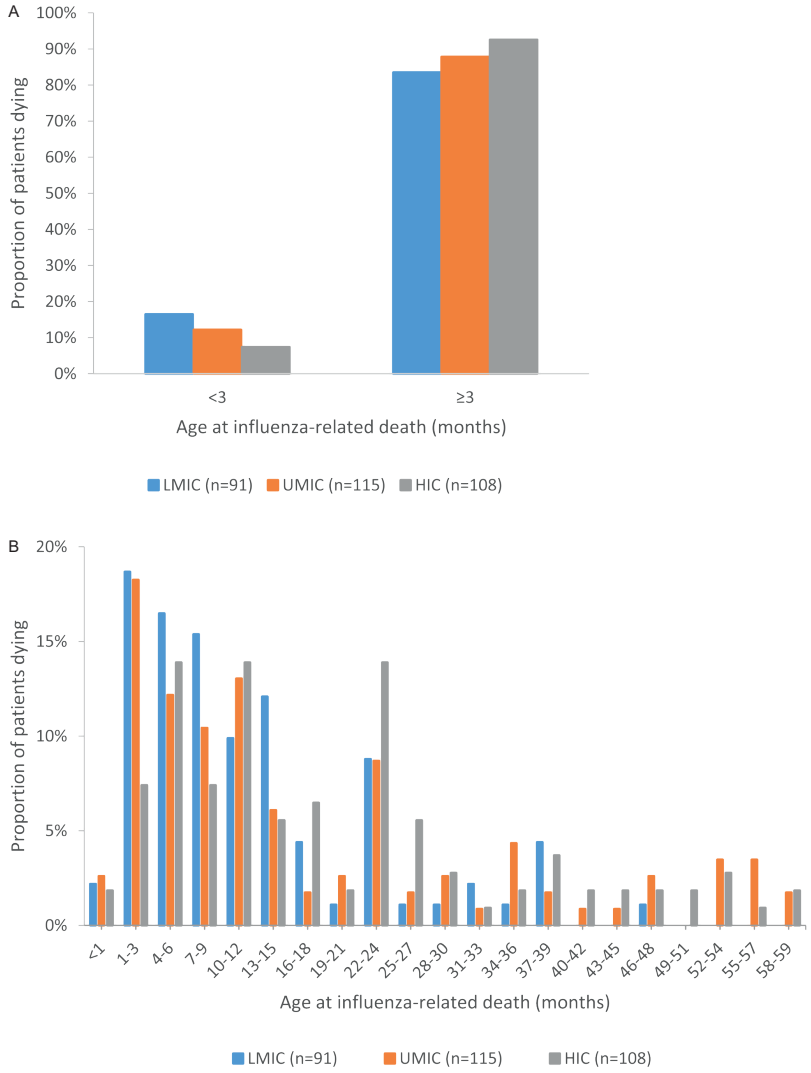

Fig. 3. A) Age younger than 3 months or 3 months and older at time of influenza-related in-hospital death for children younger than 5 years from low- and lower-middle-income countries (LMIC), upper-middle-income countries (UMIC), and high-income countries (HIC). B) Age distribution at time of influenza-related in-hospital death for children younger than 5 years from low- and lower-middle-income countries (LMIC), upper-middle-income countries (UMIC), and high-income countries (HIC).

3.2. Age distribution

Globally, more than 80% of paediatric influenza-related deaths occurred after the first 3 months of life. The proportion of children younger than 3 months at time of death was 16·5% in LMICs, 12·2% in UMICs and 7·4% in HICs (Fig. 3A). The proportions of children younger than 6 months at time of death were 33·0% in LMICs, 30·4% in UMICs, and 19·4% in HICs. Median age at death was 8·6, 11·5 and 15·5 months for children from LMICs, UMICs, and HICs, respectively (Fig. 3B). Children from LMICs were younger at time of death than children from HICs ($p<0·001$). The age distribution for preterm children without comorbidities, term children with comorbidities, and healthy term children is shown in Supplemental Figure 1.

3.3. Hospital-acquired influenza-related death

We separately described variables for 38 (12·1%) children with hospital-acquired influenza-related death and compared these to children with community-acquired influenza-related death (Supplemental Table 5). Age at death, and time between onset of influenza-related symptoms and death did not differ significantly between both groups ($p = 0·09$ and $p = 0·14$, respectively). More children with

Table 2
Global paediatric influenza-associated in-hospital deaths in children younger than 5 years.

	Estimated number of global annual influenza-related ALRI deaths in children younger than 5 years[1]	Proportion of children younger than 3 months (FLU GOLD registry)	Potentially prevented influenza-related ALRI in-hospital death*
LMIC	17,000 (6900–45,100)	17·2%	2924 (1187–7757)
UMIC	3200 (800–14,400)	11·6%	371 (93–1670)
HIC	600 (100–6200)	7·4%	44 (7–459)
Overall			**3339 (1287–9886)****

Data are provided in numbers and uncertainty ranges. ALRI=acute lower respiratory infection. LMIC=low-income and lower-middle-income countries. UMIC=upper-middle-income countries. HIC=high-income countries.
*Percentage of children younger than 3 months with in-hospital, community-acquired influenza-related death from the FLU GOLD registry (n = 276, Supplemental Table 6) was multiplied with the number of global estimated deaths as reported by Wang et al. [1] for each income group. By analyzing the income groups separately, we accounted for the fact that the proportion of children from LMICs in the FLU GOLD registry is not representative for the total global burden in this population.
**Overall number was calculated by summing up the number of potentially prevented influenza-related ALRI in-hospital deaths for each World Bank income level group.

hospital-acquired influenza-related death were born preterm as compared to children with community-acquired influenza-related death (36·8% versus 9·8%, $p<0·001$). Although timing of intensive care unit (ICU) admission in relation to influenza infection was unknown, all children with hospital-acquired influenza-related death had been admitted to the ICU. This differed from children with community-acquired influenza-related death, of which 70·1% had been admitted to the ICU ($p<0·001$).

3.4. Potential impact of maternal vaccination on influenza-related in-hospital death

Based on published global mortality estimates, we calculated that 3339 (UR 1287–9886) influenza-related in-hospital deaths occurred annually in children younger than 3 months (Table 2).

3.5. Sensitivity analyses

When excluding children with hospital-acquired influenza-related mortality (n = 38) from the analyses, differences in characteristics between income groups remained unchanged, except for the length of hospital and ICU stay, and the presence of comorbidities between children from LMICs and UMICs (Supplemental Table 6).

We also analyzed our data after excluding children with missing data for prematurity and comorbidities (n = 150). Results for age distribution remained unchanged, including a higher age at death for children from HICs (14·5 months [IQR 6·1–28·6]) than from UMICs (12·0 months [IQR 4·0–28·9]) and LMICs (9·2 months [IQR 4·0–23·2]) (Supplemental Table 7), although the difference in age at death for children from HICs compared to children from LMICs was no longer significant. Furthermore, when excluding 26 children with influenza A(H1N1)pdm09 virus infection who died within the timeframe of the WHO-declared pandemic (June 2009 - August 2010), conclusions did not change (Supplemental Table 8).

Lastly, we analysed our data after excluding children from Ecuador, Kenya, United Kingdom, Turkey, and South Africa (n = 145), as these accounted for almost half of all influenza-related deaths (Fig. 2). As these were mainly UMIC deaths (n = 89), median age at death for children from UMICs increased to 23·5 months (IQR 9·9–39·0) and there were more children with comorbidities from HICs (62·8%) as compared to children from LMICs (30·8%, $p<0·001$) (Supplemental Table 9).

4. Discussion

To our knowledge, the FLU GOLD study is the first global case series reporting on influenza-related deaths in children under 5 years of age. We collected demographic and clinical characteristics of children who died with influenza virus infection in the hospital from 31 countries. We showed that more than 80% of children were 3 months or older at time of influenza-related death. This finding implies that maternal vaccine alone would be insufficient to prevent the majority of global paediatric influenza-related in-hospital mortality, given that the duration of protection is limited to the first 3 months of life [12]. We estimated that a total of 3339 influenza-related in-hospital deaths in infants younger than 3 months could have been potentially prevented by maternal vaccination, which is 21·8% of the global influenza-related paediatric mortality burden [1]. Maternal vaccination may have a higher impact on influenza-related paediatric in-hospital deaths in LMICs as we observed a trend towards a higher proportion of children dying below the age of 3 months in LMICs compared to other income regions. Since we reported conservative p values, this difference may be significant in larger sample sizes.

Our findings are in line with a systematic review of the global burden of influenza-associated in-hospital deaths in children under 5 years in which 36% of deaths occurred in children younger than 6 months. Moreover, the highest number of deaths was estimated to occur in children aged 12–59 months, comparable to our results [1].

We found that at least 53% of children had comorbidities which is similar to results from other studies [21,22] In contrast, in a study from the United Kingdom that evaluated all influenza-related paediatric ICU admissions between 2003 and 2015, nearly four fifths of children had high-risk conditions [23]. The Advisory Committee on Immunization Practices (ACIP) from the United States Centers for Disease Control and Prevention (CDC) recommends vaccination for children aged 6 months and older and recognises that children with the following underlying conditions are at increased risk of developing medical complications attributable to severe influenza infection: chronic pulmonary disease, hemodynamically significant cardiovascular disease, immunosuppression, renal, hepatic, neurological, hematologic, or metabolic disorders [4]. In our registry, congenital heart disease and neurological disease were the most reported comorbidities. Prematurity was present in 13% of children, but data were missing for 48% of all children included in the study. Comorbidities were less often reported in children from LMICs than from UMICs. This may have been caused by lack of available clinical data or limited access to adequate healthcare resources resulting in a higher number of influenza-related deaths in previously healthy children.

Children from LMICs were younger at time of influenza-related death, had a longer time interval between onset of symptoms and hospital admission and were less often admitted to the ICU as compared to children from UMICs and HICs. These findings might be due to poor access to healthcare and limited availability of ICU beds rather than underlying susceptibility of these children to influenza.

In contrast, in UMICs and HICs, children may die at an older age due to pre-existing susceptibility from underlying conditions.

Paediatric deaths associated with influenza A(H1N1)pdm09 virus were proportionately higher in HICs than LMICs. This is likely caused by publication bias since there were more studies on influenza A (H1N1)pdm09 virus available from HICs than from LMICs. Furthermore, information on influenza subtype in LMICs was not always available, which also may have contributed to this difference.

Beyond the scope of this paper, we compared our data to 358 previously reported cases from the RSV GOLD I study of children under 5 years of age with RSV-related in-hospital mortality [15], as RSV is currently the most common viral pathogen causing ALRI in young children. Children with influenza-related death were substantially older than children with RSV-related death (Supplemental Table 10, Supplemental Figure 2). This difference was also demonstrated in other studies [24]. In a prospective Dutch birth cohort of 4072 premature infants [25], 181 infants were hospitalised with RSV infection and 2 with influenza infection in the first year of life (data not shown), indicating that severe ALRI due to influenza is less prevalent than RSV in younger children. Reasons for this age difference are likely multifactorial. In contrast to influenza, RSV predominantly affects the airways and causes greater epithelium damage [26], leading to significant airway obstruction and fatality in infants who have smaller airways than older children. Additionally, coinfections, which could cause mortality also in older children, may occur less often in children with RSV infection compared to children with influenza infection [27].

For the first time, we report global age-stratified data for children dying with influenza. This enabled us to assess the potential impact of maternal vaccination on paediatric influenza-related mortality. Furthermore, we verified each case through direct communication with the local collaborators.

There are also limitations to this study. First, our results reflect only a small proportion of paediatric influenza-related mortality occurring worldwide, since limited data are available due to lack of diagnostic testing, in particular from children who died outside of the hospital. Our database included only 18 (5·7%) children from LICs, and this proportion should be much higher based on global mortality burden estimates. Since children dying in the community may be younger and are mainly from LMICs, our results are an underestimation of the potential impact of maternal vaccination on paediatric influenza-related mortality in LMICs. Furthermore, since we only included individual-level patient data, it was not possible to use larger published case series from the US for which only aggregated data could be provided [3,20,28–31]. The percentage of children younger than 6 months within the under-five age group in these case series ranged between 19% and 35%, which is slightly higher than we report for HICs (19%). Second, we were unable to assess the potential impact of maternal vaccination on stillbirths associated with in utero influenza exposure. Furthermore, we were unable to assess the indirect impact of maternal vaccination on paediatric mortality due to bacterial pneumonia following influenza infection, all-cause ALRI, deaths prevented due to herd immunity including young siblings, or impact on postpartum maternal death and subsequent effects on the health of the child. This limitation resulted in an underestimation of the potential impact of maternal influenza vaccination on paediatric mortality. Third, we did not account for maternal vaccination coverage, vaccine efficacy and potential programmatic limitations. Data on maternal immunization during pregnancy was available for only 31 patients. For this reason we may show an overestimation of vaccine impact. Fourth, our literature search terms were more limited than those used for the global influenza burden study [1] resulting in missed published deaths (91 deaths from 18 studies). From the missed published deaths, age distribution data were available for 15 children, of which 3 (20%) were younger than 6 months at the time of influenza-related death, which is similar to what we observed (27%). We therefore expect that this limitation does not have a major impact on our study. Data were unavailable on the proportion of children younger than 3 months in the missed deaths. Fifth, we have based our estimations on the global influenza estimates from the global burden study, which has its own limitations such as heterogeneity of studies, several forms of bias and scarcity of data, which could have caused both overestimations as underestimations of paediatric influenza-related death burden according to the authors and therefore could lead to over- or underestimation of vaccine impact on mortality [1]. Moreover, data from LMICs were limited in this study which likely leads to an underestimation of mortality burden. Sixth, some authors did not respond to our invitation to collaborate, which could have led to non-response bias. Seventh, data on location of death were not always available. We assumed that these deaths occurred within the hospital due to the limited number of studies and lack of available influenza testing within the community. Furthermore, the majority of children with missing data for location of death were from HICs. It is unlikely that these children would not have been admitted to hospital. Finally, data for prematurity and comorbidities were often missing, in particular from LMICs, which could have caused an underestimation of the number of children with prematurity and comorbidity. However, when we excluded children with missing data in the sensitivity analyses, the main results from this study remained unchanged.

There are important factors to support maternal influenza vaccination: it has proved to be safe [11] and effective in preventing influenza-associated ALRI in both infants and pregnant women, the latter forming a substantial risk group for influenza-associated hospitalisation [32] and death [33]. Furthermore, maternal influenza vaccination decreased all-cause ALRI hospitalisations in infants during the first 3 months of life [34]. A study on maternal influenza vaccination among HIV-infected and HIV-uninfected pregnant women in South Africa showed that antenatal influenza vaccination campaigns in South Africa would be cost-effective [35]. However, we showed that the impact on paediatric influenza-related death may be limited since more than 80% of global paediatric influenza-related in-hospital deaths occurred after the first 3 months of life, which is beyond the timeframe in which maternally-derived anti-influenza antibodies are expected to have protective effect. Although maternal influenza vaccination may have more impact in LMICs where children with influenza infection die at a younger age, additional immunisation strategies and more high-quality patient data from LMICs are required to prevent global paediatric influenza-related death. Studies to develop a safe and effective influenza vaccine for children younger than 6 months could be considered. In the context of SDG 3·1 and 3·2, maternal vaccination should continue to be emphasized and additional strategies are needed to target influenza-related under-five mortality. The missing data, global coverage, and data quality in this study should be taken into consideration for further interpretation of the results.

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5. Data sharing statement

The FLU GOLD team believes data sharing is important for the global scientific community. Our data are available upon request to the corresponding author, except for personally identifiable data due to privacy reasons.

6. Authors' contributions

NIM, IR, LJB, HN and MN conceptualized the study. IR and FSV collected the data. YNL and FSV Analyzed the data. NIM, YNL and LJB interpreted the results. YNL wrote the initial draft. All authors critically reviewed and revised the manuscript. YNL, FSV, NIM and LJB had full access to all the data in the study and the corresponding author had final responsibility for the decision to submit for publication.

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Declaration of Competing Interest

LJB reports grants from Bill and Melinda Gates Foundation, during the conduct of the study. LJB has regular interaction with pharmaceutical and other industrial partners. He has not received personal fees or other personal benefits. The University Medical Centre Utrecht (UMCU) has received major funding obtained by LJB (>€100,000 per industrial partner) for investigator initiated studies from AbbVie, MedImmune, Janssen, the Bill and Melinda Gates Foundation, Nutricia (Danone) and MeMed Diagnostics. UMCU has received major cash or in kind funding as part of the public private partnership IMI-funded RESCEU project from GSK, Novavax, Janssen, AstraZeneca, Pfizer and Sanofi. UMCU has received major funding by Julius Clinical for participating in the INFORM study sponsored by MedImmune. UMCU has received minor funding for participation in trials by Regeneron and Janssen from 2015 to 2017 (total annual estimate less than €20,000). UMCU received minor funding for consultation and invited lectures by AbbVie, MedImmune, Ablynx, Bavaria Nordic, MabXience, Novavax, Pfizer, Janssen (total annual estimate less than €20,000). LJB is the founding chairman of the ReSViNET Foundation. HN reports grants and personal fees from the World Health Organisation, personal fees from the Bill & Melinda Gates Foundation, personal fees from Sanofi, outside the submitted work. MCN reports grants from the Bill & Melinda Gates Foundation, personal fees from Sanofi Pasteur, personal fees from Pfizer, outside the submitted work. NIM has regular interaction with pharmaceutical and other industrial partners. She has not received personal fees or other personal benefits. UMCU received minor funding for NIMs consultation and invited lectures by Abbvie and Merck. All other authors have nothing to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2021.100945.

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Part 3

MUCOSAL ANTIBODY TRANSFER & LOCAL PROTECTION

6

BREAST MILK PREFUSION F IMMUNOGLOBULIN G AS A CORRELATE OF PROTECTION AGAINST RESPIRATORY SYNCYTIAL VIRUS ACUTE RESPIRATORY ILLNESS

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An understanding of the natural world is a source of not only great curiosity but great fulfilment.
David Attenborough (1926)

The Journal of Infectious Diseases

MAJOR ARTICLE

Breast Milk Prefusion F Immunoglobulin G as a Correlate of Protection Against Respiratory Syncytial Virus Acute Respiratory Illness

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Background. Transplacental respiratory syncytial virus (RSV) antibody transfer has been characterized, but little is known about the protective effect of breast milk RSV-specific antibodies. Serum antibodies against the prefusion RSV fusion protein (pre-F) exhibit high neutralizing activity. We investigate protection of breast milk pre-F antibodies against RSV acute respiratory infection (ARI).

Methods. Breast milk at 1, 3, and 6 months postpartum and midnasal swabs during infant illness episodes were collected in mother–infant pairs in Nepal. One hundred seventy-four infants with and without RSV ARI were matched 1:1 by risk factors for RSV ARI. Pre-F immunoglobulin A (IgA) and immunoglobulin G (IgG) antibody levels were measured in breast milk.

Results. The median breast milk pre-F IgG antibody concentration before illness was lower in mothers of infants with RSV ARI (1.4 [interquartile range {IQR}, 1.1–1.6] $\log_{10}$ ng/mL) than without RSV ARI (1.5 [IQR, 1.3–1.8] $\log_{10}$ ng/mL) ($P = .001$). There was no difference in median maternal pre-F IgA antibody concentrations in cases vs controls (1.7 [IQR, 0.0–2.2] $\log_{10}$ ng/mL vs 1.7 [IQR, 1.2–2.2] $\log_{10}$ ng/mL, respectively; $P = .58$).

Conclusions. Low breast milk pre-F IgG antibodies before RSV ARI support a potential role for pre-F IgG as a correlate of protection against RSV ARI. Induction of breast milk pre-F IgG may be a mechanism of protection for maternal RSV vaccines.

Keywords. breast milk; maternal vaccination; IgG and IgA antibodies; respiratory syncytial virus; acute respiratory infection.

Maternal vaccination against respiratory syncytial virus (RSV) is a promising intervention to protect young infants against RSV infection through transfer of antibodies from mother to infant [1]. Transplacental transfer of RSV immunoglobulin G (IgG) antibodies via the neonatal Fc receptor has been characterized in mother–infant pairs in different populations [2–5]. Transplacental transfer ratio and decay kinetics of maternal IgG are considered cornerstones of protection of the infant through maternal vaccination [6]. However, other routes of antibody transfer may also be important to protect infants from RSV disease.

A novel route of RSV antibody transfer directly to the respiratory tract via RSV-specific IgG and immunoglobulin A (IgA) in amniotic fluid was recently described [7]. The acquired amniotic fluid antibodies show neutralizing activity against RSV and provide protection to the neonate for at least 1 week postpartum in vivo, demonstrating the role of mucosal immunity in protection of infants.

Postnatal antibody transfer to the mucosal surfaces occurs via breast milk [8–12]. A better understanding of the role of RSV-specific antibodies in breast milk may give further insight into mucosal antibody transfer from mother to infant in the context of maternal vaccination and may serve as a correlate of protection against RSV disease. Correlates of protection for RSV remain a knowledge gap and priority for RSV vaccine development [13]. Despite the lack of a clear correlate of protection [14], recent insights into the structure of viral envelope proteins have led to the distinction in antibody function on the basis of target epitopes. RSV F protein mediates RSV entry and fusion with the host cell membrane. Antibodies that target prefusion F (pre-F) protein account for the majority of neutralizing activity

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against RSV in human sera of infected individuals [15–17] and modify disease severity in young children [18]. Thus, antibodies directed against pre-F play an important role in protection against RSV infection. No previous studies have evaluated pre-F RSV antibody in breast milk in relationship to RSV disease risk in infants.

The aim of this study was to characterize the relationship between pre-F antibodies in breast milk and RSV acute respiratory infection (ARI) in infants.

METHODS

Study Site, Design, and Population

From mid-April 2011 to mid-April 2013, 3693 women in the second to third trimester of pregnancy were enrolled in a maternal influenza immunization trial in rural southern Nepal [19]. Weekly home-based visits were conducted until 180 days after birth for respiratory symptom surveillance of mother–infant pairs based on maternal report of symptoms each day in the past week. Nasal swabs were collected from infants if respiratory illness was noted; samples from mothers were collected for febrile respiratory disease. Breast milk was collected from a subset of 827 women living in the 3 study regions closest to the study clinic. Within this subset of mother–infant pairs, infants who had RSV-confirmed respiratory illness in the first 6 months of life were matched 1:1 to controls (infants with no RSV ARI) based on the following risk factors for RSV ARI: maternal influenza vaccination, maternal education, infant month of birth, number of siblings, use of an indoor biomass cook stove, and preterm birth (<37 weeks gestational age). Healthy infant controls were matched to have at least 4 months of respiratory surveillance.

Data Collection and Case Definition

A respiratory illness was defined as fever, cough, wheezing, rapid breathing, or a draining ear on any 1 day in the past week. Breastfeeding was not exclusive if anything other than breast milk was given to the baby. Illness episodes were considered distinct when separated by 7 symptom-free days. Clinical and sociodemographic data were collected at enrollment, birth, and weekly respiratory surveillance visits. Midnasal swabs were collected from infants who met criteria for respiratory illness in the past 7 days and were tested for RSV by reverse-transcription quantitative polymerase chain reaction (PCR) [20]. Breast milk was collected at 1, 3, and 6 months postpartum. Participants were asked to wash their hands and self-express 15 mL of breast milk into a sterile container. Samples were transported on wet ice to the field laboratory and centrifuged to remove the lipid layer, aliquoted, and frozen at −80°C prior to shipment to the University of Washington (Seattle) for testing.

Laboratory Testing

Breast milk IgA and IgG antibody concentrations against RSV-stabilized pre-F (DS-Cav1) protein were quantified by enzyme-linked immunosorbent assay (ELISA). DS-Cav1 is an RSV F protein that is stabilized by a T4 fibritin-trimerization domain (foldon) at the C-terminal, S155C, and S290C cysteine mutations to form an additional disulfide bond, and S190F and V207L cavity-filling mutations. DsCav-1 is expressed by transient transfection of HEK293F cells and purified by affinity purification (NTA resin and StrepTactin resin) and a Superose 200 gel filtration column [21]. Nunc MaxiSorp 96-well plates (Thermo Scientific) were coated overnight at 4°C with either pre-F (100 ng/mL, DS-Cav1, for pre-F IgA or pre-F IgG ELISA). In between steps, plates were washed 3 times with phosphate-buffered saline (PBS) containing 0.05% Tween-20 (Sigma-Aldrich) (PBS-T) using a microplate washer (Biotek 405 LS). Plates were blocked for 1 hour at room temperature with 1% bovine serum albumin (Roche Diagnostics) in PBS-T. Breast milk was added (100 µL/well) in duplicate, at 2–3 dilutions and incubated for 1.5 hours at room temperature. Recombinant palivizumab IgA1 and recombinant palivizumab IgA2 were synthesized by cloning variable heavy and light chain sequences of palivizumab into Lonza expression vector, followed by production in HEK293T cells, and purification by KappaSelect and high pressure size exclusion chromatography [22]. Recombinant palivizumab IgA1 and IgA2 and palivizumab (Synagis, MedImmune) were used to generate a standard curve on every plate. Horseradish peroxidase–labeled goat antihuman IgA and horseradish peroxidase–labeled goat-anti-human IgG (both Jackson ImmunoResearch) were added at a concentration of 0.5 µg/mL and 0.16 µg/mL, respectively, as detection antibodies and incubated for 1 hour at room temperature. Plates were developed with ABTS substrate (Roche) and absorbance was measured at 415 nm with a microplate spectrophotometer (Biotek Epoch). Data were captured and exported using Gen5 software (Biotek).

Statistical Analyses

Continuous variables were described using mean (standard deviation [SD]) or median (interquartile range [IQR]). Differences in the mean or median of continuous variables were tested with a 2-sided *t* test or a nonparametric Mann–Whitney test when appropriate. Log_{10} transformation was performed for all antibody measurements. For our primary analysis, we compared antibody titers prior to infection using a Mann–Whitney test; for infections that occurred before 1 month, we used the antibody titer at 1 month. We used the corresponding time point for controls as used for the matched cases. A linear mixed-model analysis was performed to compare the difference of antibody titers over time for cases and controls. We included time of breast milk collection (month 3 or 6 vs month 1) as covariates and RSV status of children in the first 6 months of life,

as well as the interaction terms of collection time by RSV positivity, to test the hypothesis of whether RSV antibody levels in breast milk increased or decreased differently by RSV status of the infant. We used a Spearman correlation to perform a correlation of RSV antibody titer to time of infection in cases only, as well as a correlation of pre-F antibody to total antibody by isotype and pre-F IgA to pre-F IgG in both cases and controls. The statistical analysis was performed using Stata/SE 13.1 software (StataCorp) and sinusoid function to examine seasonal variation using SPSS Statistics 25 (IBM) software.

Ethical Considerations

Ethical approval for the primary trial (ClinicalTrials.gov identifier NCT01034254) was obtained from the institutional review boards at the Institute of Medicine at Tribhuvan University, the Nepal Health Research Council, John Hopkins University Bloomberg School of Public Health, Seattle Children's Hospital, and Cincinnati Children's Medical Center.

RESULTS

Clinical and Sociodemographic Characteristics

Clinical and sociodemographic characteristics were compared for 174 children (87 cases and 87 controls). One hundred six of the 174 children (61%) were female. No significant differences for clinical or sociodemographic characteristics of mothers or infants were observed between cases and controls (Table 1). The mean age at primary RSV ARI in cases was 3.1 (SD, 1.5) months.

Quantification of Pre-F IgA, Pre-F IgG, Total IgA, and Total IgG

Pre-F IgA, pre-F IgG, total IgA, and total IgG antibodies were measured in 454 breast milk samples from 174 mothers at 1 month (n = 150), 3 months (n = 151), and 6 months (n = 153) postpartum. The median concentration of pre-F IgA (77.7 [IQR, 22.3–200.7] ng/mL) was higher than the median concentration of pre-F IgG (36.5 [IQR, 21.0–62.8] ng/mL). Likewise, the median concentration of total IgA was higher (0.2 [IQR, 0.15–0.27] mg/mL) than total IgG (0.04 [IQR, 0.03–0.05] mg/mL) (Supplementary Table 1). In Table 2 the $\log_{10}$ median concentrations of pre-F IgA, pre-F IgG, total IgA, and total IgG for all breast milk samples for both cases and controls are described in addition to the raw values in Supplementary Table 1.

Correlation Between Specific and Total Antibody Levels

Pre-F IgG concentration showed a moderate positive correlation to total IgG at 1 month ($\rho = 0.38$; $P < .0001$; Supplementary Figure 2), 3 months ($\rho = 0.38$; $P < .0001$), and 6 months ($\rho = 0.40$; $P < .0001$) postpartum. Pre-F IgA showed a lower positive correlation to total IgA at 1 month ($\rho = 0.22$; $P = .007$; Supplementary Figure 2) and at 3 months ($\rho = 0.27$; $P = .0009$), but not at 6 months ($\rho = 0.09$; $P = .29$). Pre-F IgG was positively correlated with pre-F IgA at 1 month ($\rho = 0.18$; $P = .03$; Supplementary Figure 3), at 3 months ($\rho = 0.37$; $P < .0001$), and at 6 months ($\rho = 0.22$; $P = .008$) postpartum.

Table 1. Maternal and Pediatric Clinical Characteristics of Cases and Controls

Characteristic	Cases (n = 87)	Controls (n = 87)	*P* Value
Maternal			
Median age, y (IQR)	22 (19–27)	22 (20–26)	.64
Mean body mass index, kg/m^2 (SD)	21.0 (2.5)	20.7 (2.9)	.55
Literacy	47/82 (57.3)	47/81 (58.0)	.93
Nulliparous	31/87 (35.6)	35/87 (40.2)	.53
Exclusive breastfeeding	57/86 (66.3)	49/87 (56.3)	.21
Household smoking	3/82 (3.7)	4/81 (4.9)	.72
Influenza vaccination[a]	40/87 (46.0)	40/87 (46.0)	.99
No. of respiratory episodes during pregnancy	5/87 (5.8)	6/87 (6.9)	.76
No. of respiratory episodes after delivery	6/87 (6.9)	4/87 (4.6)	.52
Pediatric			
Mean age at RSV illness, mo (SD)	3.1 (1.5)	NA	NA
Mean birth weight, g (SD)	2767 (401)	2802 (488)	.63
Low birth weight	15/75 (20.0)	20/74 (27.0)	.31
Median gestational age, wk (IQR)	40 (39–41)	40 (39–41)	.33
Small for gestational age	35/75 (46.7)	30/74 (40.5)	.45
Preterm[a]	6/87 (6.9)	6/87 (6.9)	.99
Female sex	40/87 (46.0)	38/87 (43.7)	.76

Data are presented as no./No. (%) unless otherwise indicated. Baseline characteristics of children with and without RSV acute respiratory infection in the first 6 months of life (cases and controls) are shown. Maternal and pediatric clinical and sociodemographic characteristics were compared between cases and controls. The Intergrowth-21 criteria [46] were used to calculate small for gestational age. Differences in mean/median of continuous variables were tested with the 2-sided *t* test or a nonparametric Mann–Whitney test when appropriate. Categorical variables were described with frequencies and percentages and compared between groups using χ^2 test.

Abbreviations: IQR, interquartile range; NA, not applicable; RSV, respiratory syncytial virus; SD, standard deviation.

[a]Variables used to match controls to cases.

Pre-F IgG and Pre-F IgA in Cases and Controls Before Infection

We compared pre-F antibody titers at the time point prior to RSV ARI in cases and matched controls. If there was no breast milk sample before infection, then we used the closest time point after RSV ARI. The median time gap between antibody measurement used and RSV ARI was 1.1 (IQR, 0.45–1.6) months. Eight infants had RSV ARI before 1 month of age, and the median time at RSV ARI in these infants was age 0.64 (IQR, 0.49–0.80) months. The median $\log_{10}$ pre-F IgG antibody titer before infection was significantly lower in breast milk of mothers of cases (median, 1.4 [IQR, 1.1–1.6] $\log_{10}$ ng/mL) than in mothers of controls (median, 1.5 [IQR, 1.3–1.8] $\log_{10}$ ng/mL) ($P = .001$; Figure 1A). The effect was more pronounced after excluding 8 children who had RSV ARI before 1 month of age and their matched controls: The $\log_{10}$ pre-F IgG antibody titer was significantly lower in breast milk of mothers of cases (median, 1.4 [IQR, 1.1–1.5] $\log_{10}$ ng/mL) compared with mothers of controls (median, 1.6 [IQR, 1.3–1.8] $\log_{10}$ ng/mL) ($P = .0002$; Supplementary Figure 5A).

Table 2. Antibody Measured in Breast Milk at All Time Points Combined, Log-Adjusted Data

Breast Milk Antibody Measured	All (N = 454), Log_{10} ng/mL		Cases (n = 227), Log_{10} ng/mL		Controls (n = 227), Log_{10} ng/mL	
	Median	(IQR)	Median	(IQR)	Median	(IQR)
Pre-F IgA (n = 450)	1.9	(1.3–2.3)	2.0	(1.4–2.3)	1.8	(1.3–2.2)
Pre-F IgG (n = 449)	1.6	(1.3– 1.8)	1.5	(1.3– 1.7)	1.6	(1.4–1.8)
Total IgA (n = 452)	5.3	(5.2–5.4)	5.3	(5.2–5.4)	5.3	(5.2–5.4)
Total IgG (n = 447)	4.5	(4.4–4.7)	4.5	(4.4–4.7)	4.6	(4.4–4.7)

Table shows median antibody titers in breast milk of all 174 mothers, and cases and controls separately for all time points combined. Log_{10} pre-F IgA, pre-F IgG, total IgA, and total IgG concentrations are shown for all children, and cases and controls separately. Pre-F IgA and pre-F IgG antibodies are expressed as nanograms per milliliter. Total IgA and total IgG antibodies are measured in milligrams per milliliter.

Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; IQR, interquartile range; Pre-F, prefusion F.

The median log_{10} pre-F IgA antibody titer of the breast milk sample at the latest time point prior to infection did not differ significantly in breast milk of mothers of cases (median, 1.7 [IQR, 0.0–2.2] log_{10} ng/mL) compared with mothers of controls (median, 1.7 [IQR, 1.2–2.1] log_{10} ng/mL) (P = .58; Figure 1B). Similarly, when excluding children with RSV ARI <1 month of age, there was no significant difference in pre-F IgA antibody in breast milk of mothers of cases (median, 1.7 [IQR, 0.0–2.1] log_{10} ng/mL) compared with mothers of controls (median, 1.7 log_{10} ng/mL [IQR, 1.1–2.1] log_{10} ng/mL) (P = .50; Supplementary Figure 5*B*).

We evaluated the ratio of pre-F antibody titers to total antibody titer by IgG or IgA isotype. For the ratio of pre-F IgG to total IgG and pre-F IgA levels to total IgA, the same trends were observed (Figure 1C and 1D). Pre-F IgG/total IgG was lower in cases than in controls (0.89 [IQR, 0.58–1.1] log_{10} ng/mL vs 1.0 [0.83–1.2] log_{10} ng/mL; P = .001), whereas pre-F IgA/total IgA did not differ between cases and controls (2.4 [0.0–2.9] log_{10} ng/mL vs 2.4

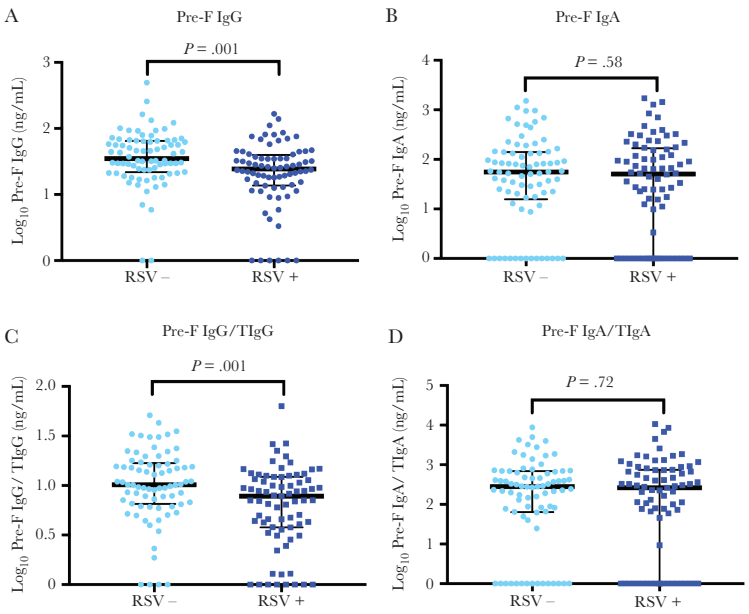

Figure 1. Prefusion F (pre-F) antibody titers prior to time of infection in cases (respiratory syncytial virus positive [RSV+]) and matched controls (respiratory syncytial virus negative [RSV–]). Mann–Whitney test was performed to compare medians of cases and controls. Pre-F antibody titer was compared for measurement prior to infection. For healthy controls, antibody measurement at time of infection for age-matched cases was used. Ratio of pre-F immunoglobulin A (IgA) to total IgA (TIgA) was multiplied by 1×10^6 to ensure values on the y-axis were >0. Ratio of pre-F immunoglobulin G (IgG) and total IgG (TIgG) was multiplied by 1×10^4 for the same reason. *A*, Log_{10} pre-F IgG. *B*, Log_{10} pre-F IgA. *C*, Log_{10} pre-F IgG divided by log_{10} TIgG. *D*, Log_{10} pre-F IgA divided by log_{10} TIgA.

[1.8–2.8] $\log_{10}$ ng/mL; $P = .72$). We performed a sensitivity analysis for infants who were exclusively breastfed in the first few days of life (n = 106), and found that pre-F IgA prior to infection did not differ significantly between cases and controls (1.7 [1.1–2.2] $\log_{10}$ ng/mL vs 1.9 [0.1–2.1] $\log_{10}$ ng/mL; $P = .85$), but did differ for pre-F IgG (1.3 [1.1–1.6] $\log_{10}$ ng/mL vs 1.6 [1.3–1.8] $\log_{10}$ ng/mL; $P = .01$).

Mixed-Model Analysis of Pre-F Antibodies Over Time

We used a mixed-effects linear regression model to compare pre-F IgG and pre-F IgA antibody concentrations in breast milk over time in mothers of cases compared to controls. The mean $\log_{10}$ difference of pre-F IgG concentration in breast milk of mothers of cases compared to controls was –0.21 (95% confidence interval [CI], –.35 to –0.06; $P = .004$) at 1 month postpartum, –0.12 (95% CI, –.26 to .02; $P = .09$) at 3 months postpartum, and 0.00 (95% CI, –.14 to .14; $P = .99$) at 6 months postpartum (Figure 2A and 2B). The mean $\log_{10}$ difference of pre-F IgA in breast milk of mothers of cases compared to controls was –0.10 (95% CI, –.38 to .17; $P = .46$) at 1 month postpartum, 0.11 (95% CI, –.17 to .38; $P = .44$) at 3 months postpartum, and 0.28 (95% CI, .01–.55; $P = .046$) at 6 months postpartum (Figure 2C and 2D). Antibody level was found to increase at 6 months relative to month 1 only for cases (0.27 $\log_{10}$ ng/mL increase in titer for pre-F IgG, $P = .001$; 0.44 $\log_{10}$ ng/mL increase for pre-F IgA, $P = .003$). There was no evidence of increase over time for either antibody level among controls ($P = .45$ and $P = .21$ for pre-F IgA and pre-F IgG, respectively). Consequently, for pre-F IgG, the difference found at 1 month between cases and controls was no longer present at 6 months of age ($P = .99$).

Antibody Concentration and Time to Infection

Among cases, there was a low negative correlation between pre-F IgG concentration in breast milk at 1 month postpartum and time to RSV ARI in cases, which is marginally significant ($\rho = -0.22$; $P = .06$), indicating that higher antibody levels may

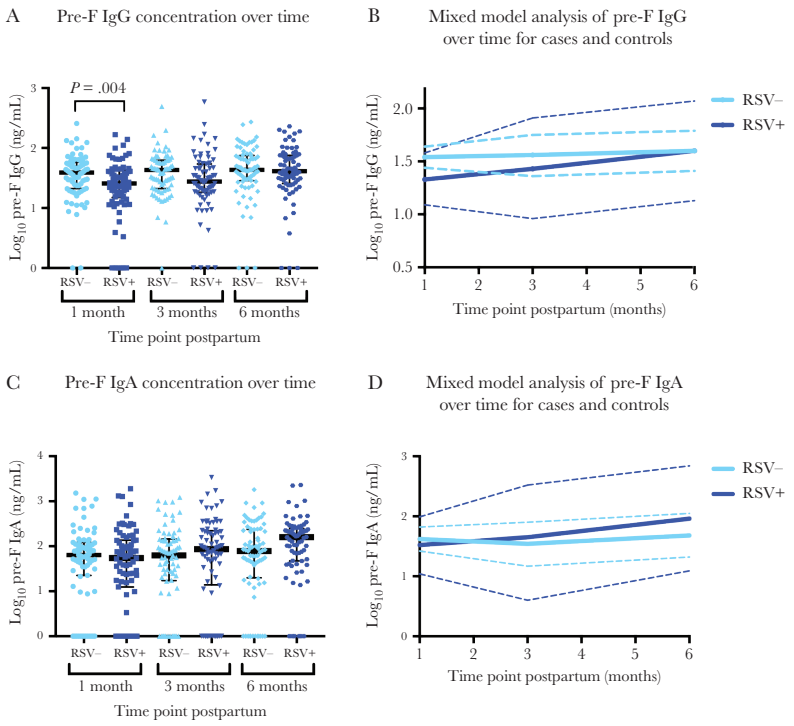

Figure 2. Mixed-model analysis of prefusion F (pre-F) antibody in cases and controls over time. A linear mixed-model analysis was used to examine the effect of respiratory syncytial virus (RSV) on pre-F antibodies at different time points. *A*, $\log_{10}$ pre-F immunoglobulin G (IgG) antibody concentration at 1, 3, and 6 months postpartum for cases (RSV positive [RSV+], dark blue) and controls (RSV negative [RSV–], light blue), with medians indicated in black. *B*, Linear mixed-model analysis for $\log_{10}$ pre-F IgG in cases and controls. Solid line is the mean, and dashed line indicates the 95% confidence interval (CI). *C*, $\log_{10}$ pre-F immunoglobulin A (IgA) antibody concentration at 1, 3, and 6 months postpartum for cases and controls, with medians in black. *D*, Linear mixed-model analysis for $\log_{10}$ pre-F IgA in cases and controls. Solid line is the mean, and dashed line indicates the 95% CI.

be associated with shorter time to infection. However, there was no detected correlation between pre-F IgA antibody concentration at 1 month postpartum and time to RSV ARI in cases ($\rho = 0.10$; $P = .40$).

Seasonal Fluctuation of Pre-F IgA and Pre-F IgG Titers

We applied a sinusoidal model to the pre-F IgG and pre-F IgA concentrations in breast milk of all mothers at 1 month postpartum. All RSV-infected infants in this substudy were born between June and September, as were the controls who were matched by birth month. Therefore, no children in this substudy were born in October through January, corresponding to the peak of the RSV season in Nepal [23], which resulted in a poor fit of the model (goodness-of-fit measure: $r = 0.008$ for pre-F IgG; $r = 0.02$ for pre-F IgA) (Supplementary Figure 4*A* and 4*B*).

DISCUSSION

We provide evidence that IgG antibodies in breast milk against RSV pre-F are lower in mothers of children who develop RSV ARI in the first months of life compared with children who do not. In the context of RSV maternal vaccine development with no established correlate of protection against RSV [24], we conclude that breast milk pre-F IgG antibodies may be a correlate of protection against RSV ARI. The importance of these findings is underscored by the fact that premature infants, who are disproportionately affected by RSV disease [25] and have reduced transplacental antibody transfer, may still be potentially protected by maternal immunization via breast milk [11].

One strength of this study was the development of a novel breast milk RSV antibody assay targeting the RSV fusion protein in a pre-F–stabilized conformation, which permitted measurement of antibodies known to be an important target for RSV neutralizing antibodies [15]. Additionally, the use of recombinant palivizumab IgA allowed for accurate measurement with an IgA standard. Palivizumab IgA1 and IgA2 were used in a 3:2 ratio as found in human breast milk [26].

The results show a potential protective role against RSV ARI for breast milk pre-F IgG but not pre-F IgA antibodies. The difference in pre-F IgG between cases and controls is small, though statistically significant. The difference in protection across antibody isotypes is in accordance with studies specific to RSV and other pathogens, such as human immunodeficiency virus [27] and cytomegalovirus [28]. Likewise, recombinant palivizumab IgA offers less effective protection following intranasal administration than IgG in mice [22].

The relationship between breast milk pre-F IgG and time to infection was an exploratory analysis; the negative correlation merits further study in a larger population powered to look at this effect using more frequent sequential breast milk samples collected over time and a comparison to serologic assays. When looking at seasonality of breast milk pre-F antibodies in breast milk, IgA but not IgG decreased in the summer months, possibly due to the shorter half-life [29] and lack of exposure to RSV. Increases in breast milk pre-F IgG at 6 months postpartum may have reflected exposure and infection of the mothers. However, in our study we did not sample for respiratory viruses in asymptomatic or afebrile illnesses in women, therefore limiting our ability to detect these by molecular diagnosis.

There is consensus on the protective effect of breastfeeding on infant respiratory morbidity and mortality [30], with lower risk of RSV hospitalization and reduced disease severity when comparing breastfed to nonbreastfed infants [31–33]. However, evidence for the mechanisms by which breast milk antibodies may enter the neonatal circulation is limited. Breast milk antibodies have been shown to reach the neonatal circulation in 3 children who were given antibody-rich human colostrum via a nasogastric tube [34]. After closure of the gut, uptake of IgG may occur via the neonatal Fc receptor, which has been identified in the human intestine [35] and is involved in bidirectional transport across the enterocyte, allowing for defense at the mucosal level [36]. Secretory IgA plays a role at the mucosal surface by neutralizing pathogens in the intestinal lumen in humans [36].

Boosting breast milk antibody via maternal vaccination may help protect infants from RSV disease. In a subunit RSV vaccine trial, increased IgA and IgG antibodies against RSV in breast milk were measured in vaccinated compared to nonvaccinated women [4]. Increased concentrations of breast milk antigen-specific antibodies have been measured following maternal vaccination against influenza, pertussis, RSV, and *Streptococcus pneumoniae* [37]. Only 1 study examined the association between respiratory pathogen–specific antibodies and clinical outcomes in 57 infants [38]. In this study, maternal influenza vaccination and increased influenza-specific IgA in breast milk correlated with decreased episodes of infant respiratory illness, though IgG was not measured. Finally, there is evidence that high virus-specific IgA may interfere with vaccine response for rotavirus vaccination [39], which may be a consideration for RSV maternal vaccination.

The most important limitation of this study was that we did not measure pre-F antibodies in serum of all mothers of these infants or in cord blood. No blood was drawn from infants during this study, so further study in infants was not possible. An alternative explanation for protection may be serum pre-F antibody titers in women and their infants. However, in a subset of 310 maternal infant pairs within the maternal vaccination cohort, neutralizing RSV antibody titers in cord blood were not shown to protect against RSV ARI [40]. For 44 maternal infant pairs that overlapped with the cohort in this study, we examined the correlation between breast milk pre-F IgG at 1 month postpartum and cord blood antibody titers and found a positive correlation between breast milk pre-F IgG antibodies and neutralizing antibody titers in cord blood ($r(^2) = 0.29$; $P = .05$). We found no relationship between

breast milk pre-F IgA and cord blood neutralizing antibody titers (r^2) = –0.07; P = .6). In an exploratory analysis, we found no relationship between disease severity and breast milk pre-F IgG and IgA antibody titers from samples collected closest to the time of infection (data not shown). Furthermore, we found no relationship between breast milk pre-F antibody levels at the time points closest to infection and nasal swab PCR cycle threshold values (data not shown). Another limitation of this study is a facet of the study design. Although RSV ARI often occurs after 6 months of age [25, 41], in this study we were limited to early RSV infection in subjects <6 months of age by design. We did not measure antibody titers in the first month postpartum. The study population was small (N = 174), though larger than almost all studies measuring antigen-specific antibodies against respiratory pathogens (n = 5–258) [37] and larger than any study measuring RSV antibodies in breast milk (n = 57–130) [4, 42, 43]. These results should be replicated in a larger group in a different population with longer follow-up time. An additional limitation was the choice to measure antibodies against pre-F but not to exclude antibodies that bind epitopes present on postfusion F protein (sites II and IV). Antibodies that target only antigenic site ø show high neutralizing activity [44] and may correlate even better with protection from RSV. The protective effect of breast milk pre-F–specific antibodies against respiratory disease may have been underestimated because antibodies against all pre-F epitopes were measured, which included less potent RSV-neutralizing or nonneutralizing antibodies. Finally, we did not measure antibodies against G protein, which have recently also been shown to display neutralizing activity and correlate with decreased disease severity in infants and young children [45] and should be assessed in future studies.

In conclusion, the current study provides evidence that pre-F IgG antibodies in breast milk may play a protective role against RSV-confirmed ARI in the first 6 months of life. Induction of pre-F IgG in breast milk may be a potential mechanism of protection of maternal RSV vaccine candidates.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. All co-authors contributed to the final manuscript. M. C. S., J. M. T., J. K., S. K. K., and J. A. E. contributed to the design of the original trial. M. C. S. conceived of and secured funding for the original trial. S. K., S. L., J. M. T., and J. K. supervised the conduct of the study in the field for the original trial.

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INTRANASAL PALIVIZUMAB TO PREVENT RESPIRATORY SYNCYTIAL VIRUS INFECTION IN HEALTHY PRETERM INFANTS

Submitted Manuscript

Do not hire a man who does your work for money,
but him who does it for love of it.
Henry David Thoreau (1817-1862)

Intranasal Palivizumab to Prevent Respiratory Syncytial Virus Infection in Healthy Preterm Infants

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ABSTRACT

Background
Mucosal administration of monoclonal antibodies (mAbs) against respiratory pathogens is a promising alternative for systemic administration since lower doses are required. Clinical development of mucosal mAbs is a highly active field. Clinical proof-of-concept is not yet available. We aimed to investigate the efficacy of mucosally administered palivizumab, a mAb targeting respiratory syncytial virus (RSV).

Methods
In this investigator-initiated, double-blind, randomized placebo-controlled trial (NTR7403) we evaluated intranasal palivizumab for the prevention of RSV infection in preterm infants after determining an acceptable safety profile in a phase I cross-over trial (NTR7378) in healthy adults. We randomized infants 1:1 to receive intranasal palivizumab (1 mg/ml) or placebo daily during the RSV season. The primary outcome was any RSV infection with RSV hospitalization as key secondary outcome.

Results
We recruited 268 infants after which the trial was stopped for futility following the planned interim analysis. Adverse events were similar in both groups. In total, 168/268 infants were excluded from the efficacy analyses due to absent RSV circulation during the pandemic. Lab-confirmed RSV infection was similar in 18/47 (38.3%) infants in the palivizumab arm and 11/47 (23.4%) in the placebo arm (adjusted OR (aOR): 2.2; 95%CI: 0.7-6.5, p=0.14). The proportion of infants hospitalized for RSV was similar in the palivizumab arm (7/47, 14.9%) and placebo arm (3/47, 6.4%) (crude OR: 2.6; 95%CI 0.6-10.6).

Conclusions
Daily intranasal palivizumab prophylaxis did not show protection against RSV infection in late preterm infants. The nose may not be the first site of RSV viral replication in infancy or nasal antibody half-life may not be sufficient for clinical protection. Our findings of lack of efficacy have important implications for the clinical development of other antibodies for intranasal administration.

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INTRODUCTION

Globally, RSV is the second cause of death in the infant period[21], yet there is no vaccine or treatment available[22]. Of the sixteen vaccine candidates and monoclonal antibodies (mAbs) in late-stage clinical trials, none target the LMIC market, where RSV mortality is highest. Palivizumab, a humanized mAb against the surface F protein of RSV, has been market-approved for more than twenty years, but access is limited to high-risk infants due to prohibitive costs. Furthermore, administration is burdensome, via monthly intramuscular (i.m.) injections and not maximally effective with breakthrough infections occurring at low trough antibody levels.

According to Ku, *et al*.[23], circulating IgG antibodies lack efficient access to mucosal compartments as antibody levels in the lung are 200-500 times lower after intravenous infusion resulting in the need for high doses (up to 8g) of potent neutralizing monoclonal antibodies with only a small antiviral effect in the respiratory tract. The need for high doses of systemic mAb to reach a therapeutic dose may be overcome through local administration. Mucosal administration of mAbs may offer a key solution for major respiratory pathogens: this approach offers a dose-sparing highly targeted prevention by stopping infection at the site of viral entry. Monoclonal antibodies need to be administered at high doses systemically to reach a therapeutic dose locally in the respiratory tract. Mucosal mAb administration overcomes this challenge by stopping infection at the site of viral entry and is therefore a key solution for major respiratory pathogens. Preclinical studies support efficacy of local administration of mAbs against respiratory pathogens[24]. Previously, we demonstrated that intranasal (i.n.) palivizumab provides full protection against experimental RSV infection in mice in a dose-dependent manner for at least a week after administration[25]. Another recent study has shown that low doses of i.n. hyper-enriched anti-RSV IgG inhibit infection in mice[26]. Recently, multiple preclinical studies showed that i.n. administered SARS-CoV-2 neutralizing mAbs provide protection in mice[23,27].

Currently, intranasal antibody drug development is highly active with 7 drug candidates in development for SARS-CoV-2, RSV, and influenza. Development is led by pharmaceutical companies, public private partnerships and non-profit networks (total market capital >20.6 billion USD) with several preclinical candidates, 1 phase I trial ongoing, a phase I/II trial and a phase II trial expected to start soon. Therefore, the results of this study have important implications for mucosal antibody development including the necessity of a timely interim analysis to evaluate efficacy to avoid wasted time and capital.

Intranasal palivizumab offers a child-friendly, affordable and effective alternative for intramuscular palivizumab. Previously, we and others have shown 80% efficacy against RSV hospitalization through intramuscular administration of palivizumab in late preterm infants 32-25 weeks gestational age[28]. We hypothesized that local administration to the airways will be at least as effective because it is delivered directly to the main port of entry and decreases the chance of break through infection.

We describe product development, a phase I trial followed by the first report of a phase IIb trial to evaluate the efficacy of daily i.n. administration of palivizumab during the RSV season to prevent RSV infection in otherwise healthy late preterm infants during the first year of life.

METHODS

Study design

We conducted a double-blind, randomized, placebo-controlled cross-over phase I safety trial (Narsyn Study A, NTR7378, Supplementary Appendix) in 2018. Subsequently, we intended to obtain proof–of-concept that intranasal palivizumab prevents RSV infection in infants. Study B (NTR7403, Supplementary Appendix), a double-blind, randomized, placebo-controlled proof-of-concept phase IIb trial was initiated based on overall safety profile of Study A upon recommendation of an independent data safety and monitoring board (DSMB). Recruitment for study B was conducted at 39 hospitals (1 academic, 38 regional) in the Netherlands from November 2018 through January 2021. The trial was approved by the institutional review board of the University Medical Center Utrecht, the Netherlands (NL66735.041.18).

Study population

Study A included 20 healthy adult volunteers between 18-60 years of age. We excluded adults with a nasal cold or nasal obstruction that could interfere with administration of the study medication, respiratory symptoms 4 weeks prior to study medication administration, simultaneous use of other nose drops or spray, use of other nasal medication (including any cocaine use ever) or use of tobacco. For study B, we included infants with a gestational age between 32+0 and 35+6 weeks who were younger than 6 months at the start of the RSV season (October 1st). To limit the required**3** sample size, the trial was performed in a high-risk population of infants with at least one older sibling. We excluded infants with congenital heart disease, bronchopulmonary dysplasia, Down's syndrome, or other serious congenital disorders for which regular palivizumab was indicated. Simultaneous use of nose drops or spray other than normal saline was also an exclusion criterion.

Study medication

Study medication consisted of nasal drops with a concentration of 1 mg/ml palivizumab (ATC code J06BB) in 0.9% sodium chloride. Commercial saline nasal drops (0.9% sodium chloride, Fagron) were used as placebo. We showed the drug formulation is stable at 4C (intended use) and room temperature for at least 52 weeks. We included the 24 month shelf life at 4C in the investigational medicinal product dossier [Supplementary Appendix]. Study staff and study participants were blinded to study arm assignments. Study participants were randomized 1:1 in a non-stratified manner using blocks of 2 and 4 using Castor Electronic Data Capture (EDC) platform. Study medication and placebo were identically packaged and indistinguishable by sight or smell. For study A, participants administered one nasal drop (50ul) daily to the right nostril for 7 days before wash-out (14 days) and crossover to the alternate treatment for 7 days. For study B, parents were instructed to administer one drop of study medication in each nostril daily from the beginning of the RSV season for a maximum duration of 5 months. Eligible infants who were born during the RSV season were enrolled until the end of January and received minimally 2 months of study medication.

Study definitions and follow-up

Primary endpoints. For study A, the primary outcome was self-reported local and systemic adverse events (AEs) according to the FDA scorecard. Participants were instructed to use a log to record any solicited local or systemic AEs and notify study staff. In the case of objectifiable

symptoms, researchers performed a home visit. For study B, the primary outcome was any lab-confirmed RSV infection.

Secondary endpoints. Secondary outcomes included RSV hospitalization (key secondary outcome), medically attended RSV infection without hospitalization, non-medically attended RSV infection, respiratory tract infection (RTI) hospitalization, medically attended RTI without hospitalization, non-medically attended RTI, RSV-associated intensive care unit (ICU) stay, mechanical ventilation and supplemental oxygen, otitis media, and wheeze in the first year of life. Parents were instructed to take a nasal swab (Copan universal transport medium (UTM)) in case of respiratory symptoms lasting more than 1 day. Parents recorded medication adherence, presence of respiratory symptoms, doctor visits, and the use of airway medication in a daily log through April and presence of wheezing until their infant was 1 year of age. Weekly follow-up calls were performed. Study B endpoints are further defined in the Statistical Analysis Plan [Supplementary Appendix]. For both trials, objective adherence was measured by weighing study medication before and after use. Subjective adherence was measured by self-reported administration in a diary.

Laboratory tests

Nasal swabs were transported in UTM by regular mail to the laboratory and were stored at –80°C until analysis. Polymerase-chain-reaction (PCR) assays were performed. The presence of RSV RNA was determined as described previously with minor modifications[29]. Samples, spiked with a fixed dose of murine encephalomyocarditis virus as internal control, were extracted using the MagNA Pure 96 DNA and Viral NA Large Volume Kit (Roche Diagnostics, Mannheim, Germany). Copy DNA synthesis and real-time PCR were performed using the one-step Taqman Fast Viral Master Mix (Applied Biosystems) in an ABI Prism 7500 real-time PCR system.

Statistical analysis

Detailed information about the statistical procedures for this study is provided in the predefined Statistical Analysis Plan [Supplementary Appendix]. Nearly all analyses were performed according to the statistical analysis plan; we specified if the analysis was performed post-hoc in the Results section The predefined target sample size of 408 infants provided 85% power to detect a relative risk reduction for RSV infection of 62.5% assuming an RSV infection rate of 16% in the placebo group[30] and 80% power to detect a relative risk reduction for RSV hospitalization of 80% assuming 8% RSV hospitalizations in the placebo group. An interim analysis was performed according to protocol to assess futility or efficacy when 50% of the expected events had been observed. The predetermined stop criterion for futility was a conditional power <20% for the primary endpoint analysis. The primary endpoint was compared between trial arms using a mixed effect logistic regression on the modified intention-to-treat (mITT) population as described in the Statistical Analysis Plan. Post-hoc analyses were performed to assess wheezing in RSV-infected and non-infected infants. The analyses were performed in R (version 4.0.3 or higher), SAS Enterprise Guide (version 8.2) and SPSS (version 25.0.0.2). All reported effect sizes are for palivizumab relative to placebo.

RESULTS

Phase I

Safety. In September 2018, 20 subjects were enrolled. One subject was excluded before any study medication was administered due to symptoms of a respiratory infection at the start of the study. Airway patency after 10 minutes was 100% in both the palivizumab (10/10) and placebo (9/9) arm. Self-reported local and general symptoms were tabulated per arm per study participant [Table S2A]. There were no SAEs in either trial arm and no AEs were considered to be treatment-related by the study staff or DSMB [Table S2B-C].

Phase IIb

Study population. Of 4403 eligible patients, 268 were enrolled in the study [Figure 1]. Eleven (11) infants were enrolled during season 1 (2018/2019), 89 during season 2 (2019/2020) and 168 during season 3 (2020/2021). Parents administered nasal drops for an average duration of 4.1 months (SD: 1.2); 4.2 months (SD: 1,2 months) in the intervention group and 4.0 months (SD: 1.2 months) in placebo group. As there was complete absence of RSV circulation in the winter of 2020/2021 due to the COVID-19 pandemic, infants enrolled during season 3 were excluded from the efficacy analyses. The interim analysis performed on May 19th, 2021, showed that the conditional power of the trial was 1%. The trial was stopped on June 7, 2021 after the DSMB confirmed the stopping rule for futility was met (conditional power < 20%). [Supplementary Appendix].

Clinical characteristics. The mITT population (n=94) used for the interim and final primary analysis consisted of all infants for whom the primary endpoint was known. We excluded 6/100 infants who discontinued the study early (3 in the intervention group and 3 in the placebo group). One child in the intervention arm who discontinued the trial early, but was hospitalized with RSV before trial discontinuation, was included in the mITT population.

Baseline characteristics were similar in both study arms [Table 1]. Median age of infants at time of inclusion in the study was 2.3 months (IQR 0.7-4.3) and median gestational age was 34.3 weeks (IQR 33.4-35.1). Twenty-nine (29%) infants were part of a multiple birth. Subjective and objective adherence was high and similar in both study arms. [Figure S3].

Safety. Adverse events were determined to be unrelated to study medication in the all-subjects treated population [Table S10]. No SAEs were determined to be related to study medication; the number of SAEs was similar in the palivizumab arm (22) and placebo arm (26).

Efficacy. In the mITT population, 29 infants (30.9%) had any laboratory-confirmed RSV infection; 18/47 (38.3%) in the palivizumab arm and 11/47 (23.4%) in the placebo arm [Table 2]. Trial arms were similar in terms of any RSV infection (adjusted OR (aOR): 2.2; 95%CI: 0.74-6.5) in the mITT population and per protocol population (aOR: 2.0; 95%CI: 0.60 – 6.3) [Table 2]. Sensitivity analyses assessing the impact of missing outcome [Table 3], different CT-value cut-offs and adherence [Table S3] also showed no difference between both trial arms. The key secondary outcome, number of infants hospitalized with lab-confirmed RSV was similar in the palivizumab arm (7/47, 14.9%) and placebo arm (3/47, 6.4%). Other secondary outcomes were also similar in both trial arms [Table 2, Table S5].

Wheeze. There was no difference in any wheezing, fraction of wheezing days [Table S6], or wheezing episodes between the trial arms (incidence rate ratio (IRR): 1.15; 95%CI 0.8-1.7)

[Table S7-S8]. The proportion of infants with recurrent wheezing and physician-diagnosed wheeze were similar across trial arms [Table S7], also when analyzed separately for infants of parents with and without an atopic history. Occurrence of any wheezing was similar for RSV-infected and non-infected infants [Table S9] (post-hoc analysis).

DISCUSSION

In this study we evaluated the safety and efficacy of daily i.n. palivizumab administration against RSV infection in late preterm infants through a phase I and IIb clinical trial. Clinical development of the investigational product was fully investigator-initiated and conducted without funding from the pharmaceutical industry. This is the first study determining efficacy of nasal administration of antibodies with a non-human target to prevent respiratory infection. Intranasal prophylaxis in late preterm infants was not effective against lab-confirmed RSV infection despite high rates of adherence.

The rate of total RSV infection (30.8%) and RSV hospitalization (10.6%) was higher than expected at the time of sample size calculation (16% and 8% respectively) but similar to the rate reported in a recently published RSV burden study[31]. The proportion of infants with any wheezing in the placebo arm (43.5%) was similar to the MAKI trial (47%)[28].

Limitations of the study include no circulation of RSV during the third season of the trial due to the COVID-19 pandemic, resulting in a relatively small sample size for the final efficacy analysis. The small sample size resulted from early termination of the trial due to futility before the final season of enrollment. Baseline incidence of RSV infection was sufficiently high to support our negative conclusion. Second, administration of study medication was dependent upon parental adherence. Parental-reported subjective adherence may have been subject to social desirability bias, underreporting missed doses resulting in overestimating adherence. Objective adherence based on weight of dosage was high, indicating that insufficient adherence is not the main driver of lack of efficacy. Third, symptoms of RSV infection and nasal swabs were collected by parents. To mitigate the risk of missed outcomes, parents were contacted on a weekly basis by dedicated study staff. We therefore expect a low number of missed infections this study.

The observed lack of efficacy may be explained by several non-exclusive mechanisms. First, there is currently no sampling technique in the nasal cavity to measure an effective medication dosage by measuring trough antibody concentrations; therefore, it has not been possible to define the half-life of palivizumab in the airways. For this reason, half-life of palivizumab in the airways has also never been shown for intramuscular palivizumab. The half-life of IgG in the nasal epithelial lining fluid is not well established but due to mucociliary clearance it is expected that it is substantially shorter than in serum. Half-life of bovine IgG in the nose of mice was determined to be 4 hours[32]. However, it remains unclear whether measurement of half-life corresponds to the clinical outcome of interest, namely, protection against RSV infection. Furthermore, it is unclear whether antibody levels in the epithelial lining fluid of the nasal cavity are a good correlation of protection. In vivo we showed that despite being fully protected against RSV for at least one week[25], monoclonal RSV-neutralizing antibodies (0.5 mg/kg) palivizumab administered into the lungs of naïve wild-type BALB/c mice are detected at low levels or not at all on the mucosal level 7 days after administration in the nasal airway or lungs [unpublished data]. Second, it is known that the eyes (mouth less so) are routes of inoculation for RSV[33,34] and local nasal administration did unlikely protects against these infection routes. Lastly, both inadequate dosing or antibody-dependent enhancement (ADE) might have contributed to lack of efficacy. In ADE, suboptimal RSV medication concentrations may enhance viral replication[35]. However, the trial dosage of i.n. palivizumab is expected to be higher than airway medication concentrations achieved through

i.m. administration. The dosing regimen of i.m. palivizumab has been designed such that trough concentrations are minimally 30 $ugml^{-1}$ and ideally greater than 40 $ugml^{-1}$ (as a margin of safety for person-to-person variability) for clinical efficacy[36]. The daily dose of 50 ug per nostril in this study is easily above the minimal threshold needed for protective efficacy (0,064ug per nostril). In summary, lack of efficacy may be explained by a short half-life of study medication in the nasal cavity, overestimated adherence, inadequate protection for viral inoculation routes other than the nose, and inadequate dosing. This study also shows a local correlate of protection is required.

We show that an IgG mAb administered once daily to preterm infants does not prevent RSV infection. In future trials, it will be important to measure antibody half-life in the nose as improved sampling devices such as a synthetic absorptive matrix (SAM) strip have recently become available to collect neat mucosal lining fluid. The neonatal Fc receptor (FcRn), which is critical to extended half-life of antibodies, is present on fixed nasal tissue[37] and transepithelial delivery of therapeutic extended half-life mAbs has been shown in human nasal epithelial cells in vitro[38] transgenic mice expressing human FcRN [39] and ex vivo porcine olfactory mucosa[40]. Intranasal extended half-life mAbs have recently been shown to block COVID-19 infection after experimental infection in mice[23]. A controlled human challenge model may be used to determine the half-life of these mAbs in the nose. In vitro models of human nasal epithelium show high expression of FcRn in ciliated cells and trans epithelial delivery of extended half-life IgG mAbs[38]. IgA is a mucosal antibody which has been associated with protective immunity against RSV[11]. Potentially, mucosally delivered IgA mAbs may play a more important role in prevention of infection in the airways than IgG. However, we previously showed that reformatting palivizumab into anti-RSV monomeric and secretory IgA is a less effective intranasal prophylaxis in mice[41]. Finally, in the case respiratory pathogens primarily infect through nasal inoculation as opposed to other routes (i.e., eyes, mouth), they may more effectively block infection.

In conclusion, daily intranasal palivizumab prophylaxis did not show protection against RSV infection in late preterm infants.

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Index of Primary Tables and Figures

Figure 1. Study B enrollment

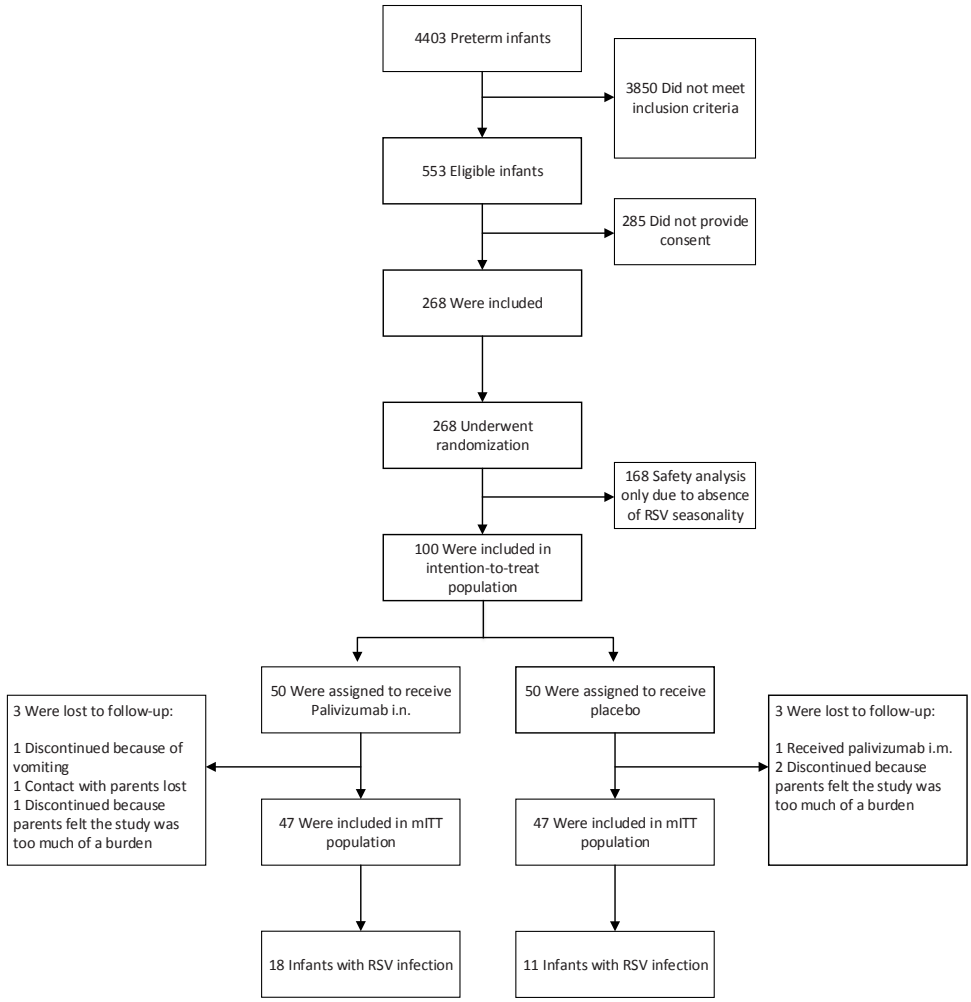

Table 1. Baseline characteristics of Study B participants

	Palivizumab (*N* = 50)	**Placebo (*N* = 50)**	**Total (*N* = 100)**
Female (%)	22 (44)	26 (52)	48 (48)
Age in months (median, IQR)	2.3 (0.7–4.4)	2.4 (0.6–4.2)	2.3 (0.7–4.3)
Gestational age in weeks (median, IQR)	34.3 (33.1–35.3)	34.5 (33.6–35.1)	34.3 (33.4–35.1)
Birth weight in grams (median, IQR)	2325 (1947–2602)	2364 (2000–2575)	2342 (1953–2590)
Multiple birth (%)	16 (32)	13 (26)	29 (29)
Complication(s) during pregnancy (%)	23 (46)	27 (54)	50 (50)
Antenatal corticosteroids (%)	20 (40)	22 (44)	42 (42)
Complication(s) during delivery (%)	26 (52)	29 (58)	55 (55)
Antibiotics during delivery (%)	8 (16)	7 (14)	15 (15)
Vaginal Delivery (%)	24 (48)	27 (54)	51 (51)
Apgar score 5 min (median)	9 (8–10)	9 (8–10)	9 (8–10)
Respiratory support after birth (%)	26 (52)	24 (48)	50 (50)
Received antibiotics after birth (%)	19 (38)	22 (44)	41 (41)
Maternal age at birth child (median, IQR)	32 (30–36)	32 (30–35)	32 (30–35)
Breastfeeding (%)	21 (42)	23 (46)	44 (44)
Breastfeeding and formula (%)	22 (44)	17 (34)	39 (39)
Formula (%)	7 (14)	10 (20)	17 (17)
Parental level of education – postgraduate			
Maternal	46 (92)	46 (92)	92 (92)
Father	42 (87.5)	46 (92)	88/98 (89.8)

Maternal smoking during pregnancy (%)	5 (10)	4 (8)	9 (9)
Smoking inside (%)	1 (2)	0 (0)	1 (1)
Total number of persons in household (median, IQR)	4 (4–5)	4 (4–5)	4 (4–5)
Number of older siblings (median, IQR)	1 (1–2)	1 (1–1)	1 (1–1)
Day care attendance (%)	28 (56)	27 (54)	55 (55)
Sibling attending day care (%)	1 (1–2)	1 (1–1)	1 (1–1)
Atopy mother (%)	26 (52)	16 (32)	42 (42)
Physician diagnosis asthma (%)	8 (16)	5 (10)	13 (13)
Physician diagnosis hay fever (%)	20 (40)	10 (20)	30 (30)
Physician diagnosis eczema (%)	10 (20)	8 (16)	18 (18)
Atopy father (%)	20/47 (42.6)	18 (36)	38/97 (39.2)
Physician diagnosis asthma (%)	5/47 (10.6)	2 (4)	7/97 (7.2)
Physician diagnosis hay fever (%)	12/48 (25)	13 (26)	25/98 (25.5)
Physician diagnosis eczema (%)	8/48 (16.7)	6 (12)	14/98 (14.3)

Table 2. Efficacy of intranasal prophylaxis against RSV infection and all-cause respiratory tract infections

Variable	Palivizumab (*N* = 47)	Placebo (*N* = 47)	Adjusted odds ratio [1] (95% CI)	P value	Crude odds ratio (95% CI)	Risk difference (%) (95% CI)	Relative risk reduction (%)[2] (95% CI)
Primary Endpoint							
RSV infection (%)	18 (38.3)	11[3] (23.4)	2.2 (0.74 – 6.5)	0.14	2.0 (0.83 – 5.0)	14.9 (-3.5 – 33)	-64 (-208 – 13)
Secondary Endpoints							
Hospitalization for RSV infection (%)	7 (14.9)	3 (6.4)			2.6 (0.62 – 11)	8.5 (-3.8 – 21)	-133 (-748 – 36)
Medically attended RSV infection without hospitalization (%)	8 (17.0)	2 (4.3)			4.6 (0.92 – 23)	12.7 (0.1 – 25)	-300 (-1685 – 10)
RSV infection without medical attention (%)	3 (6.4)	7 (14.9)			0.39 (0.094 –1.6)	-8.5 (-21 – 3.8)	57 (56 – 88)
All-cause RTI							
Any RTI (%)	46 (97.9)	45 (95.7)			2.0 (0.18 – 23)	2.1 (-5.0 – 9.2)	-2.2 (-10 – 5.0)
RTI hospitalization (%)	9 (19.2)	6 (12.8)			1.6 (0.53 – 5.0)	6.4 (-8.4 – 21)	-50 (-288 – 42)
Medically attended RTI	26 (55.3)	22 (46.8)			1.4 (0.62 – 3.2)	8.5 (-12 – 29)	-18 (-76 – 21)
Non medically attended RTI	43 (91.5)	45 (95.7)			0.48 (0.083 – 2.7)	-4.3 (-14 – 5.6)	4.4 (-6.2 – 14)

[1] Adjusted analyses using a mixed effect logistic regression with treatment arm, having more than 1 sibling, date of birth between August 14 and December 1, neonatal respiratory support as fixed effects and random intercept for family. Adjusted analyses were not performed for secondary outcomes because there was only a very small number of infants with this endpoint. Placebo group is the reference group.
[2] The following formula was used to calculate relative risk reduction: RRR=((CER-EER))/CER
[3] One child had an RSV hospitalization (November) followed by a case of non-hospitalized medically-attended RSV (December). One participant had two medically-attended RSV infections and two participants had two non-medically attended RSV infections within one RSV season.
RTI, respiratory tract infection.
*100%.

Table 3. Sensitivity Analyses including infants with missing outcome

Analysis	Population	Palivizumab	Placebo	aOR (95% CI) or RR (95%)
aOR for primary analysis with all missing outcomes replaced with no RSV	ITT (*N* = 100)	18/50 (38.3)	11/50 (23.4)	2.2 (0.76 – 6.3)[1]
aOR for primary analysis with all missing outcomes replaced with RSV	ITT (*N* = 100)	21/50 (42.0)	14/50 (28.0)	2.0 (0.71 – 5.5)[1]
aOR for primary analysis with all missing outcomes replaced with RSV in the placebo group and no RSV in the treatment group	ITT (*N* = 100)	18/50 (36.0)	14/50 (28.0)	1.5 (0.55 – 4.0)[1]
aOR for primary analysis with all missing outcomes replaced with no RSV in the placebo group and RSV in the treatment group	ITT (*N* = 100)	21/50 (42.0)	11/50 (22.0)	2.8 (0.97 – 7.8)[1]
aOR for primary analysis in the per protocol population	Per Protocol Population (*N* = 86)	16/42 (38.1)	11/44 (25.0)	1.9 (0.59 – 6.3)[1]
Relative risk	mITT (*N* = 94)	18/47 (38.3)	11/47 (23.4)	1.6 (0.87 – 3.1)[2]

[1] Mixed effect logistic regression with treatment arm, having more than 1 sibling, date of birth between August 14 and December 1, neonatal respiratory support as fixed effects and random intercept for siblings was used to calculate OR for CT-value sensitivity analyses. [2]Crude relative risk for treatment arm.

8

LOWER RESPIRATORY TRACT INFECTION CAUSED BY RESPIRATORY SYNCYTIAL VIRUS: CURRENT MANAGEMENT AND NEW THERAPEUTICS

The Lancet Respiratory Medicine, 2015

Simplify, Simplify, Simplify!
Henry David Thoreau (1817-1862)

Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics

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Respiratory syncytial virus (RSV) is a major worldwide cause of morbidity and mortality in children under five years of age. Evidence-based management guidelines suggest that there is no effective treatment for RSV lower respiratory tract infection (LRTI) and that supportive care, ie, hydration and oxygenation, remains the cornerstone of clinical management. However, RSV treatments in development in the past decade include 10 vaccines and 11 therapeutic agents in active clinical trials. Maternal vaccination is particularly relevant because the most severe disease occurs within the first 6 months of life, when children are unlikely to benefit from active immunisation. We must optimise the implementation of novel RSV therapeutics by understanding the target populations, showing safety, and striving for acceptable pricing in the context of this worldwide health problem. In this Review, we outline the limitations of RSV LRTI management, the drugs in development, and the remaining challenges related to study design, regulatory approval, and implementation.

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Introduction

Respiratory syncytial virus (RSV) bronchiolitis contributes greatly to mortality in children under 5 years of age,[1] and has implications for long-term respiratory health.[2] Nearly all children in the world will be infected with RSV by 2 years of age.[3]

Several evidence-based guidelines for the management of bronchiolitis exist, with differing recommendations, but all agree on supportive management in the inpatient setting. A guideline published by the American Academy of Pediatrics[4] reported insufficient evidence for any intervention except respiratory support and hydration. In view of the paucity of therapeutic alternatives, it is essential to understand the existing challenges to the development of prevention and treatment options for RSV.

Burden of disease

In the USA, RSV is the leading cause of hospital admission in children under 1 year of age, causes about 150 000 hospital admissions per year in children under 2 years of age, and accounts for 18% of all emergency department visits in children under 5 years of age.[5–7] Beyond the substantial disease burden during acute infection, evidence suggests that RSV bronchiolitis plays a causal part in the development of recurrent wheeze, and is associated with the development of asthma and subsequent respiratory morbidity.[5,8–10] Evidence supports a transient association of RSV lower respiratory tract infection (LRTI) and recurrent wheeze, which subsides after the school years,[11,12] and a more permanent effect on long-term respiratory health and asthma in the adult years.[10] If the consequences of RSV LRTI are more permanent and extend to adult asthma, then RSV vaccination will have repercussions into adulthood, which underscores the importance of developing preventive and therapeutic strategies, such as vaccination, beyond prevention or treatment of acute infection.

The pathogenesis of long-term RSV morbidity is incompletely understood. Evidence supports the role of both a genetic and physiological predisposition for severe disease and recurrent wheeze, and a role for RSV in respiratory epithelium damage with subsequent development of recurrent wheeze.[2,13,14] Biological mechanisms that might explain the association between RSV infection and the development of asthma include persistent airway hyper-responsiveness after RSV infection, impaired T-regulatory function, persistent activation of the innate immune response, T-helper-2 activation leading to airway remodelling, and increased susceptibility to allergen sensitisation because of reduced airway epithelial barrier function.[15] Differential persistence of RSV recurrent wheeze might be

Key messages

- RSV LRTI is a worldwide health problem; it is a major cause of morbidity and mortality in children under 5 years old and has a high socioeconomic burden, yet the mortality burden is still poorly understood
- A rigorous analysis confirms that there are no effective evidence-based therapeutic or preventive interventions for RSV, and supportive care (hydration and oxygenation) remain the cornerstone of clinical management
- The past decade has been characterised by new therapeutics in clinical development including 10 vaccines and 11 antivirals
- We are now challenged to optimise these new therapeutics, with remaining challenges to development and implementation, including the need for regulatory guidance on drug testing, establishment of clinically relevant outcomes for vaccine and therapeutic efficacy, establishment of target populations and subpopulations, acceptable pricing, and logistic barriers to distribution in regions where mortality is highest

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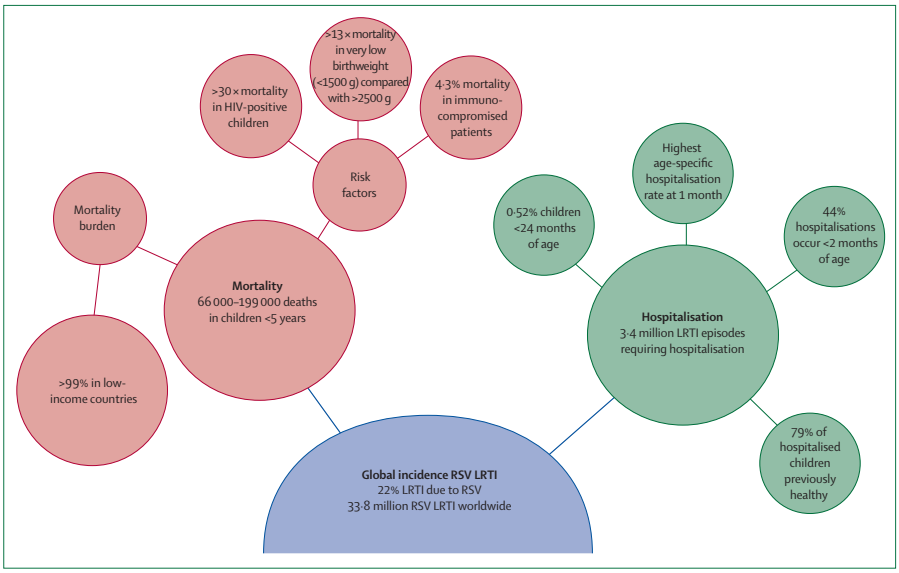

Figure 1: **RSV burden of disease: key facts and figures**
LRTI= lower respiratory tract infection. RSV=respiratory syncytial virus.[18–22]

explained by the severity of the initial episode, with these long-term sequelae occurring more frequently in children admitted to hospital than in children treated as outpatients with RSV infection.[16] These respiratory sequelae result in a disproportionate health-care and financial burden for children under 5 years of age.[17]

More than 99% of deaths associated with RSV occur in low-income countries.[18] In all low-income countries, LRTI is the leading cause of death, and RSV is one of the most common pathogens causing LRTI.[1] Two estimates of mortality from RSV have been reported using different modelling approaches.[1,18] A systematic review of epidemiology data reported the estimated incidence of RSV-associated LRTI of 33·8 million cases in children under 5 years old worldwide in 2005, of which 3·4 million (10%) were admitted to hospital and an estimated 66 000–199 000 died (figure 1). This estimate assumed that RSV causes negligible mortality in children older than 2 years of age. The lower bound estimate was generated using pooled case fatality ratios from hospital-based data, which probably underestimate true mortality rates. The upper bound was estimated under the assumption that all excess LRTI mortality during the RSV season was RSV-associated, after extrapolation from a single study.[18] The second mortality estimate was derived from the Institute for Health Metrics and Evaluation global all-cause of death analysis compiling mortality data from 1990 to 2013, in which RSV pneumonia was reported to cause an estimated 41 100 deaths in children under 5 years of age in 2013 (95% CI 23 000–65 500).[1] High-risk groups include premature infants, HIV-infected children, children with other immunocompromised status, and infants with very low birthweight.[19–21] Although risk factors for severe disease have been identified, most children admitted to hospital with RSV LRTI were previously healthy (figure 1).[22] Obstacles limiting the ability to compile an accurate worldwide estimate of disease burden of RSV LRTI include absence of a universal definition, quality of monitoring methods, paucity of monitoring outside the hospital setting, and scarcity of diagnostic confirmation of RSV infection.

Clinical management: less is more

Bronchiolitis is a variable but usually self-limiting disease, and it is estimated to resolve in 90% of children about 21 days after symptom onset.[23,24] However in the case of severe disease (defined by respiratory distress or dehydration) children need to be managed with intravenous fluids and supplemental oxygen as inpatients.

The American Academy of Pediatrics (AAP) bronchiolitis guideline[4] restricts the use of therapeutic interventions that are not evidence based. Moreover, the Cochrane reviews[25–27] support the absence of efficacy of systemic corticosteroids and bronchodilators as

	Recommended	Not recommended
American Academy of Pediatrics, 2014[4]	Supplemental oxygen optional if SpO_2 is greater than 90%, nebulised hypertonic saline optional for hospitalised children with expected length of stay longer than 72 h, nasogastric or intravenous fluids if oral hydration cannot be maintained	Albuterol, epinephrine, nebulised hypertonic saline in emergency department, systemic corticosteroids, antibacterial medicine (unless concomitant bacterial infection), chest physiotherapy, continuous pulse oximetry
Royal Australian College of General Practitioners, 2008[28]	Supplemental oxygen, saline nasal drops, nasal suctioning, comfortable positioning (prone or supine if unable to position self), continuous pulse oximetry monitoring if in prone position, oral feeding can continue unless respiratory distress increases, trial of β_2 agonist bronchodilators for children older than 9 months (discontinue if no response), antibiotics if clinical signs or symptoms of bacterial infection, paracetamol or ibuprofen can be used if pyrexia is present	Chest physiotherapy, routine mist, routine steam, routine nebulised saline, routine nebulised adrenaline, routine β_2 agonist bronchodilators, routine ipratropium bromide, routine antibiotics, routine corticosteroids, routine ribavirin, routine immunoglobulin, routine oral antitussives, oral expectorants or oral decongestants
Scottish Intercollegiate Guidelines Network, 2006[29]	Supplemental oxygen if SpO_2 is less than 92% or if severe respiratory distress or cyanosis, nasogastric feeding if child cannot maintain hydration or oral intake, nasal suction for hospitalised infants showing respiratory distress, pulse oximetry 8 to 12 h after supplementary oxygen is discontinued	Nebulised ribavirin, antibiotic therapy, inhaled β_2 agonist bronchodilators, nebulised ipratropium or epinephrine, inhaled or oral corticosteroids, chest physiotherapy
NICE, 2015[30]	Supplemental oxygen if SpO_2 is less than 92%, continuous positive airway pressure if impending respiratory failure, upper airway suctioning in children who have respiratory distress or feeding difficulties because of upper airway secretions or children who present with apnoea, fluids by nasogastric or orogastric tube if children cannot take fluid orally, intravenous isotonic fluids to children who do not tolerate nasogastric or orogastric fluids or have impending respiratory failure, consider capillary blood case testing in children with severe worsening respiratory distress or impending respiratory failure	Chest physiotherapy for children who do not have relevant comorbidities, antibiotics, hypertonic saline, nebulised adrenaline, salbutamol, montelukast, ipratropium bromide, systemic or inhaled corticosteroids and nebulised adrenaline, routine upper airway suctioning, routine blood gas testing

Peripheral capillary oxygen saturation=SpO_2. Guidelines included are either accepted on a national level (not hospital based) and apply a clearly defined evidence-based framework to recommendations

Table 1: **Treatment recommendations based on current evidence-based global management guidelines**

suggested by the guidelines.[4] Tables 1 and 2 outline differences between the AAP and three additional evidence-based guidelines[4,28–30] for the management of bronchiolitis; the main differences between the new and old AAP guidelines are summarised in the panel.[4,35] Oxygen supplementation is recommended when pulse oximetry shows peripheral capillary oxygen saturation (SpO_2) less than 90%.[35] When oxygen supplementation is not sufficient, invasive or non-invasive ventilatory support might be necessary. High-flow nasal cannula (HFNC) for oxygen delivery generates a positive airway pressure in bronchiolitis and is emerging as a potentially interesting delivery method. Respiratory support using HFNC is a promising strategy, because it seems safe for children that are managed in a general paediatric ward and might decrease the need for intubation or paediatric intensive care unit admission.[4,36,37] However, there are no randomised controlled trials for HFNC, so this method still lacks sufficient evidence for recommendation. There are various theoretical risks of using HFNC for babies with RSV LRTI, including the risk of delaying intubation and increased mortality because of HFNC failure.[38,39] The AAP guideline pre-dates evidence from a randomised controlled trial[39] that challenges the role of oximetry as an identifying criterion for bronchiolitis admissions. Pulse oximetry readings were artificially elevated, displaying 3% higher than true SpO_2—as recorded by pulse oximetry with non-artificially elevated levels. Artificial elevation resulted in a 16% decrease in the probability of hospital admission in two groups with similar outcomes, controlled for disease severity.[40] Although lower oxygen saturation thresholds seem safe, clinicians should not value oxygen saturation too highly as a single marker of disease severity and need for admission to hospital.

The European Respiratory Society 2004 Task Force assessed therapeutics often used to treat acute viral bronchiolitis using the Grades, Assessment and Evaluation method[41] and reported that nebulised hypertonic saline might be useful, but no other interventions are useful and should therefore not be used.[42] The AAP guidelines do not recommend giving nebulised hypertonic saline to infants in the emergency department and only weakly recommend its use in patients admitted to hospital with an average length of stay greater than 3 days. Evidence has been compiled from a meta-analysis of 11 trials[43] and data from four more recent trials[31–34] that compare various concentrations of nebulised hypertonic saline with normal saline. A reduction in length of hospital stay of 1·2 days was reported in the meta-analysis,[43] but has been contradicted by results of trials that reported no relevant reduction in length of hospital stay.[31–34] There is evidence that adverse effects after treatment with hypertonic saline are similar with or without concomitant bronchodilator use, but with

	2014 American Academy of Pediatrics[4]	2008 Royal Australian College of General Practitioners[28]	2006 Scottish Intercollegiate Guidelines Network[29]	NICE Guideline 2015[30]
Inhaled bronchodilators	Level B: albuterol (salbutamol) should not be given	Level A: β_2 agonists not recommended Level D: trial β_2 agonists if older than 9 months, discontinue if no response Level A: ipratropium bromide not recommended	Level B: β_2 agonists not recommended Level X: nebulised ipratropium not recommended	Not recommended
Systemic corticosteroids	Level A: not recommended	Level A: not recommended	Level A: not recommended	Not recommended
Ribavarin	No recommendation	Level A: not recommended	Level B: not recommended	No recommendation
Antibiotics (only if indications for bacterial co-infection present)	Level B: recommended	Level A: not recommended Level D: consider for secondary bacterial infection	Level X: not recommended	Not recommended
Chest physiotherapy	Level B: should not be used	Level A: not recommended	Level A: not recommended	Not recommended if children do not have relevant comorbities
Maintaining hydration and fluid balance	Level X: nasogastric or intravenous fluids if unable to maintain oral hydration	Level D: maintain oral feeding unless feeding increases respiratory distress	Level D: nasogastric feeding if child cannot maintain oral intake	Nasogastric or orogastric tube recommended when children cannot take enough fluid orally Intravenous isotonic fluids recommended for children who do not tolerate nasogastric or orogastric fluids, or have impending respiratory failure
Supplemental oxygen	Level D: choice not to administer if SpO_2 >90%	No recommendation	Level D: should be given for SpO_2 ≤92% or severe respiratory distress or cyanosis Level X: CPAP should be considered for severe respiratory distress or apnoea	Recommended for SpO_2 <92%
Pulse oximetry	Level C: continuous pulse oximetry not recommended	Level D: continuous pulse oximetry if in prone position	Level C: should be performed for every child attending hospital with acute bronchiolitis Level X: monitor 8-12 h after discontinuation of supplemental oxygen therapy	No recommendation
Epinephrine	Level B: should not be given	Level A: nebulised adrenaline not recommended	Level A: not recommended	Not recommended
Nebulised hypotonic saline, Normal Saline	Level B: can be given during hospitalisation*	Level D: mist, steam, nebulised saline not recommended	No recommendation	Not recommended
Paracetamol or ibuprofen	No recommendation	Level D: may be given	No recommendation	No recommendation
Antitussives, expectorants, decongestants	No recommendation	Not recommended	No recommendation	No recommendation
Capillary blood gas	No recommendation	No recommendation	No recommendation	Consider in children with severe worsening respiratory distress or impending respiratory failure Not recommended as routine
Nasal suctioning	No recommendation	Level D: may be trialled	Level D: should be used for children who exhibit respiratory distress due to nasal blockage	Recommended if respiratory distress or feeding difficulties or apnoea

Guidelines compared from table 1 based on level of evidence for each intervention. Level A: well designed randomised controlled trials; Level B: randomised controlled trials with minor limitations or overwhelming evidence from observational studies; Level C: observational studies (case-control and cohort); Level D: expert opinion, case reports; Level X: validating study not possible but clear benefit or harm or recommended practice by development group. CPAP=continuous positive airway pressure. *4 trials published after the publication of the 2014 American Academy of Pediatrics guidelines found no benefit of hypertonic saline therapy.[33-34]

Table 2: **Level of evidence per recommended intervention**

the possibility of bronchospasm with hypertonic saline, the addition of a bronchodilator might ensure treatment safety.[4,43] Furthermore, reduction in length of hospital stay was restricted to a few patients with moderate bronchiolitis and length of hospital stay greater than 72 h. Most trials included in the Cochrane review[43] had a relatively long length of stay (>3 days) in the trial group without hypertonic saline, which limits generalisation to settings in which length of stay is less than 3 days. The effect of continued nebulised hypertonic saline treatment in settings with shorter length of stay and treatment in the outpatient setting has yet to be examined.

In view of widespread use of non-evidence-based therapies for bronchiolitis, reduction of unnecessary therapies in the inpatient setting is essential. In the USA, a temporal association exists between the introduction of the 2006 AAP bronchiolitis guidelines and a reduction of therapeutic interventions, except for antibiotics.[44] The 2014 guidelines further restrict therapeutic intervention, which means the management of bronchiolitis can be summed

***Panel*: Main changes in the American Academy of Pediatrics guidelines[4,35] between 2006 and 2014**

- Carefully monitored trial of bronchodilators no longer recommended
- Continuous pulse oximetry no longer recommended
- Nebulised hypertonic saline not recommended in the emergency department, weakly recommended for hospitalised children
- Discussion of high-flow nasal cannula without recommendation due to limited evidence
- Hydration support may be administered via nasogastric route as well as intravenously

up in three words: less is more. Nevertheless, further controlled studies stratifying children with bronchiolitis into subpopulations according to aetiology, age, and severity might uncover groups of children who could benefit from specific interventions that showed no benefit in the evidence-based guidelines for the treatment of bronchiolitis as a whole.

New therapeutics

RSV is a negative-sense single-stranded RNA virus encoding 11 proteins. RSV mainly infects the ciliated airway epithelial cells of the respiratory tract and causes both damage and inflammation of the bronchioles. Two surface proteins (G and F) play a part in RSV binding and fusion respectively. The RSV viral envelope protein, SH (small hydrophobic), is an ion channel whereas the inner envelope is formed by the M (matrix) protein. Inside the viral envelope, four proteins make up the nucleocapsid: N (nucleoprotein [protein that is conjugated with a nucleic acid]),[45] which binds the RNA; P (phosphoprotein [protein that can be modified post-translationally by attaching a phosphate group or a complex phosphate molecule]),[46] which is an important polymerase cofactor; L (polymerase); and M2–1, which is a transcription factor. M2–2 is postulated to have a regulatory role in RNA replication, and NS1 and NS2 are non-structural proteins that might downregulate RNA synthesis by inhibiting type I interferon responses.[47] Of all the RSV proteins, F and G are the most important surface epitopes for neutralisation and thus the most frequent targets for vaccine induced protective immunity and antivirals (figure 2).

There are only two licensed drugs for treatment of RSV infection. Inhaled ribavirin, a nucleoside analogue and virostatic, is approved by the Food and Drug Administration (FDA) for treatment of children with severe RSV-associated disease. However, this antiviral is no longer recommended in the AAP guideline because of insufficient evidence of effectiveness.[4] Palivizumab, a humanised monoclonal antibody that targets the RSV F protein, was approved by the FDA and European Medicines Agency for immunoprophylaxis in high-risk infants after the Impact trial[64] showed a 55% reduction in hospital admission attributable

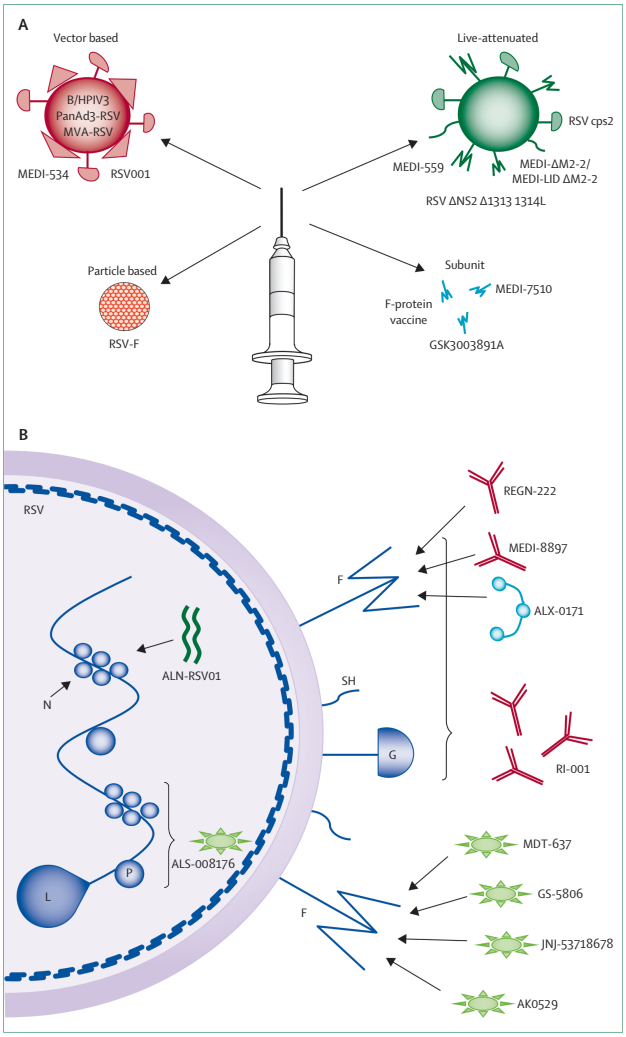

Figure 2: **Vaccines, antivirals, and RSV targets**
(A) Vaccines. (B) Antivirals and RSV targets. A) RSV vaccines in clinical development. Vector-based vaccines MEDI-534 and RSV001 are delivered through humanised bovine parainfluenza type 3 chimeric vectors (B/HPIV3), simian adenovirus vectors (PanAd3-RSV) and modified vaccina virus ankara vectors (MVA-RSV).[48,49] RSV-F is a particle-based vaccine that expresses post-fusion F protein in baculovirus which forms nanoparticles.[50,51] Live-attenuated vaccine candidates include MEDI-559,[52] MEDI-ΔM2-2/MEDI-LID ΔM2-2, RSV ΔNS2 Δ1313, 1314L, RSV cps2.[53–55] MEDI-7510, F-protein vaccine (NCT02298179),[51] GSK3003891A[56] are subunit vaccines that display the RSV F protein.[52] (B) Antivirals are shown with arrows showing the RSV protein targets. RI-001 targets various surface epitopes, as it is a polyclonal antibody.[57] ALS-008176 targets the P, N, L polymerase complex in its entirety[58] whereas ALN-RSV01 is an siRNA targeting the N protein.[59] F protein is the target for most antivirals (MDT-637, GS-5806, JNJ-53718678, AK0529)[60] and antibodies (REGN-222, MEDI-8897, ALX-0171)[61–63] in clinical development. RSV=respiratory syncytial virus. SH=small hydrophobic protein. F=fusion protein. G=surface protein important for attachment. N=nucleoprotein. P=phosphoprotein. L=large polymerase. M=matrix protein.

***Figure 3*: Overview of RSV treatment in development**

(A) Vaccines. (B) immunoglobulins. (C) antivirals. Company and product name, if available, are classified by development stage (discovery, preclinical, phase 1–3, marketed). The image is up to date through April, 2015. Courtesy of GlobalData. RSV=respiratory syncytial virus. STAT=signal transducer and activator of transcription. See appendix for a more detailed description of methods.

See Online for appendix

to RSV in high-risk children. With patent expiration for palivizumab expected as early as mid-2015, the opportunity arises for lower pricing, which will contribute to greater access for groups and populations with the greatest burden of disease, ie, low-income countries.

Vaccines

Vaccine development has been slower than expected, after use of a formalin-inactivated whole virus vaccine in the 1960s resulted in RSV-enhanced disease with 80% hospitalisation and two deaths.[65] Four target populations that might benefit from an RSV vaccine have been identified: infants under 6 months, children older than 6 months, pregnant women, and elderly people (65 years or older).[66] Older siblings have emerged as a potentially effective target for vaccination. Transmission dynamics studied at the community level in Kenya show that transmission mainly occurs through introduction of RSV into the family unit via school-aged siblings, supporting the viability of indirect immunity in the household.[67] Identifying the most appropriate target population to vaccinate will be an important step in future immunisation strategies against RSV. Four vaccine approaches (live-attenuated, subunit, particle based and vector based) are in development, all of which have advantages for particular target populations (figure 2A).

Live-attenuated vaccines aim to achieve a tenuous double goal: safe attenuation of the virus while inducing maximum immunogenicity. In other words, a safe attenuated vaccine should avoid the immunological pitfalls of enhanced T-helper-2 responses and the development of non-neutralising antibodies, as induced by formalin-inactivated RSV, and mimic exposure to wild-type virus. Live-vaccine candidates are attenuated through reverse genetics using mutations to limit the chances of reversion to wild-type while containing mutations that have been shown to increase immunogenicity by augmenting host responses.[68] Mutations in the RNA sequences encoding M2–2, SH, NS2, and L are used in vaccine candidates.

Subunit vaccines provide a safe alternative to live-attenuated vaccine candidates with no chance of reversion to wild-type, but offer little immunogenicity in young children.[66] The F surface protein on the viral envelope and the N protein represent important vaccine antigens for subunit vaccines intended for maternal immunisation. Insight into pre-fusion and post-fusion conformational changes of the F protein presents the question of which epitope to target to provide greater immunogenicity and long-term protection in the development of subunit vaccine candidates.[69] Antibodies against metastable pre-fusion F are highly neutralising, whereas the post-fusion F protein is more stable and contains important neutralising epitopes, including the binding site for palivizumab.[70] Subunit vaccines would probably be more useful in adults or pregnant women for the protection of infants, as they do not carry the potential risks associated with mother to fetus transmission of live-attenuated vaccines.

Finally, two vector vaccine candidates aim to deliver RSV viral proteins using a more stable vector, although anti-vector immunity could pose a problem. Viral vectors, specifically adenovirus and human parainfluenza virus 3, and one particle-based vaccine through baculovirus nanoparticles (small stabilised structures consisting of viral antigens that are produced through Sf9–baculovirus recombinant technology), have been used to deliver RSV F, N, and M2–1 and elicit protective immunity.[71] Figure 3A gives an overview of RSV vaccines in preclinical and clinical trials and table 3 summarises the ten vaccines in clinical trials only.

With the approval of vaccines on the horizon it is important to make the most of emerging clinical interventions. Both maternal and paediatric immunisation could be powerful interventions to prevent severe RSV infection in early childhood. Maternal RSV vaccination studies are in progress to establish placental transfer of neutralising antibodies and postnatal half-life of these antibodies. These studies will be instrumental to optimise timing of vaccination. Limitations of active immunisation include the risk of enhanced disease, restricted immunogenicity of subunit vaccines, and possible attenuation of effectiveness because of interference by natural maternal derived antibodies. Maternal vaccination, although promising, might be limited by placental transfer, antibody decay rates, and safety in pregnant women. In view of the role of RSV LRTI in the pathogenesis of recurrent wheeze, the importance of vaccine development could extend beyond the prevention of hospital admission of infants to long-term respiratory health.

Antivirals

Because of the low immune responsiveness of young children who are at the highest risk of severe disease following RSV infection, and the need to induce a level of protection higher than natural immunity, vaccine development has been complemented by the development of therapeutic antiviral drugs.

11 antivirals for RSV are being investigated in clinical trials. These new compounds belong to four main therapeutic classes: immunoglobulins, siRNA-interference (post-transcriptional gene silencing), fusion inhibitors, and small molecules. These modalities target five of the 11 proteins encoded by the RSV genome including F (fusion), G (viral attachment), and N, P, and L (RNA polymerase) (figure 2B).

Both monoclonal and polyclonal antibodies neutralise RSV. Monoclonal antibodies show higher neutralising activity and fewer adverse effects than plasma-derived polyclonal antibodies, although this can be minimised with substantial purification. However, polyclonal antibodies targeting many epitopes are less susceptible to viral escape mechanisms. MEDI-8897 is a monoclonal

	Company	Trial number	Target	Mechanism of action	Route of administration	Development status	Results summary	Target population
Vaccines: live-attenuated								
MEDI-559	MedImmune	NCT00767416	N/A	Attenuated with point and deletion mutations A2 cp248/404/1030/ΔSH	Intranasal	Phase 2c	Biologically active and immunogenic in seronegative children, increase in MA-LRIs require further safety studies, no enhanced disease	Paediatric[52]
MEDI-ΔM2–2/ MEDI-LID ΔM2–2	NIAID	NCT01459198	N/A	Deletion of RNA regulatory factor, M2–2	Intranasal	Phase 1	Restricted in replication, immunogenic after single dose in RSV-seronegative children	Paediatric[53]
RSV ΔNS2 Δ1313 1314L	NIAID	NCT01893554	N/A	Attenuating NS2 gene deletion, 1313 deletion, 1314L substitution and phenotypic stabilisation	Intranasal	Phase 1	Phase 1 ongoing	Paediatric[54]
RSV cps2	NIAID	NCT01852266	N/A	Codon-stabilised version MEDI-559 (at positions 248 and 1030 of the L gene)	Intranasal	Phase 1	Phase 1 ongoing	Paediatric[55]
Vaccines: vector								
MEDI-534	MedImmune	EudraCT2008-002651-24	N/A	Humanised bovine parainfluenza type 3 chimeric (B/HPIV3) vector displaying the RSV F protein	Intranasal	Phase 2c	Highest dose associated with increased MA-LRI but no increase in disease severity; suppression of viral shedding; no enhanced disease in seronegative infants	Paediatric[48]
RSV001	ReTheira Srl (formerly Okairos, acquired by GSK)	NCT01805921	N/A	F, N, M2–1 expressed in simian adenovirus (PanAd3-RSV) and modified vaccinia virus ankara (MVA-RSV)	PanAd3-RSV: Intranasal MVA-RSV: intramuscular	Phase 1	Safety demonstrated in adults, PanAd3-RSV and MVA-RSV are safe and immunogenic candidates	Paediatric[49]
Vaccines: particle-based								
RSV-F	Novavax	NCT02247726	N/A	Post-fusion F expressed in baculovirus, forms nanoparticles	Intramuscular	Phase 2	Starting phase 2 in pregnant women Well tolerated, no serious adverse event, high RSV antibody levels within 14 days, persist for 91 days in women of childbearing age	Maternal[50,72,73]
Vaccines: subunit								
MEDI-7510	MedImmune (together with Immune Design GLAAS)	NCT02289820	N/A	RSV F protein with GLA as adjuvant, selective binding to TLR-4	Intramuscular	Phase 1	Phase 1 ongoing	Paediatric[51,74]
F-protein Vaccine	Novartis	NCT02298179	N/A	Post-fusion F protein with aluminium hydroxide adjuvant	Intramuscular	Phase 1	Phase 1 ongoing	Maternal
NCT02360475 (Formulations 1–6)	GSK	NCT3003891A, NCT01905215	N/A	Passive immunisation via maternal transfer using purified recombinant F protein engineered to maintain pre-fusion F conformation as vaccine antigen	Intramuscular	Phase 2	Starting Phase 2 in healthy women First in human trial in healthy men ongoing, interim results: a rapid anamnestic anti-RSV neutralising antibody, acceptable adverse event profile in healthy men	Maternal[56]
Antivirals: antibodies								
RI-001	ADMA Biologics	NCT00632463, NCT01814800	Various viral epitopes	Polyclonal RSV neutralising antibody	Intravenously	Phase 2c	Significant improvement in RSV titre from baseline to D18; 9-24x in high dose group (n=21)[57] compassionate use (n=13): 4-fold rise in antibody titres RI-002 Ph3c for indication PIDD	..
Motavizumab (MEDI-524)	MedImmune	NCT00421304, NCT00435227	F	RSV neutralising monoclonal antibody	Intravenously	Interrupted	No effect on viral load, difference in hospital stay duration or severity score, more intensive care admissions in motavizumab arm[75]	..

(Table 3 continues on next page)

	Company	Trial number	Target	Mechanism of action	Route of administration	Development status	Results summary	Target population
(Continued from previous page)								
MEDI-8897 (derived from AIMM D25)	MedImmune	NCT02114268, NCT02290340	Prefusion F	RSV neutralising monoclonal antibody with extended half-life	Intramuscular or intravenously	Phase 2	Target population healthy infants. Ongoing RCT in healthy preterm infants	..
ALX-0171	Ablynx	NCT02309320	F	Antibody nanobody	Inhalation	Phase 2	In healthy male volunteers: no dose-limiting toxicity, no significant change lung function, opportunity for once daily dose[61,63] Phase 1 and phase 2a ongoing in toddlers and infants with RSV LRTI	..
REGN-2222	Regeneron	NCT02325791	F	Monoclonal antibody anti-RSV F	Intramuscular	Phase 1	Recruitment to start June, 2015	..
Antivirals: antisense								
ALN-RSV01	Alynam Pharmaceuticals	NCT00496821, NCT00658086, NCT01065935	N	Small-interfacing RNA's (siRNA)	Intranasal	Phase 2c	Safe and well tolerated in healthy adults[59] Phase 2a experimental infection: 40% relative reduction in infection rate (p<0·01)[76] Phase 2a lung transplant: 85% reduction in bronchiolitis obliterans syndrome (p<0·02)[76] Phase 2b: Treatment effect D90 and D180 Bronchiolitis Obliterans Syndrome 52–65%[76]	..
Antivirals: fusion inhibitors								
MDT-637 (VP014637)	Teva Pharmaceuticals (MicroDose Therapeutx)	NCT01355016	F	Prohibits cell entry	Inhalation	Phase 2	No significant adverse events in all three phase 1 trials (single and multiple dose in healthy adults or single dose in asthmatics), desirable pharmacokinetic profile[77]	..
GS-5806	Gilead	NCT01756482	F	Prohibits cell entry	Oral	Phase 2	Achieved lower viral load, lower mucus weight, lower symptom scores; adverse events include low neutrophil counts and increased alanine aminotransferase[60]	..
JNJ-53718678	Janssen	NCT02398591, NCT02387606	F	Prohibits cell entry	Oral	Phase 1	No study results available	..
AK0529	Ark Biosciences Inc	NCT02297594	F	Prohibits cell entry	Oral	Phase 1	Phase 1 ongoing	..
Antivirals: nucleoside analogue								
ALS-008176	Alios Biopharma	NCT01906164	RSV polymerase	Nucleoside analogue	Oral	Phase 2	Good safety profile, rapid decline of viral load and clearance of RSV RNA, decreased mucus weight and symptom score in healthy adults[58] Phase 1 ongoing in RSV hospitalised children	..
Antivirals: other								
Danirixin (GSK1325756)	GSK	NCT02201303	CXCR2	Selective, reversible CXCR2 antagonist	In vitro	Phase 1	Trial evaluating concentration necessary to inhibit neutrophil activation after in-vitro whole blood incubation[78,79]	..

MA-LRI=medically attended lower respiratory illness. RSV=respiratory syncytial virus. N/A=not available. RCT=randomised controlled trial. LRTI=lower respiratory tract infection. PIDD=primary immunodeficiency diseases.

Table 3: **Overview of RSV antivirals, therapeutics, and vaccines in clinical trials**

antibody targeting the antigenic "site zero", an epitope unique to the pre-fusion RSV F protein. It is a promising drug candidate that has moved onto phase 2 trials as a passive immunisation strategy.[80] Using YTE technology (antibody half-life extension technology using three mutations to the fragment crystallisable domain of an

Search strategy and selection criteria

References for this Review were identified through a search of PubMed and the Cochrane Library for original research and reviews, with no date or language restrictions, on Aug 1, 2015. We did not intend to do a systematic review of the literature with evidence grading. No inclusion or exclusion criteria were used. Instead, we selected articles that were most relevant to the subheadings used in this Review. We searched for original research and reviews using the terms "respiratory syncytial virus" or "viral" and "management", "therapeutics", "vaccines", "antivirals", and "treatment." Earlier landmark publications that are cited in these articles were added if judged to be relevant. ClinicalTrials.gov, the World Health Organization International Clinical Trials Registry Platform, and the European Union clinical trials register were searched for any drug with the indication "Respiratory Syncytial Virus" or "RSV".

antibody [M252Y, S254T, T256E]), this potent antibody has an extended half-life of 70–100 days, making a single injection a possibility.[71,81] Development has been discontinued for motavizumab, a higher affinity variant of palivizumab with greater neutralising activity. A phase 3 clinical trial[82] showed similar efficacy between both monoclonal antibodies but a 2% greater incidence of cutaneous adverse events in motavizumab recipients compared with palivizumab recipients. Moreover, a phase 2 randomised clinical trial[75] in which motavizumab was used as treatment in children with RSV LRTI showed no effect on viral load or clinical severity.

One therapeutic agent, ALN-RSV01, uses antisense technology (siRNA) to interfere with protein synthesis by targeting mRNA encoding the N protein. Of the four fusion inhibitors, GS-5806 was studied in a phase 2 randomised clinical trial[60] and showed an ability to reduce viral loads and disease severity in healthy adults. Finally, there are two small molecules inhibitors; ALS-008176 targets the RNA polymerase to interfere with protein synthesis, and danirixin is a CXCR2 antagonist. Figures 3B and 3C give an overview of all antivirals and other drugs in development and table 3 outlines the 11 antivirals and other therapeutics in clinical trials, including motavizumab, for which development has ended.

Nucleolin has emerged as a novel potential therapeutic target after being identified as a functional human receptor for the RSV F protein in vivo.[83] AS1411, a guanosine-rich oligonucleotide, is in phase 2 clinical trials for cancer patients and might be a potential therapeutic agent because it binds to the cell-surface nucleolin. It is patented for antiviral use for RSV but clinical trials for this indication have not started.[84]

Remaining challenges

Although the investment in RSV therapeutics has injected new hope in emerging RSV pharmaceuticals, challenges remain for their clinical development and implementation—namely absence of consensus on the most clinically relevant outcomes, the definitions of clear target populations, and barriers to drug access.

Consensus among academics, developers, and regulators is needed on clinical trial design, including identifying relevant endpoints and criteria of vaccine and therapy efficacy. In the absence of a universal severity score for RSV bronchiolitis and clinical, virological, and immunological endpoints to objectively assess RSV immune responses and disease severity, assessment of RSV interventions remains a challenge. Surrogate markers of disease severity and protection need to be better defined and clear endpoints established for successful clinical trials. Legal and regulatory guidance on clinical testing in RSV-naive infants, young children, and pregnant women are needed because of the risk of vaccine-enhanced disease or adverse effects in these vulnerable populations. Greater transparency and agreement is needed in the development chain to assess therapeutic efficacy, preferably in the form of an international protocol or guideline.

Different subpopulations with RSV LRTI should be defined and considered when testing therapeutic efficacy. For children with asthma, a hyper-reactive inflammatory immune response to viral infection might result in enhanced disease. Higher rates of bacterial co-infection, HIV exposure, and HIV infection should be taken into consideration in populations in low-income countries.[66] Patient subpopulations for therapeutic testing should be established for clinical trials to accurately measure therapeutic efficacy. Further advances in personalised medicine will help to identify the subset of children that could benefit from these interventions.

A more accurate characterisation of disease burden that includes active surveillance data and an understanding of the long-term consequences of RSV will be essential in establishing target populations for RSV prevention and therapeutics, and a comprehensive cost-effectiveness estimate. As the burden of disease disproportionately affects low-income countries, trials that establish a safe and effective profile within this population are essential to combat RSV.

Once approved, practical barriers remain to ensuring that new therapeutics address the worldwide burden of disease. Economic and logistic barriers are greatest in regions where the RSV disease burden is highest, and mechanisms such as differential pricing agreements and collaboration with local stakeholders can help with distribution in low-income countries.

Conclusion

RSV bronchiolitis represents a worldwide health problem, with a substantial disease burden in children less than 5 years of age and 66 000–199 000 estimated deaths worldwide per year. Beyond the acute disease, RSV is implicated in the pathogenesis of recurrent

wheeze and possibly in the development of asthma. Evidence-based guidelines offer no obviously effective therapeutic interventions, leaving the standard management of RSV bronchiolitis dependent on adequate hydration and respiratory support. Active paediatric and passive immunisation via maternal vaccination are emerging preventive strategies. Antivirals and other novel molecules in clinical trials will hopefully offer clinicians new therapeutic options in a doctrine of non-intervention. The definition of optimum clinical and laboratory endpoints to assess the efficacy of these preventive and treatment interventions against RSV is needed. Furthermore, there is a pressing need to characterise the morbidity and mortality of RSV worldwide, to define target populations for prevention and treatment, to have the mechanisms in place to ensure acceptable pricing, and to undertake trials that show safety and effectiveness in this young and vulnerable population.

Contributors

NIM, LB, and FM-T contributed to the concept and plan for this Review. Literature review was done by NIM in collaboration with LB. All authors contributed to the final manuscript. The Respiratory Syncytial Virus Network (ReSViNET) contributed figures 3A–C.

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9

THE RESPIRATORY SYNCYTIAL VIRUS VACCINE LANDSCAPE: LESSONS FROM THE GRAVEYARD AND PROMISING CANDIDATES

The Lancet Infectious Diseases, 2018

Breng de dingen terug tot hun essentie,
maar laat de poëzie bestaan.
Leonard Cohen (1934-2016)

The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates

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The global burden of disease caused by respiratory syncytial virus (RSV) is increasingly recognised, not only in infants, but also in older adults (aged ≥65 years). Advances in knowledge of the structural biology of the RSV surface fusion glycoprotein have revolutionised RSV vaccine development by providing a new target for preventive interventions. The RSV vaccine landscape has rapidly expanded to include 19 vaccine candidates and monoclonal antibodies (mAbs) in clinical trials, reflecting the urgency of reducing this global health problem and hence the prioritisation of RSV vaccine development. The candidates include mAbs and vaccines using four approaches: (1) particle-based, (2) live-attenuated or chimeric, (3) subunit, (4) vector-based. Late-phase RSV vaccine trial failures highlight gaps in knowledge regarding immunological protection and provide lessons for future development. In this Review, we highlight promising new approaches for RSV vaccine design and provide a comprehensive overview of RSV vaccine candidates and mAbs in clinical development to prevent one of the most common and severe infectious diseases in young children and older adults worldwide.

Introduction

Acute lower respiratory infection (ALRI) caused by respiratory syncytial virus (RSV) has gained recognition as a global health problem with a high burden of disease, and no vaccine licensed for prevention. In children under 5 years, it is estimated that 33·1 million episodes of ALRI, 3·2 million hospital admissions, and as many as 118 200 deaths were attributable to RSV worldwide in 2015 (figure 1).[1] Although often characterised as a paediatric disease, RSV infection in adults represents a substantial health burden. Mortality attributable to RSV in adults aged 65 years or older is estimated to be 7·2 per 100 000 person-years,[7] and 8% of RSV ARLI among older adults admitted to hospital was reported to result in death[8] in the USA. RSV vaccine candidates aim to protect at least three target populations that are at risk for severe RSV disease: (1) young infants (0–6 months), (2) older infants and young children (2 months or older) through active immunisation, and (3) older adults (65 years or older).

Development of effective RSV vaccines and monoclonal antibodies (mAbs) presents both opportunities and challenges. First, concerns of enhanced respiratory disease (ERD) following vaccination with the formalin-inactivated RSV (FI-RSV) vaccine in the 1960s have complicated the design and testing of RSV vaccines.[9] Second, an absolute correlate of protection against a clinically relevant RSV infection remains elusive, although cell-mediated immunity,[10] mucosal IgA,[11] and potent neutralising antibodies[12] have been associated with decreased disease severity.

Between 2016, and 2017, three phase 2b or phase 3 trials (two vaccine trials in older adults[13,14] and one mAb trial in infants[15]) did not meet clinical endpoints. In addition to possible inadequacies in trial design and implementation, the failure of these candidates shows the continued gaps in knowledge regarding immunological mechanisms of protection in the different target populations. Another challenge to RSV vaccine design is the lack of consensus regarding clinical endpoints, which might differ according to the target population. Finally, a consideration in RSV vaccine development is the limited protection conferred by immune responses elicited by natural RSV infection. Natural immunity provides only transient protection against subsequent infection, and re-infection occurs frequently,[16] although the most severe RSV disease is usually observed during the primary infection. mAbs circumvent the problem of transient immunity to RSV and an immature immune response to vaccination in young infants at risk of severe disease. An ideal RSV vaccine candidate should prevent severe disease in at-risk populations and might also lessen person-to-person transmission.[17]

Despite these obstacles, there are several opportunities for RSV vaccine and mAb development. First, RSV disease burden has received increasing attention from international stakeholders such as WHO[18] and the Bill & Melinda Gates Foundation, based on better estimates of RSV-associated mortality worldwide.[19] Second, the discovery and stabilisation of the prefusion (pre-F) conformation of the RSV surface fusion (F) glycoprotein provided a new target for vaccines and mAbs[20,21] as pre-F specific antibodies might be more potent than postfusion (post-F) antibodies in protecting against RSV ALRI. Third, pharmaceutical companies have recognised the urgent unmet need of RSV prevention and prioritised the development of RSV vaccines and mAbs.

In 2015, RSV prevention and therapeutic strategies were reviewed, identifying ten vaccines in clinical

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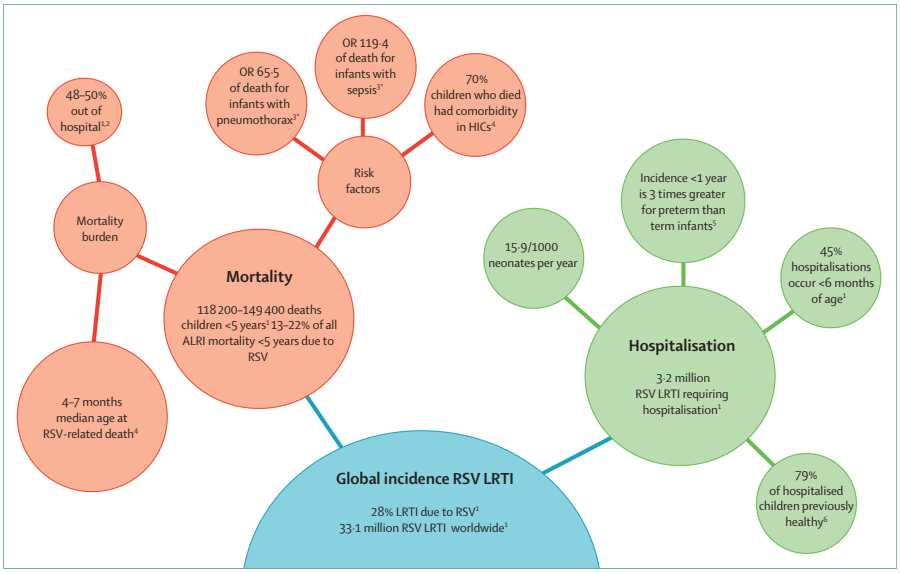

Figure 1: **Global burden of RSV in children under 5 years of age[1–6]**
Incidence is shown worldwide for children less than 5 years of age unless otherwise stated. The hospital admission rate of 15·9 hospital admissions per 1000 neonates per year is in developing countries. The RSV ALRI hospital admission rate of 15·9 among neonates is reported per 1000 individuals per year in developing countries. OR=odds ratio. LRTI=lower respiratory tract infection. RSV=respiratory syncytial virus. HIC=high-income country. *Compared with children who survived RSV hospitalisation and were mechanically ventilated.

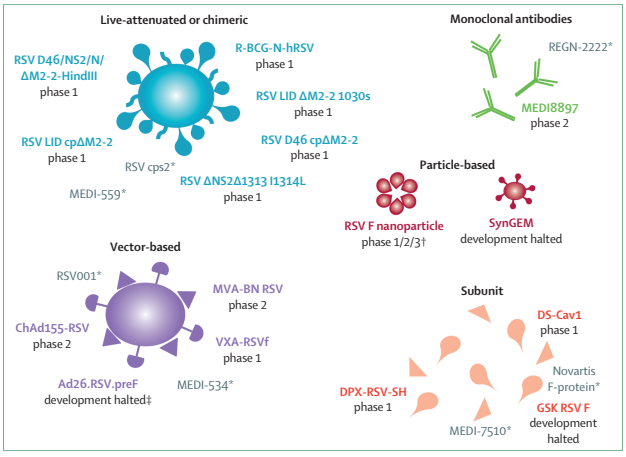

Figure 2: Overview of vaccine candidates and monoclonal antibodies in clinical trials per preventive approach including candidates for which development was halted
RSV=respiratory syncytial virus. *Development has been halted since the last RSV therapeutics review performed in 2015.[22] Candidates for which development is halted but are not indicated with an asterisk are still listed in clinical development according to the PATH snapshot.[23] †Three candidates. ‡Two candidates.

development.[22] An update of the 2015 review is necessary in light of the recent failures and new candidates in the years since 2015. In this Review, we show that only 40% (four of ten) of the vaccine candidates from 2015 are continuing in clinical trials and 14 additional new vaccine candidates have entered clinical trials (figure 2). A single vaccine candidate can be in clinical development both in different populations and in different clinical phases; in these instances, they are considered to be additional candidates. Therefore, the RSV F nanoparticle is considered to be three candidates and Ad26.RSV.preF to be two. Throughout the manuscript we have adhered to the 19 vaccine candidates and mAbs in clinical development according to the PATH Vaccine Snapshot.[23]

RSV vaccine history

RSV vaccine development started shortly after the first identification of the virus in humans in 1957.[24] However, ERD upon natural RSV infection after vaccination with a FI-RSV candidate in a series of trials in the 1960s severely hindered inactivated virus and subunit vaccine development for many years. Nevertheless, work continued on the development and human testing of live-attenuated RSV vaccine candidates. In the following 60 years, only two products were licensed for prevention of RSV: (1) RSV

intravenous immunoglobulin (RSV-IVIG) and (2) palivizumab. Over the past 10 years, development of preventive interventions for RSV has rapidly expanded. Currently, 19 vaccine candidates and mAbs for different target populations are in clinical trials, and many more are in preclinical development.[23] The history of RSV vaccine development is discussed in more detail in the appendix.

Lessons from the vaccine and mAb graveyard

There have been three late-phase vaccine and mAb trial failures between 2016, and 2017 (table 1). It is important to distil lessons learned from these results to inform future vaccine development. First, a phase 3 trial in 1149 healthy preterm infants was done to evaluate REGN2222 (suptavumab), a mAb against antigenic site V on the RSV pre-F protein.[25] The trial did not meet its primary efficacy endpoint to prevent medically attended RSV infections up until day 150 of life.[26] REGN2222 was accelerated from phase 1 to phase 3 because of promising results and the US Food and Drug Administration (FDA) granted fast-track designation in October, 2015. Ultimately, the basis for failing to meet the primary clinical endpoint is not known, as analyses of this late-stage failure have not yet been made public.

Second, an RSV F nanoparticle vaccine candidate based on aggregates of full-length post-F did not meet the predefined study endpoint in older adults. The results of the preceding phase 2 trial showed modest efficacy[27] and promising immunogenicity measures, as identified by a rise in geometric mean titre for IgG antibodies against the F protein and palivizumab competing antibodies (PCA).[28] The trial was granted fast-track designation by the FDA in 2016.[29] In the phase 3 trial, 11850 participants were enrolled over a single season. However, the vaccine candidate did not show efficacy against RSV moderate–severe lower respiratory tract disease (ms-LRTD) in phase 3 results.[14] Compared with the previous season, RSV acute respiratory disease (RSV-ARD) and ms-LRTD attack rates were lower than expected in the 2015–16 season (RSV-ARD 2·0% *vs* 4·9% and RSV-msLRTD 0·4% *vs* 1·8% during the vaccine and previous season, respectively). The vaccine manufacturer speculates that the difference in vaccine efficacy observed might in part be due to this lower attack rate and high pre-existing immunity in the study population.[27] Another proposed explanation for failure of this vaccine candidate is that the quantity of the immune response to vaccination might not represent effective immunity. PCA titres might not correspond to effective immunity as non-neutralising antibodies can also bind the palivizumab binding site and can interfere with the binding of neutralising antibodies.[30] In a post-hoc subgroup analysis, the vaccine candidate showed efficacy against hospital admissions for all-cause chronic obstructive pulmonary disease (COPD) exacerbations.[27] There was a non-statistically significant trend towards higher RSV microneutralisation titres in adults without RSV-ARD when compared with adults with RSV-ARD.

	Company/ sponsor	Vaccine type	Mechanism of action	Clinical trial phase	NCT	Trial design, name	Dates	Study population	Administration/ dosing	Location	Clinical endpoint
REGN2222 (suptavumab)	Regeneron	mAb	mAb against antigenic site V	3	NCT02325791	Double-blind, placebo-controlled trial (NURSERY)	July, 2015–July, 2017	1149 healthy preterm infants<6 months of age with a gestational age ≤35 weeks, not eligible to receive palivizumab prophylaxis	Administered once or twice during the RSV season	250 sites in 19 countries	Medically attended RSV infections through day 150 of life
RSV F nanoparticle for older adults	Novavax	Particle-based	Aggregates of full-length post-F	3	NCT02608502	Double-blind placebo-controlled trial (RESOLVE, RSV-E-301)	Nov, 2015–Dec, 2016	11850 participants ≥60 years of age	135 µg via IM injection	60 US sites	RSV ms-LRTD for 182 days follow-up
MEDI-7510	MedImmune	Subunit	Soluble (unaggregated) postfusion (post-F) conformation of the F protein with a TLR4 agonist adjuvant	2b	NCT02508194	Double-blind placebo-controlled trial	Sept, 2015–Nov, 2016	1900 adults ≥60 years	Single IM injection	61 study sites in 7 countries (North America, Europe, South Africa, and Chile)	RSV-associated respiratory illness between 14 days post vaccination throughout the end of the surveillance period, approximately 7 months

IM=intramuscular. ms-LRTD= moderate–severe lower respiratory tract disease. RSV=respiratory syncytial virus. mAb=monoclonal antibody.

Table 1: **Recent vaccine candidates that failed to meet efficacy endpoints in late-phase clinical trials**

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	Target Population	Pre-F Immunity[35]	Immune response	Mucosal/systemic
Particle-based				
RSV F nanoparticle (Novavax)	M	Pre-F<post-F	Broadly neutralising antibodies	Systemic
RSV F nanoparticle (Novavax)	O	Pre-F<post-F	Broadly neutralising antibodies	Systemic
RSV F nanoparticle (Novavax)	P	Pre-F<post-F	Broadly neutralising antibodies	Systemic
SynGEM (Mucosis)	O and P	Unclear F conformation	Activation of B and T cells; local secretion of neutralising IgA in the nose; production of IgG neutralising IgG in the blood	Mucosal and systemic
Vector-based				
MVA-BN RSV (Bavarian Nordic)	O	Pre-F<post-F	B and T cell response; antibodies against 5 RSV antigens	Systemic
ChAd155-RSV (GSK)	O	Pre-F>post-F	B and T cell response; neutralising antibodies against F antigen; CD8 T cells against F, N and M2-1 antigens	Systemic
VXA-RSVf oral (Vaxart)	O	Pre-F<post-F	B and T cell immunity, protection at mucosal surface	Mucosal>systemic
Ad26.RSV.preF (Janssen)	P	Pre-F	B and T cells	Systemic
Ad26.RSV.preF (Janssen)	O	Pre-F	B and T cells	Systemic
Subunit				
GSK RSV F (GSK)	M	Pre-F	B and T cell response	Systemic
DPX-RSV (Dalhousie University, Immunovaccine, and VIB)	O	None	B cell response specific to SHe antigen	Systemic
RSV F DS-Cav1 (NIH/NIAID/VRC)	O and M	Pre-F	Pre-F-specific serum neutralising antibodies, and CD4 T cells	Systemic
Live-attenuated				
rBCG-N-hRSV (Pontificia Universidad Catolica de Chile)	P	Pre-F and post-F	B and T cell response; Th1 polarised response; antibodies against N, F, G	Systemic
RSV D46 cp ΔM2-2 (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F and post-F	B and T cell response; enhanced antibody production due to increased antigen production from M2-2 deletion	Mucosal and systemic
RSV LID ΔM2-2 1030s (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F and post-F	B and T cell response; enhanced antibody production due to increased antigen production from M2-2 deletion	Mucosal and systemic
RSV ΔNS2 Δ1313/I1314L (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F and post-F	B and T cell response	Mucosal and systemic
RSV D46 ΔNS2 N ΔM2-2-HindIII (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F and post-F	B and T cell response; enhanced antibody production due to increased antigen production from M2-2 deletion	Mucosal and systemic
RSV LID cp ΔM2-2 (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F and post-F	B and T cell response; enhanced antibody production due to increased antigen production from M2-2 deletion	Mucosal and systemic
Monoclonal antibody				
MEDI8897 (MedImmune)	P	NA	NA	NA

Pre-F=prefusion conformation of the RSV F protein. Post-F=postfusion conformation of the RSV F protein. N=RSV nucleocapsid protein. F=RSV fusion protein. G=RSV attachment protein. O=older adults. M=maternal. P=paediatric. VIB=Flemish Institute of Biotechnology. NIH=National Institutes of Health. NIAID=National Institutes of Allergy and Infectious Diseases. VRC=Vaccine Research Center. LID=Laboratory of Infectious Diseases. RSV=respiratory syncytial virus. NA=not applicable or not available. BLP=bacterium-like-particle. MVA=modified vaccinia Ankara.

Table 2: **Expected immune response for vaccine candidates and monoclonal antibodies**

One conclusion that can be drawn from this trial is that late-phase clinical research for RSV vaccine candidates should include evaluation across more than one RSV season.

Third, development of the MEDI-7510, a subunit vaccine candidate using soluble (unaggregated) post-F conformation of the F protein with a TLR4 agonist adjuvant, was discontinued after a phase 2b trial in 1900 adults aged 60 years or older. Safety and increased B and T cell responses in the vaccine compared with the placebo group were shown in a phase 1 clinical trial[31] after safety and improved immunogenicity with an

adjuvant was demonstrated in a first-in-human trial.[32] The study did not meet its primary efficacy objective; the incidence of RSV-associated respiratory illness as diagnosed by PCR was 1·7% and 1·6% in the vaccine and placebo groups respectively, for a vaccine efficacy of –7·1.[31] On day 29, 93% of vaccinees had an anti-F IgG antibody seroresponse and there was a 4·6 geometric mean times rise in anti-F IgG titre at the end of the RSV season in vaccine recipients compared with the placebo group.[31] One proposed explanation for the negative results could be that the selected post-F antigen induced antibodies without appropriate epitope specificity.[33] Other proposed explanations include a low incidence of laboratory-confirmed RSV in the study population, or selection of the study population, which included high-risk and low-risk older adults. Considerations for the future include selection of an older study population at higher risk of severe RSV infection.

Vaccine antigens

Vaccine antigens included in RSV vaccine candidates are diverse. The majority of vaccines in clinical trials (11 of 18) use the F protein, a class I viral fusion protein, as an antigenic target. The RSV F protein is highly conserved and facilitates viral fusion with host cells. Understanding the structural differences between pre-F and post-F conformations, and stabilisation of the pre-F soluble forms, has resulted in advances in vaccine antigen design.[21,34] Current vaccine candidates use pre-F and post-F as vaccine antigens (table 2). The predominant conformation displayed on the FI-RSV vaccine candidate was the post-F conformation.[36] It remains unclear as to whether there is a trigger for the pre-F to post-F conformational change, but it does occur spontaneously, making it difficult to ensure that a wildtype F vaccine antigen maintains a pre-F conformation. However, stabilising mutations have been identified that can preserve the pre-F-specific epitopes.[34,37] The antigenicity of some stabilised pre-F constructs has not been rigorously investigated, and remains an open question as to whether particular stabilising mutations affect the conformation of antibody binding sites. Assays to assess antigen conformation are needed. There is no consensus on cellular receptors that determine viral tropism.[38]

Other less frequent vaccine antigens, used alone or in combination with other antigens, include the RSV envelope associated glycoproteins G (one of 18) and small hydrophobic (SH) protein (one of 18), as well as internal proteins: nucleocapsid (N) (three of 18), M (one of 18), and M2-1 (one of 18). Besides the F protein, the G protein is the only other target for neutralising antibodies on the viral surface. The G protein is most important for viral attachment and is less frequently used as a vaccine antigen due to high variability across RSV strains,[39] and little knowledge of its surface structure.[40] The G protein exists as an oligomer on the surface of RSV particles and as a monomer when secreted from infected cells in soluble form.[41] There is evidence that the soluble form of the G protein can act as a decoy that helps the virus to evade the antibody response.[42] Another possible vaccine target, the SH protein, is not well understood, but data suggest that it has a role in viral replication in vivo[38] and inflammasome activation.[43] The SH protein contains transmembrane and extracellular domains;[44] the latter has been used as a vaccine antigen.[45] Internal proteins are particularly relevant to induce T cell-mediated immunity.[40] As such, three non-membrane RSV proteins have been included in RSV vaccine design. The N protein is the major nucleocapsid protein that encapsidates the RNA genome of the virus.[46] The M2-1 and M2-2 proteins are specific to RSV and other pneumoviridae. M2-1 is essential for viral transcription,[47] and M2-2 deletion is used in live vaccine candidates for viral attenuation. Finally, the M protein is a membrane-associated protein that gives virions their filamentous shape.[48,49] In summary, different viral proteins are being used as antigens in RSV vaccine design. Viral surface glycoproteins such as F and G are known to induce antibodies with differing neutralisation capacity. The SH protein might be important for induction of

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For more on **ReSViNET Foundation** see www.resvinet.org

See **Online** for appendix

	Vaccine type
Pregnant mothers	
RSV F nanoparticle (Novavax)	Particle-based
GSK RSV F (GSK)	Subunit
RSV F DS-Cav1 (NIH/NIAID/VRC)	Subunit
Paediatric	
RSV F nanoparticle (Novavax)	Particle-based
ChAd155-RSV (GSK)	Vector-based
SynGEM (Mucosis)	Particle-based
Ad26.RSV.preF (Janssen)	Vector-based
rBCG-N-hRSV (Pontificia Unversidad Catolica de Chile)	Chimeric
RSV D46 cp ΔM2-2 (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
RSV LID ΔM2-2 1030s (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
RSV ΔNS2 Δ1313 I1314L(Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
RSV D46/NS2/ N/ΔM2-2-HindIII (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
RSV LID cp ΔM2-2 (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
MEDI8897 (MedImmune)	Monoclonal antibody
Older adults	
RSV F nanoparticle (Novavax)	Particle-based
SynGEM (Mucosis)	Particle-based
MVA-BN RSV (Bavarian Nordic)	Vector-based
VXA-RSVf oral (Vaxart)	Vector-based
Ad26.RSV.preF (Janssen)	Vector-based
DPX-RSV-Protein (Immunovaccine, VIB and Dalhousie University)	Subunit
RSV F DS-Cav1 (NIH/NIAID/VRC)	Subunit

NIH=National Institutes of Health. NIAID=National Institutes of Allergy and Infectious Diseases. LID=Laboratory of Infectious Diseases. VIB=Flemish Institute of Biotechnology. VRC=Vaccine Research Center. RSV=respiratory syncytial virus.

***Table 3*: Overview of vaccines and monoclonal antibodies by target population**

antibody dependent cell-mediated cytotoxicity (ADCC), whereas non-membrane proteins are especially important to induce a robust T-cell response.[40]

Target populations

RSV prophylactic interventions are designed to protect at least two populations most susceptible to severe RSV disease: RSV-naive young infants and children, and older adults, although other high-risk populations are important to consider. It is estimated that 45% of hospital admissions and in-hospital deaths due to RSV-ALRI occur in infants younger than 6 months of age,[1] an age at which vaccines are generally less immunogenic. Older adults and adults with chronic cardiopulmonary conditions have emerged as an important target for RSV prevention owing to an increased understanding of RSV burden in this population. An overview of all RSV vaccine candidates per target population is shown in table 3 and strategies to prevent RSV in different target populations are discussed in more detail in the appendix.

Immunological endpoints

Antibodies are thought to be the key players in limiting RSV ALRI as evidenced by proven protection in immunoprophylaxis trials in children.[50–52] Evidence from experimental human infection in adults suggests a protective role for nasal RSV-specific IgA against RSV infection,[11] underscoring the importance of mucosal immunity. A limited ability to generate memory IgA responses after RSV infection could be in-part responsible for incomplete immunity and subsequent RSV re-infection. Antibodies directed against different antigenic sites of the F protein display different neutralisation capacities with the most neutralisation-sensitive epitopes exclusive to the pre-F conformation. Antibodies with specificity for antigenic sites ø and V show high neutralising activity and are exclusive to the pre-F conformation.[25,53] Antigenic site ø is located at the apex of the pre-F conformation, the most variable region of the highly conserved F protein.[21] Antibodies against antigenic site III prefer the pre-F conformation and have high neutralising activity.[54] Antibodies directed against site II and IV, present on both pre-F and post-F, have medium to high neutralisation potency.[53,55] Finally, antibodies against antigenic site I, present primarily on post-F, show weak or no neutralisation. Escape mutants of these antigenic sites have been identified, but global RSV genetic data are needed to assess the molecular heterogeneity of RSV and the subsequent susceptibility or resistance to mAbs targeting RSV among circulating viruses.

The mechanisms of protection could differ according to vaccine type, and, therefore, many different immunological assays are used in clinical trials. Neutralising activity of serum is a frequent immunological endpoint of vaccine trials. A measure of functional antibody response can be elucidated by the ratio of times-increase in RSV-binding antibodies to times-increase in RSV-neutralising antibodies (ELISA-to-neutralisation response ratio). A ratio of less than 1 might be an important correlate of protection.[56] Furthermore, rather than a definitive protective threshold for antibodies, times-rise in antibody titre could be a relevant correlate of protection for live-attenuated vaccines, since that might be the best indicator of B-cell priming. In 2017, efforts by PATH, WHO, and the National Institute for Biological Standards and Control (NIBSC) examined the variability of RSV neutralisation assays across laboratories and recommended steps for improved standardisation globally,[57] resulting in the development of a new WHO International Standard for Antiserum to RSV with 1000 IU of RSV subtype A neutralising activity per vial now available through the NIBSC.[58] Standardisation of other frequently used immunological assays such as PCA, ELISA, and T-cell assays has not yet taken place.

Once infection of the lower airways is established, CD8 T cells play an important part in viral clearance.[35] Th2-biased responses have been associated with animal models of RSV ERD and measurement of Th1 and Th2 responses are considered important to predict safety of vaccine candidates other than live-attenuated vaccines in clinical trials in young children.

Animal models are important for preclinical development of vaccine candidates and assessing the possibility of enhanced disease. Alveolitis in the cotton rat and priming of a Th2 response in mice are considered markers to assess possible ERD. However, there is no consensus on the ability to reproduce ERD in calves.[59]

Although we discuss several potential immunological correlates of protection for vaccine trials, we considered cell-mediated immunity beyond the scope of the manuscript. The different aspects of the expected immune response for all 19 vaccine candidates and mAbs in clinical development are highlighted in table 2. A definitive threshold for protection against RSV disease remains elusive. So far, no vaccine candidates have been tested in the experimental human infection model, but the model provides a unique opportunity to test vaccine candidates in the natural host despite practical and ethical challenges.[60] Ultimately, the outcome of large-scale vaccine trials will inform which immunological measures correspond to protection from clinical RSV disease.

Vaccine strategies

We have divided vaccines in clinical development into four categories in accordance with the PATH RSV vaccine and mAb snapshot: particle-based, vector-based, subunit, and live-attenuated or chimeric vaccines.[24] We have also included mAbs in clinical development for the prevention of RSV ALRI. In the snapshot there are 43 vaccines and four mAbs in development, of which 19 are in clinical stage development. In table 4 we provide a comprehensive overview and more detailed comparison of all characteristics of the 19 vaccine candidates and mAbs in clinical development. Other approaches, which

	Manufacturing process	Antigen, adjuvant	Mechanism of action	Target population	Animal models	Phase 1	Phase 2	Phase 3	Result summary
Particle-based vaccines									
RSV F nanoparticle, Novavax, phase 3*	Sf9/BV recombinant technology	Stabilised F protein exhibiting post-F morphology, aluminium phosphate	F forms nanoparticle in multimeric micelle format	Maternal	Cotton rats[61,62], baboons[63] guinea pigs[64]	Dec, 2010–Dec, 2011 NCT01290419 (n=150)	Oct, 2012–May, 2013 NCT01704365 (n=330) Oct, 2013–April, 2014 NCT01960686 (n=720) Sept, 2014–July, 2016 NCT02247726 (n=50)	Dec, 2015–June, 2020 NCT026249 47 (n=8618)	Phase 2: all formulations well tolerated and immunogenic; most robust Ab response with 120 µg and 0·4 mg aluminium formulation, peak day 14 and persistence through day 91; RSV infection measured by western blot was reduced by 52% (p=0·009) in healthy women of childbearing age (n=720)[65] Vaccine safe, immunogenic, and reduced RSV infection in healthy women of childbearing age (n=330)[66]
RSV F nanoparticle, Novavax, phase 2*	Sf9/BV recombinant technology	Stabilised F protein exhibiting post-F morphology, aluminium phosphate and Matrix M	F forms nanoparticle in multimeric micelle format	Older adults	Cotton rats[61,62] baboons[63]	Oct, 2012–March, 2014 NCT01709019 (n=220)[67]	Oct, 2014–March, 2016 NCT02266628 (n=1599) Oct, 2015–Nov, 2016 NCT02593071 (n=1330) Jan, 2017–July, 2018 NCT03026348 (n=1329)	Nov, 2015 – Dec, 2016 NCT02608502 (n=11850)	Phase 2: safe, VE: 41% *vs* RSV-ARD, 64% VE *vs* RSV-msLRTD[68] Phase 3: safe, no efficacy *vs* RSV-ARD and RSV-msLRTD; post-hoc efficacy *vs* all-cause hospitalisation (n=11 850) Phase 2 rollover; second immunisation protective against RSV-ARD and msLRTD (n=1329)[14]
RSV F nanoparticle, Novavax, phase 1*	Sf9/BV recombinant technology	Stabilised F protein exhibiting post-F morphology, aluminium phosphate/ Matrix M-1	F forms nanoparticle in multimeric micelle format	Paediatric	Cotton rats[61,62] baboons[63]	Nov, 2014–April, 2016 NCT02296463 (n=32)	NA	NA	Phase 1: well tolerated; anti-F IgG and PCA increase day 14, peak day 28, elevated to day 56; 10-times increase PCA and anti-F IgA adjuvanted 6-times increase in unadjuvanted[69] (n=32)
SynGEM, Mucosis, phase 1†	BLP mimopath technology carrying F proteins	F protein, unclear which conformation, BLP	BLP allows presentation of F protein and elicits mucosal IgA	Older adults and paediatric	Mice	July, 2016–Dec, 2017 NCT02958540 (n=48)	NA	NA	Phase 1: some immunogenicity in healthy adults but did not meet threshold; development suspended
Vector-based vaccines									
MVA-BN RSV, Bavarian Nordic, phase 2‡	MVA-BN technology (antigens expressed in attenuated modified vaccinia Ankara)	F, G (subtype A and B), N, M2, no adjuvant	Virus replication blocked at a late stage	Older adults	Cotton rats, BALB/c mice[69]	*Aug, 2015–May,2016 NCT02419391 (n=63) †Sept, 2018–Aug, 2019 NCT02864628 (n=96)	Sept, 2016–Aug, 2018 NCT02873286 (n=400)	NA	Phase 1; safe, 2-times increase IgG and IgA; 3–5-times increase in T cell responses (n=63)[69] Phase 2 interim results: well tolerated; broad Ab and T cell response in older adults after single vaccination (n=421)[70]
VXA-RSVf oral, Vaxart, phase 1§	Antigen and adjuvant expressed in non-replicating adenovirus vector (Ad5)	F, dsDNA that activates TLR3 receptor	Vector delivers directly to gut (ileum)	Older adults	Cotton rat	June, 2016–Dec, 2017 NCT02830932 (n=66)	2018?	NA	Preclinical: systemic anti-F Abs and protection against RSV infection in cotton rat model[71]

(Table 4 continues on next page)

	Manufacturing process	Antigen, adjuvant	Mechanism of action	Target population	Animal models	Phase 1	Phase 2	Phase 3	Result summary
(Continued from previous page)									
Ad26.RSV.preF, Janssen, phase 2*	Antigen expressed in human adenovirus type 26 produced in PER.C6 human cell line	Pre-F (previously FA2), no adjuvant	Ad26 vector is replication incompetent but expresses immunogenic F antigen	Older adults	Mice, cotton rats[72]	Nov, 2016—Dec, 2018 NCT02926430 (n=73)	Dec, 2017–Jul,y 2018 NCT03339713 (n=180)	NA	Phase 2: well tolerated; durable humoral and cellular immune response for FA2 candidate; comparable or higher for preF candidate in older adults[73]
Ad26.RSV.preF, Janssen, phase 1*	Antigen expressed in human adenovirus type 26 produced in PER.C6 human cell line	Pre-F (previously FA2), no adjuvant	Ad26 vector is replication incompetent and expresses immunogenic F antigen	Paediatric	Mice, cotton rats[72]	Nov, 2017–March, 2019 NCT03303625 (n=60)	Nov, 2017– March, 2019 NCT03303625 (n=60)	NA	NA
ChAd155-RSV, GSK, phase 2*	Chimpanzee adenovirus ChAd155-RSV with F, N, M2-1 insert and E1 deletion	F, N, M2-1, no adjuvant	Intracellular RSV antigen expression; replication incompetent vector	Paediatric	Mouse, cotton rat, calves[74]	July 2015–Feb, 2017 NCT02491463 (n=73)	Jan, 2017–Sept, 2020 NCT02927873 (n=96)	Plan to start post 2020 with age de-escalation seronegative infants	Phase 1: safe, B cell and RSV-neutralising antibodies in RSV-seropositive adults (n=73)[75]
Subunit vaccines									
GSK RSV F, GSK, phase 2*	Pre-F produced in CHO cells	Pre-F, with or without aluminium hydroxide	Pre-F antigen induces neutralising antibodies that are transferred to infant	Maternal	Mice, cotton rats, guinea pigs, cows	Dec, 2014–March, 2017 NCT02298179 (n=288) July, 2013–March, 2015 NCT01905215 (n=128)[76]	March, 2015–June, 2016 NCT02360475 (n=507) April, 2016–June, 2016 NCT02753413 (n=102) Nov, 2016–March, 2018 NCT02956837 (n=406) July, 2017–Jan, 2021 NCT03191383 Halted due to instability of pre-F antigen during manufacturing	NA	Phase 1: safe, RSV-A neutralising Ab titres increased 3·2–4·9 times; remained high to day 60, decreased on day 180 and 360 in healthy men (n=128)[76] Phase 2: increased RSV-A neutralising Ab 30 days post-vaccination in healthy non-pregnant women[77]
DPX-RSV, Immunovaccine and VIB, Dalhousie University, phase 1*	DepovaxTM delivery in 100% oil-based platform preventing release at injection site	SHe, DepovaxTM or aluminium hydroxide	Depovax gives controlled and prolonged exposure of antigen and adjuvant	Older adults	Mice, cotton rats	May, 2015–June, 2017 NCT02472548 (n=40)	NA	NA	Phase 1: well tolerated, antigen-specific Ab response durable to day 421, low immunogenicity with alum adjuvant in healthy older adults[78]
RSV F DS-Cav1, NIH/NIAID/VRC, phase 1*	Prefusion stabilised trimeric RSVF expressed in CHO cell line	Pre-F, alum/ TLR4 agonist (E)	Pre-F antigen elicits highly neutralising antibodies against pre-F epitopes	Maternal and older adults	Cotton rats, mice, calves,[79] macaques[37]	Feb, 2017–Jan, 2020 NCT03049488 (n=100)	NA	NA	Preclinical: induction of highly neutralising Abs and differential adjuvant-induced enhancement[79] Immunisation of mice and macaques induces RSV-neutralising Ab many times protective threshold[37]

(Table 4 continues on next page)

	Manufacturing process	Antigen, adjuvant	Mechanism of action	Target population	Animal models	Phase 1	Phase 2	Phase 3	Result summary
(Continued from previous page)									
Live-attenuated or chimeric vaccines									
rBCG-N-hRSV, Pontificia Universidad Catolica de Chile, phase 1¶	Recombinant BCG expressing N antigen	N, no adjuvant	Paired BCG and RSV vaccine induces Th1 response	Paediatric	Mice[80–83]	June, 2017–May, 2018 NCT03213405 (n=24)	NA	NA	Preclinical: protective T cell immune response and recruitment of Th1 cells[81,82]
RSV D46 cpΔM2-2, Sanofi Pasteur/LID/NIAID/NIH, phase 1†	M2-2 deletion via reverse genetics and 5 aa substitutions in 3 proteins called the "cp" mutations, originally identified in a cold-passaged vaccine candidate cpRSV	Native RSV, no adjuvant	Deletion of regulatory factor M2-2 causes inefficient replication but high immunogenicity; further attenuation with cp mutations	Paediatric	African green monkeys	Oct, 2015–May, 2018 NCT02601612 (n=45)	NA	NA	NA
RSV LID ΔM2-2 1030s, Sanofi Pasteur/LID/NIAID/NIH, phase 1†	M2-2 deletion via reverse genetics and temperature sensitivity mutation 1030s	Native RSV, no adjuvant	Deletion of regulatory factor M2-2 causes inefficient replication but high immunogenicity; temperature sensitive mutation at position 1030 of L gene	Paediatric	Mice, African green monkeys	June, 2016–July, 2017 NCT02794870, NCT02952339 (n=33)	NA	NA	NA
RSV ΔNS2 Δ1313 I1314L, Sanofi Pasteur/LID/NIAID/NIH, phase 1†	NS2 and 1313 deletion via reverse genetics, I1314L substitution	Native RSV, no adjuvant	NS2 deletion bolsters innate response; deletion at position 1313 of L protein, and I1314L substitution confers moderate temperature sensitivity	Paediatric	Mice and chimpanzees	June, 2013–May, 2017 NCT01893554 (n=75) Aug, 2017–May, 2019 NCT03227029 (n=80)	NA	NA	NA
RSV D46/NS2/N ΔM2-2 HindIII, Sanofi Pasteur/LID/NIAID/NIH, phase 1†	LID backbone without deletions or substitutions in SH gene, point mutation in NS2 and N proteins, modified M2-2 deletion, based on RSV MEDI ΔM2-2	Native RSV, no adjuvant	Deletion of regulatory factor M2-2 causes inefficient replication but high immunogenicity	Paediatric	African green monkeys	March, 2017–April, 2019 NCT03102034, NCT03099291 (n=33)	NA	NA	NA

(Table 4 continues on next page)

	Manufacturing process	Antigen, adjuvant	Mechanism of action	Target population	Animal models	Phase 1	Phase 2	Phase 3	Result summary
(Continued from previous page)									
RSV LID cp ΔM2-2, Sanofi Pasteur/ LID/NIAID/NIH, phase 1†	M2-2 deletion via reverse genetics, and cp mutation	Native RSV, no adjuvant	Deletion of regulatory factor M2-2 causes inefficient replication but high immunogenicity; further attenuation with cp mutations	Paediatric	African green monkeys	Sept, 2016-April, 2018 NCT02890381 (n=17)	NA	NA	NA
Monoclonal antibody (mAb)									
MEDI8897, MedImmune, phase 2‖	In vitro-optimised human mAb with YTE mutation in Fc	NA	Antibody targeting site Ø of the F protein of RSV with an extended half-life	Paediatric	Cotton rats, cynomolgus monkeys[84]	April, 2014-June, 2015 NCT02114268 (n=342) Jan, 2015-Sept, 2016 NCT02290340 (n=151)	Nov, 2016–Nov, 2018 NCT02878330 (n=1454)	NA	Phase 1; well tolerated, mean half-life 85–117 days; time to max concentration 5–9 days; bioavailability 77% in healthy adults (n=136)[85]

RSV=respiratory syncytial virus. NA=not applicable or not available. ARD=acute respiratory disease. PCA=palivizumab-competing antibodies. SHe=small hydrophobic protein ectodomain. RSV ARD=all symptomatic respiratory disease due to RSV. mLRTD=moderate–severe RSV-associated lower respiratory tract disease. VIB=Flemish Institute of Biotechnology. NIAID=National Institutes of Allergy and Infectious Diseases. VRC=Vaccine Research Center. NIH=National Institutes of Health. LID=Laboratory of Infectious Diseases. Ab=antibody. aa=aminoacid. BLP=Bacterium-like-particle. *Intramuscular. †Intranasal. ‡Intramuscular or intranasal. §Oral. ¶Intradermal. ‖Intravenous or intramuscular.

Table 4: **Overview of RSV vaccines and monoclonal antibodies in clinical development**

are still in preclinical development, including nucleic acid-based vaccines, whole-inactivated vaccines, and biosimilars, are discussed in the appendix.

Particle-based vaccines

The RSV F nanoparticle-based vaccine platform is being evaluated for protection of three target populations: (1) infants through maternal vaccination, (2) children between 6 months and 5 years, and (3) older adults aged 60 years or older. These vaccine candidates use aggregates of a modified stabilised F protein which has the post-F morphology.[86] The maternal RSV F nanoparticle vaccine candidate is farthest along in clinical development and the PREPARE trial has entered the third year of a phase 3 trial to enrol up to 8618 pregnant women at 80 sites in 11 countries.[27] In January, 2018, an informational analysis of the phase 3 trial was announced in which the vaccine candidate successfully targeted an efficacy threshold against the primary endpoint in infants at day 90 of more than 40%.[87] Second in clinical development is the RSV F nanoparticle vaccine for older adults. Despite the absence of efficacy in a phase 3 trial (RESOLVE) with a non-adjuvanted vaccine candidate, development was continued in a phase 2 study initiated in January, 2017, in Australia in 300 adults. The aim of this trial is to establish whether two dose regimens with an adjuvant (Matrix-M, a saponin-based adjuvant, or aluminium phosphate) could increase the magnitude and quality of the immune response in this population. The results from the RESOLVE trial in older adults suggested vaccine efficacy in adults with COPD, leading to considerations to initiate a future trial in this older adult population at high risk for severe RSV infection.[27] Finally, the phase 1 trial was completed in young children 24–72 months of age in 2016, but no data have been published yet.[88]

SynGEM is a particle-based needle-free vaccine candidate containing the RSV F protein attached to empty bacterial particles made from *Lactococcus lactis*. In this vaccine platform, an antigen is presented by a bacterial particle. An influenza vaccine candidate in clinical trials that uses the same vaccine platform has shown both local and systemic antibody responses[89] but further optimisation is needed for RSV vaccination. The preliminary results of immunogenicity testing of SynGEM have been reported. The immunogenicity of this vaccine was evaluated after delivery as a nasal spray to healthy adult volunteers. Two intranasal doses of SynGEM were administered 28 days apart at a low or high dose in 24 individuals per group (six participants in each group receiving placebo, double-blinded). Assays of serum RSV F-specific antibodies, PCA, and F-specific IgA indicated some immunogenicity, but the results did not reach the threshold set for continuation to viral challenge and the studies were suspended in 2017 (P J Openshaw, Imperial College London; and C Chiu, Department of Laboratory Medicine, University of California San Francisco, personal communication).

Vector-based vaccines

Five vector-based vaccines are in clinical development. The first uses a modified vaccinia Ankara (MVA) virus, a replication-defective smallpox viral vector, and the remaining four vaccine candidates use an adenovirus vector to display viral antigens. The MVA vector has been safely used in vaccines for other infectious diseases.[90] This vaccine candidate, MVA-BN-RSV, induces both humoral and cell-mediated responses by displaying four vaccine antigens: F, G, N and M2-1. Phase 2 results in healthy older adults from this candidate will soon be announced.

The second vector-based vaccine candidate, VXA-RSV-f, uses an innovative platform with an adenovirus 5 based oral tablet that is stable at room temperature. Using the same oral adenovirus vaccine delivery platform, a phase 1 trial for influenza has been conducted, which showed neutralising antibody responses against influenza and no interference of pre-existing vector immunity.[91] Preclinical studies for the RSV vaccine candidate in the cotton rat model showed an increase in anti-F antibodies and protection against RSV challenge.[71] In the older adult population, immunosenescence can be characterised by impaired T-cell responses to RSV.[92,93] This vaccine candidate, which induces a strong humoral response, could be a promising intervention in this population.

Third and fourth, Ad26.RSV.preF, is a vaccine candidate being developed for older adults and the paediatric population. In this candidate, pre-F antigen is expressed in the human adenovirus strain 26, a vector with a favourable safety profile when used for other infectious diseases.[94,95] Previously, the vaccine candidate vector expressed post-F as antigen (FA2) but has now been changed to stabilised pre-F conformation. The stabilised pre-F protein has five aminoacid changes from wildtype, and is stable at 4°C and heat-stable.[34] With the expectation that this vaccine candidate will induce highly neutralising antibodies against pre-F, phase 2 trials will be conducted in RSV-seropositive children. In December, 2017, a phase 2 trial began comparing concomitant admixtion of RSV vaccine and seasonal influenza vaccine versus seasonal influenza vaccine alone in healthy older adults.[96]

Fifth, ChAd155-RSV, the replication-incompetent chimpanzee adenovirus 155 has been used as a vector for the F, N, and M2-1 proteins. The anticipated use for this paediatric vaccine is to start immunisation at 2 months of age, and to use two doses alongside the normal paediatric vaccination schedule, instead of seasonally.[74] This vaccine candidate is being evaluated in 12–23-month-old RSV seropositive children. In the future, there are plans to conduct clinical trials in seronegative children sequentially from older to younger ages (12–24 months, followed by 6–12 months, and subsequently 2–6 months of age) to ensure safety in RSV-naive populations. Results of phase 2 trials are expected to be announced in 2020.

In summary, vector-based vaccines are used to display various RSV viral proteins and three of these vaccine candidates are already in phase 2 trials.

Subunit vaccines

Owing to concerns of ERD associated with protein-based vaccines, subunit vaccines are only in development for pregnant women and older adult populations.

One subunit vaccine in development is the GSK RSV F vaccine candidate, which uses a version of soluble secreted F protein empirically engineered to maintain the pre-F conformation. Results from a phase 1 trial showed safety and immunogenicity as evidenced by RSV neutralising antibody response in healthy men.[76] However, a phase 2 trial scheduled for 2017 was halted because of instability of the pre-F antigen during manufacturing.

DPX-RSV is a vaccine candidate with a unique choice of vaccine antigen: the extracellular domain of the SH protein of RSV.[45] The DepoVax technology allows for a prolonged exposure of antigen and adjuvant, and aims to induce ADCC using a liposome and oil-based depot.[97] The antigen and adjuvant are encapsulated in a liposome, lyophilised and suspended in oil, and the process is expected to produce vaccines with long shelf-life stability.[97] Phase 1 results on safety and immunogenicity in the older adult population have been released and are expected to be published from this investigator-initiated study.

Structure-guided stabilisation of the pre-F conformation has yielded a subunit vaccine candidate, RSV F DS-Cav1. The stabilisation includes a foldon trimerisation domain, the introduction of cysteine residues to form a disulphide bond, and cavity-filling hydrophobic residues.[37] The vaccine is able to preserve neutralisation-sensitive epitopes on a functional pre-F form of the viral surface protein. In preclinical studies, the subunit vaccine induced high amounts of RSV-neutralising antibodies in mice and non-human primates.[37] Preliminary results from the phase 1 trial, VRC 317, are promising and are expected to be published soon.

Finally, another stabilised pre-F subunit vaccine candidate, which has been optimised for antigen design after screening 360 candidates with cryo-electron microscopy, is expected to enter phase 1 clinical trials soon.[99]

Live-attenuated and chimeric vaccines

In the context of historical concerns for enhanced RSV disease, live-attenuated vaccines can be considered safe for RSV-naive infants, based on consistent clinical study results showing that these candidates do not prime for ERD following subsequent exposure to wildtype RSV after vaccination.[100] Another benefit of live-attenuated vaccines against RSV in young infants is their ability to replicate in the respiratory tract despite the presence of maternally acquired antibodies, and to elicit a broad humoral and cellular response.[101] Live-attenuated vaccines are probably limited to the

paediatric population under 2 years of age, as pre-existing immunity in older populations might not permit sufficient replication to generate protective immune responses. Safety could be a concern for intranasal live-attenuated vaccines, in particular if attenuation is insufficient. However, evaluation of current vaccines has not shown evidence of increased rates of vaccine-associated ALRI or fever, though there might be increased rates of rhinorrhoea, similar to what has been observed with the live-attenuated influenza vaccines.

Five live-attenuated vaccine candidates in phase 1 clinical trials are being developed in partnership with the National Institutes of Health. Live-attenuated vaccines face the challenge of achieving sufficient attenuation to be safe, and remaining immunogenic enough to induce a protective immune response. An improved understanding of the RSV viral genome has informed the development of new vaccine candidates that could overcome this challenge. Two main modifications to the RSV genome have been engineered through reverse genetics: the ΔM2-2 deletion which attenuates viral replication and upregulates antigen expression,[102] and the ΔNS2 deletion, which reduces viral suppression of host interferon thereby boosting the innate immune response. RSV MEDI ΔM2-2 reduced viral replication while inducing a strong primary serum neutralising antibody and potent anamnestic response in RSV-seronegative infants and children.[102] Further results from phase 1 clinical trials with the other live-attenuated vaccine candidates are expected.

The only chimeric vaccine candidate in clinical development, rBCG-N-hRSV, is delivered via a BCG strain. BCG has a safe profile in newborn babies and infants, induces a Th1 response,[81,82] and allows for combined vaccination against two major respiratory pathogens: *Mycobacterium tuberculosis* and RSV. Not only is the Th1 cellular response important in protecting against lung pathology, inflammation, and viral replication[83] but the candidate also induces a humoral response. The antigen presented by this vaccine candidate is the RSV N protein.[103] So far, this candidate is the only vaccine candidate intended for administration to newborn babies.[103]

Monoclonal antibodies

A promising highly potent monoclonal antibody has emerged as a passive administration strategy to prevent severe RSV infection. MEDI8897, also known as nirsevimab, was optimised from the human antibody D25 that targets antigenic site ø on the pre-F conformation, which is more neutralisation sensitive than the palivizumab epitope, antigenic site II. Using the YTE (aminoacid substitutions Met252Tyr/Ser254Thr/Thr256Glu) technology, which extends antibody half-life and modulates ADCC,[104] the three-times increase in half-life of MEDI8897,[105] compared with palivizumab offers the possibility of passive protection for all infants for an entire season through a single intramuscular injection. The intended use is for term and preterm infants entering their first RSV season. Passive vaccination with an extended half-life antibody offers an approach to protecting infants that is safe and can be reasonably priced. Representatives of the pharmaceutical company have indicated that they expect vaccine-like pricing for MEDI8897. Given the increased potency, the extended half-life, and the required dose, it is expected that the cost to protect an infant during the RSV season can be kept relatively low.[84]

Considerations by regulatory agencies and WHO

Considerations in population selection for vaccine trials mentioned by the European Medicines Agency (EMA) include: first testing a vaccine candidate in a seropositive before testing in a seronegative population, testing a maternal vaccine in non-pregnant women of child-bearing age before testing in pregnant women, and including older adults with comorbidities in vaccine trials. No particular considerations were mentioned for population selection in studies for mAbs. In October, 2017, the EMA released draft guidelines for the clinical evaluation of RSV prophylactic interventions that included guidance regarding trial design, assessment of efficacy, and safety.[106] The draft guidelines will be revised after a period of public consultation based on comments and new publications.

WHO has recognised the importance of RSV as a global health problem and has identified the development of RSV vaccines as a priority for the WHO Initiative for Vaccine Research and for Biological Standardization. WHO has developed RSV vaccines preferred product characteristics and research and development technical roadmap documents.[107,108] Further guidance for development will contribute to adequate policy making. WHO standardisation activities led to the development and establishment of the first international standard for antiserum to RSV. Development of guidelines for evaluation of quality, safety, and efficacy of RSV vaccines has been initiated and will be part of the consultation with regulators, manufacturers, and academia in 2018, with the aim of finalisation in 2019. Further discussion on guiding principles for mAbs is needed before proceeding with the development of the WHO guidelines. These and other WHO standards serve as a basis for setting national regulatory requirements and WHO prequalification.

Finally, the WHO is now doing a surveillance pilot study in 14 countries to test the feasibility of using the Global Influenza Surveillance and Response System platform for RSV surveillance and it is expected that this pilot will contribute to our understanding of the RSV disease burden and seasonality in different geographical regions.[109]

Discussion

Challenges in RSV vaccine design include concerns of ERD post-vaccination, lack of definitive immunological correlates

of protection, lack of consensus regarding clinical endpoints, and little natural immunity following RSV infection. Despite these challenges, developments such as an understanding of the structural biology of the RSV fusion protein, as well as lessons learned from late-phase vaccine trial failures have informed the field as it moves forward.

We attempted to collect data regarding expected plans for access to a preventive intervention in low-income and lower middle-income countries (LMICs) and expected pricing for all vaccine candidates; however, this information is not publicly available. Given that the most severe RSV infection occurs in low-income and LMICs,[19] information regarding LMIC target countries and potential pricing for vaccine candidates will be essential to facilitate access to vaccines worldwide, especially in areas where the mortality burden is highest. A mechanism should be introduced to ensure that information regarding expected pricing and access to interventions is transparent and available in the public domain. RSV vaccines and mAbs will be considered in the development of the Vaccine Investment Strategy by Gavi, the Vaccine Alliance in 2018.[110]

A vaccine trial can be considered a probe study to establish whether a causal relationship exists between RSV infection and asthma, a long-standing question in the field. If long-term follow up had been undertaken during the pivotal RSV prevention trials using palivizumab, these trials would now have provided 20 years of follow up on respiratory morbidity after RSV prevention in high-risk infants. Lack of long-term surveillance for airway morbidity in vaccine trials is a missed opportunity to provide novel scientific insights, important not only to understand the pathogenesis, but also the long-term vaccine efficacy against airway morbidity following RSV infection. In addition to wheeze, objective outcomes such as lung function measurements, including demonstration of bronchial hyperreactivity and IgE measurements, will ideally be incorporated in vaccine trials to fully understand the effect of RSV prevention on asthma development.

Viral interference, in which RSV inhibits infection by other viruses, is becoming an increasingly important concept to understand in the context of an approved RSV vaccine. RSV vaccination could conceivably result in an increased prevalence of other respiratory viruses. There is evidence supporting viral interference for influenza vaccination,[111,112] for RSV prevention,[113,114] and during the RSV season in the absence of RSV.[115] It is important for vaccine trials to examine this effect by evaluating the prevalence of all-cause ALRI, as well as RSV-specific ALRI, to better understand the implications of viral interference for an RSV vaccine.

This Review provides an extensive overview of the 19 vaccine candidates and mAbs in clinical trials to prevent RSV infection. RSV vaccine development is moving rapidly and shows promise to address an unmet global health problem. Vaccines for various target populations are in clinical development. One vaccine candidate and one mAb are in late-phase trials (2b or 3) and aim to prevent the disease burden in infants. Despite some failures, RSV vaccine candidates and mAbs in clinical development hold promise that a preventive intervention for RSV is on the horizon.

Search strategy and selection criteria

A data collection template was designed for all vaccines in clinical development according to the PATH respiratory syncytial virus (RSV) vaccine and monoclonal antibody (mAb) Snapshot, updated November, 2017 (appendix). Vaccines were divided into four major groups: particle-based, vector-based, live-attenuated or chimeric, and subunit vaccines. Immunoprophylaxis with mAbs was included as a fifth category. Gaps in knowledge were identified through a search of PubMed for clinical trials with "syncytial" in the title published between Jan 1, 2013, and April 3, 2018, with no language restrictions (NIM, ACL, NH, IK, EB, JSM). Furthermore, data for this Review were systematically collected using a data collection template (appendix) at the RSV Vaccines for the World conference organised by the Respiratory Syncytial Virus Network (ReSViNET) between Nov 29 and Dec 1, 2017, in Malaga, Spain (NIM, ACL, NH, IK, EB, JSM). The goal of this meeting was to share scientific data and expertise on RSV vaccine development, and to connect stakeholders involved in RSV research. We did not intend to do a systematic review of the literature. No inclusion or exclusion criteria were used. Instead, we selected articles that were most relevant to the subheadings used in this Review. ClinicalTrials.gov, the WHO vaccine pipeline tracker for RSV, the European Medicines Agency, and pharmaceutical websites were used to identify all relevant trials for these vaccine candidates and mAbs.

Contributors

LJB and NIM were involved in the design and plan for this Review. NIM, ACL and NH were involved in the data collection, data extraction, and quality assessment and contributed to the writing of the manuscript, in collaboration with DH, MCN, JAM, ACL, NH, UJB, PJO, JSM, JAE, AM, RAK, EAFS, IK, OR, PAP, HYC, ARF, HN, LKT, AG, EB, NGP, JV, FPP, MP, AS, EEW, RTS, BSG, LJB. The manuscript was written in collaboration with the ReSViNET Foundation.

Declaration of interests

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10

RESPIRATORY SYNCYTIAL VIRUS PREVENTION WITHIN REACH: THE VACCINE AND MONOCLONAL ANTIBODY LANDSCAPE

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If a plant cannot live according to its nature, it dies; and so a man.
Henry David Thoreau (1817-1862)

Respiratory syncytial virus prevention within reach: the vaccine and monoclonal antibody landscape

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Respiratory syncytial virus is the second most common cause of infant mortality and a major cause of morbidity and mortality in older adults (aged >60 years). Efforts to develop a respiratory syncytial virus vaccine or immunoprophylaxis remain highly active. 33 respiratory syncytial virus prevention candidates are in clinical development using six different approaches: recombinant vector, subunit, particle-based, live attenuated, chimeric, and nucleic acid vaccines; and monoclonal antibodies. Nine candidates are in phase 3 clinical trials. Understanding the epitopes targeted by highly neutralising antibodies has resulted in a shift from empirical to rational and structure-based vaccine and monoclonal antibody design. An extended half-life monoclonal antibody for all infants is likely to be within 1 year of regulatory approval (from August, 2022) for high-income countries. Live-attenuated vaccines are in development for older infants (aged >6 months). Subunit vaccines are in late-stage trials for pregnant women to protect infants, whereas vector, subunit, and nucleic acid approaches are being developed for older adults. Urgent next steps include ensuring access and affordability of a respiratory syncytial virus vaccine globally. This review gives an overview of respiratory syncytial virus vaccines and monoclonal antibodies in clinical development highlighting different target populations, antigens, and trial results.

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Introduction

In the past decade, the substantial burden of respiratory syncytial virus (RSV) has received increasing recognition globally. RSV is the second leading cause of infant mortality after the neonatal period[1] with more than 99% of childhood deaths occurring in low-income and middle-income countries (LMICs).[2] Nevertheless, the RSV burden in children is likely underestimated, and major gaps in knowledge regarding RSV disease burden have been addressed only recently. More than 50% of pediatric RSV mortality occurs out of hospital (as opposed to in hospital) in LMICs[3] with poverty as a substantial risk factor (figure 1). Infants at highest risk of RSV disease in high-income countries (HICs) include the very young infants born prematurely and those with underlying congenital heart or chronic lung disease,[8] Down's Syndrome,[9] and neuromuscular disorders.[10] Maternal vaccination is insufficient to protect infants with extreme prematurity as transplacental antibody transfer only reaches mature levels towards the end of the third trimester.[11]

In older adults (aged >60 years), the burden of morbidity and mortality due to RSV was also underestimated until recently. Modelling studies now estimate that the RSV burden is similar to the burden of seasonal influenza in adults older than 65 years.[12–14] Preliminary economic evaluations have highlighted the potential value of a vaccine for older adults, especially in HICs. Key economic drivers of cost-effectiveness include RSV incidence, risk of death, and level and duration of protection.[15,16]

Natural immunity to RSV is incomplete and reinfection occurs throughout life.[17] A concern in the development of RSV vaccines is the potential for enhanced respiratory disease in which more severe illness occurs upon natural infection after vaccination of RSV-naive infants as was observed with formalin-inactivated RSV in the 1960s.[18] Enhanced respiratory disease was associated with induction of poorly neutralising antibodies in vaccine recipients[19] and animal models of enhanced respiratory disease show a T helper type 2 (Th2) biased T-cell response.[20] For this reason, an RSV vaccine for RSV-naive recipients ideally elicits potent neutralising antibodies without a Th2 bias. Although a definitive correlate of protection against RSV infection remains elusive, cell-mediated immunity,[21] mucosal IgA,[22] and neutralising antibodies[23–26] have been associated with protection from RSV infection.

Key messages

- Knowledge of neutralisation-sensitive viral epitopes informed a shift from empirical to structure-based vaccine and monoclonal antibody design
- Market access for an extended half-life respiratory syncytial virus monoclonal antibody for prophylaxis in all infants is within reach in 2023 and will likely be followed by approval of a maternal vaccine to protect all infants
- No vaccine or monoclonal antibody is within reach for resource poor areas with the highest paediatric mortality burden
- Subunit, vector-based, and nucleic acid vaccine approaches are in late-phase trials for older adults (older than 60 years)

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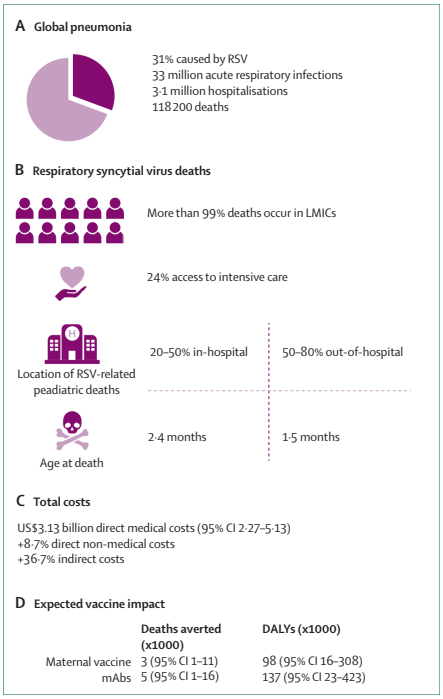

Figure 1: **Paediatric RSV disease burden key facts and figures**
(A) Contribution of RSV to worldwide pneumonia: approximately one-third of worldwide pneumonia is caused by RSV. (B) Deaths related to RSV: more than 99% of the global burden of paediatric mortality due to RSV occurs in low-income and middle-income countries.[1] Access to care is likely a key factor of the inequitable distribution of the mortality burden as less than one fourth of these children have access to an intensive care.[4] At least half of this burden was previously hidden, these deaths occur out out-of-hospital.[3] Recently the out-of-hospital burden has been characterised and is distinct from the in-hospital mortality burden which has implications for global vaccine development: children who die out of hospital die at a younger age and risk factors are linked to poverty instead of underlying conditions.[5] (C) Total costs: estimated direct associated with RSV exceed US$3 billion in low-income and middle-income countries, with additional direct non-medical and indirect costs.[6] (D) Expected vaccine impact: the cost-effectiveness and potential impact of maternal immunisation versus monoclonal antibodies has been estimated in deaths averted and discounted disability adjusted life-years.[7] RSV=respiratory syncytial virus.

Stabilisation of the pre-Fusion (pre-F) conformation of the RSV fusion (F) protein has led to the determination of viral epitopes that elicit highly neutralising antibodies. Antibodies that recognise pre-F provide most of the neutralising activity in human RSV-immune sera[27] supporting development of vaccine candidates and monoclonal antibodies (mAbs) based on stabilised pre-F antigens (figure 2).

There are three different target populations for RSV prevention: paediatric, maternal, and older adult (figure 3).[32] Leading strategies for the paediatric population include passive immunoprophylaxis with mAbs for young infants (aged <6 months) and live-attenuated vaccines (LAVs) for active immunisation of older infants (aged >6 months). Young infants might also be protected by passively transferred antibodies in immunised pregnant women. Stabilised pre-F subunit vaccines are in late-phase development for maternal vaccination. Finally, for older adults three vaccination approaches (nucleic acid, subunit, and vector-based vaccines) that employ pre-F antigen are in late phase trials.

In 2018, we did a comprehensive review of the RSV vaccine landscape in which we distilled lessons learned from late-phase vaccine failures and identified 19 vaccine candidates and monoclonal antibodies in clinical trials.[33] The review might have provided vaccine developers with guidance for future vaccine development by endorsing pre-F as a new target for RSV preventive interventions. The pre-F antigen is now the basis of six vaccine candidates and two mAbs in phase 3 trials.[34–37] Furthermore, we endorsed controlled human infection models as a unique tool to generate rapid proof of concept of protection and extensive immunological characterisation. This approach has been adopted into clinical development for six current RSV vaccine candidates (MV-012–968, RSVPre-F, MVA-BN-RSV, palivizumab biosimilar, clesrovimab, and Ad26.RSV.Pre-F). This updated review shows that 11 (58%) of 19 candidates from 2018 (and three [30%] of ten candidates from our 2015 review)[38] have continued development, with simultaneous expansion of the field with 19 additional candidates having entered clinical trials (figure 2, figure 4). Finally, after the success of mRNA SAR-CoV-2 vaccine development, vaccines delivered as mRNA are a novel preventative approach that has been rapidly accelerated to late-phase trials.

Methods

Vaccine and monoclonal antibodies (mAb) candidates in clinical phases of development were identified using the PATH (centre for vaccine innovation and access) RSV Vaccine and mAb snapshot (last updated Sept 28, 2021).[32] The data collection template from previous reviews[33] was updated (appendix p 1) and filled out by searching PubMed, clinical trial registries, WHO, European Medicines Agency, and pharmaceutical websites for each vaccine candidate, with no date or language restrictions. We did not intend to conduct a systematic review of the peer-reviewed literature but instead provide an update on the current development by capturing all recent publicly available information. No inclusion or exclusion criteria were used. Instead, for each vaccine candidate or mAb in clinical development, information was selected by date (with preference for more recent literature) and by relevance (with preference for trial data). When available, peer-reviewed publications were preferred to information from trial registries or pharmaceutical websites. To supplement the data collected and the identified gaps in

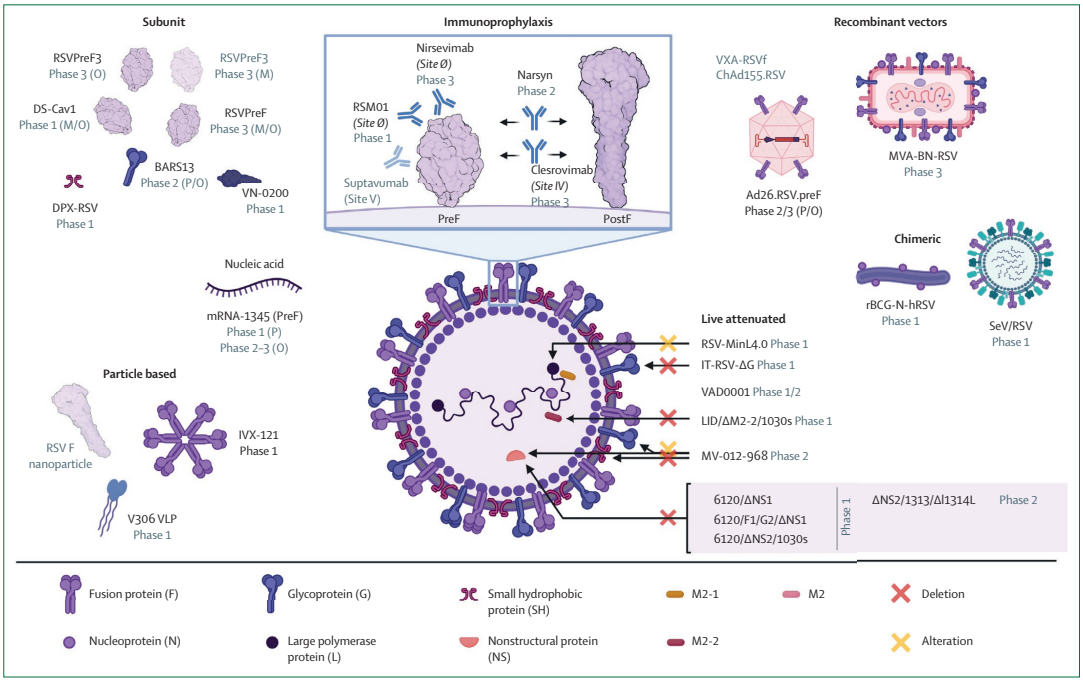

Figure 2: **Overview of vaccine candidates by preventive approach**
Pre-F protein was created with Protein Data Bank RCSB PDB 4MMU[28,29] and post-F protein was created with 3RRT.[30,31] Light grey indicates vaccine development halted or discontinued. RSV=respiratory syncytial virus. PreF=prefusion protein. PostF=postfusion protein. Ad=adenovirus. MVA=modified vaccinia Ankara virus. BCG=mycobacterium bovis. SeV=sendai virus.

knowledge, data for this review were systematically collected using the data collection template (appendix p 1) at the virtual RSV Vaccines for the World Conference organised by the Respiratory Syncytial Virus Network from Nov 10–12, 2021. The goal of this meeting was to share scientific data and expertise on RSV vaccine development, and to connect stakeholders involved in RSV research. During the meeting, information was collected from scientific presentations, posters, and discussions. Any publicly available data from this meeting has been included in this manuscript. Vaccines were divided into six major groups: recombinant vector, subunit, particle based, live attenuated, chimeric, and nucleic acid. Immunoprophylaxis with mAbs was included as a seventh category. Vaccine characteristics such as mechanism of action, adjuvants, route of administration, and summary of trial results have been compiled in the table.

Lessons learned

We examine lessons learned from three late phase clinical trial failures since our last review. The PREPARE trial[39] was a milestone: the first phase 3 trial of an RSV maternal vaccine. More than 4000 pregnant women received an RSV F nanoparticle vaccine or placebo (2:1 ratio) during the third trimester. RSV maternal vaccination was determined to be safe. Although the vaccine did not meet the primary endpoint, the candidate is the first proof-of-concept demonstration for efficacy of RSV maternal immunisation against severe RSV infection in infants. Efficacy was shown through day 90 in South Africa, where more than 50% of participants were enrolled: 56% (95% CI 33–71) against medically significant RSV lower respiratory tract infection (LRTI) and 74% (50–86) against RSV LRTI with severe hypoxemia. Moreover, there was 49% efficacy against all-cause infant pneumonia through 1 year after vaccination.[39] The difference in efficacy might be explained by hospitalisation for less severe disease and lower background rates of severe RSV infection in HICs. compared with LMICs. Lessons learned include geographical heterogeneity of RSV disease burden and potential efficacy between different countries, and the importance of timing of vaccination in relation to RSV season and gestational age.[39] Furthermore, it was shown that RSV neutralising antibodies and F surface

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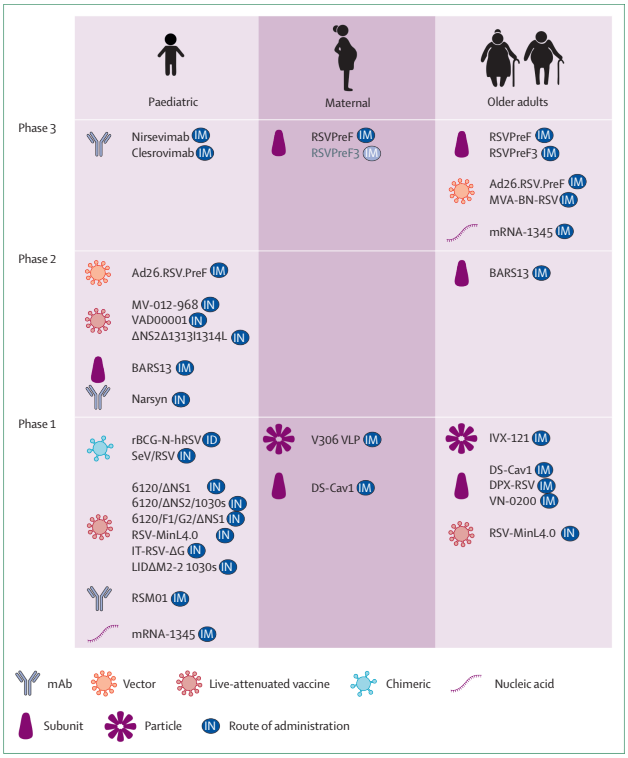

Figure 3: **RSV vaccine and monoclonal antibody agents by target population**
Vaccine candidates and monoclonal antibidies are categorised into three different target populations: paediatric, maternal, and older adults (aged >60 years) and clinical phase of development (ie, phase 1, 2, or 3). Different immunisation approaches are indicated by the key. Light grey text indicates development halted. IM=intramuscular. IN=intranasal. ID=intradermal. RSV=respiratory syncytial virus. PreF=prefusion protein. PostF=postfusion protein.

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glycoprotein binding antibodies were correlated with protection against RSV LRTI with severe hypoxemia (eg, a vaccine-induced maternal antiF IgG 16 times increase from maternal enrollment to day 14 was associated with a baseline covariate-adjusted vaccine efficacy of 75%).[40] Proven efficacy poses an ethical dilemma that a potentially life-saving vaccine might not become available in these countries as drug development was discontinued because prespecified criteria for efficacy were not met.[41] A rollover trial might be considered to confirm efficacy and develop this vaccine for LMICs.

At the time of our last review in 2018,[42] analysis of the late-stage clinical trial failure of suptavumab (REGN2222), an antigenic site 5 mAb, which did not meet its primary endpoint, had not yet been made public. In a phase 3 study[42] in 18 countries, it was shown that suptavumab did not reduce RSV hospitalisation or outpatient RSV LRTI due to a natural mutation in the predominant circulation strain of RSV subgroup B that resulted in loss of antibody binding and neutralisation. There were no changes in circulating RSV A strains and negligible anti-suptavumab antibody responses. Post-hoc analyses suggested the antibody was relatively effective against the subgroup A strains but not the new circulating B strain; the relative risk for RSV subgroup A hospitalisation or outpatient LRTI versus placebo was 0·38 (95% CI 0·17–0·86). These findings highlight the importance of characterisation of the viral fitness of monoclonal antibody resistant viral mutants in clinical development and the risk associated with targeting a single viral epitope as well as more potential variability of certain targeted antigenic sites.

Finally, ChAd155.RSV, a recombinant chimpanzee adenovirus vector vaccine expressing RSV f, N, and M2–1 proteins, was in development for the paediatric population. Development was halted after preliminary analyses of a phase 2 trial in infants aged 3–7 months showed that the target efficacy profile was unlikely to be met.[43] The published first-in-human trial in healthy adults showed adequate safety as well as increased specific humoral and cellular immune responses.[44] The results of the phase 2 study have not yet been published so further lessons learned and analysis of the results are pending. Potentially the choice of vaccine antigens was not optimal for an effective immune response.

LAVs

LAVs are designed to generate a potent immune response, including a local mucosal antibody and cellular response, by mimicking natural infection while being attenuated for reduced virulence. Genetic stability is important to limit the chance of reversion to wildtype virus. A better understanding of the RSV genome and reverse genetics has allowed the rational design of LAV candidates by deleting or modifying proteins known to be important in RNA synthesis regulation or interference with host-immune responses (eg, M2-2, NS2, SH, L, and G proteins) leading to restricted viral replication.[45]

An analysis of the compiled results of seven phase 1 trials using intranasal LAV (n=239; children aged 6–24 months) provides information on vaccine safety, efficacy, and duration of protection of RSV LAV candidates.[45] LAVs are considered safe after first exposure, because vaccine-enhanced disease has not been detected after LAV immunisation, although LAV have the potential to induce upper respiratory illness if attenuation is insufficient.[45] Estimated efficacy from compiled data of five vaccine candidates was 67% (95% CI 24 to 85) against medically-attended RSV acute-respiratory illness and 88% (–9 to 99) against medically-attended RSV LRTI. On an immunological level, a four times rise in RSV-plaque reduction neutralising antibody titre was predictive of vaccine efficacy and responses were durable through 1 year after vaccination.[45]

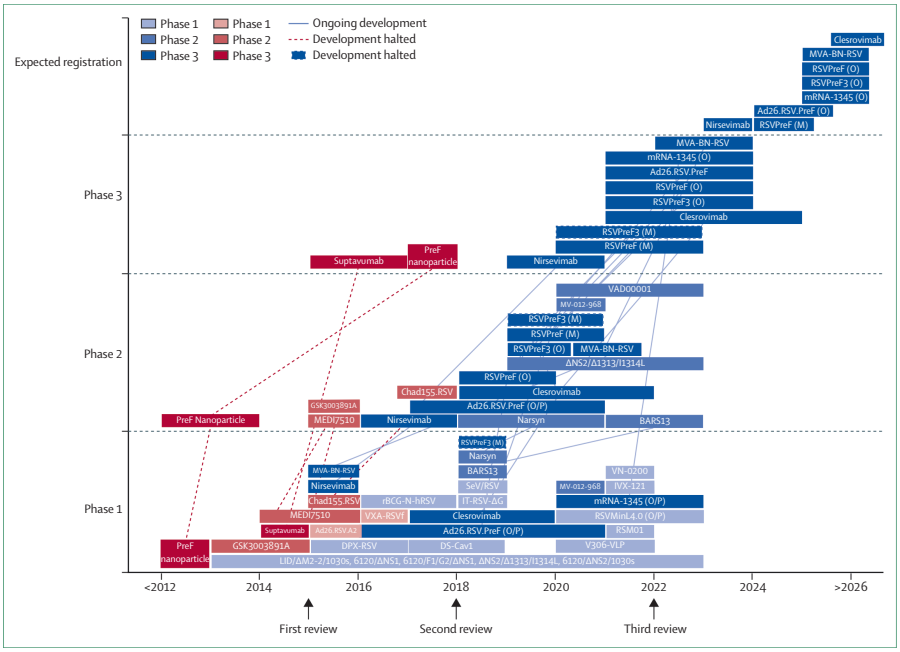

Figure 4: **Historical perspective of RSV vaccine and immunoprophylaxis development over the last 10 years and expected market access**
Candidates that are in ongoing development (blue) or no longer in development (red) are presented at the timing of the clinical trials rounded off to full years. The darkness of the colour represents the furthest development (phase 1, 2, or 3) of the candidate. Candidates with multiple clinical trials are connected with full or dotted lines to show the speed of development. Live attenuated viruses by the same manufacturer are summarised in one box as development of these candidates largely overlaps. The timing of current and previous RSV vaccine landscape reviews is shown at the bottom. RSV=respiratory syncytial virus. PreF=prefusion protein. The development of RSVPreF (M) was not discontinued but instead halted (dotted box). Expected registration was estimated by adding 1 year after published interim results, or if not available by adding 1 year to the estimated completion year published on ClinicalTrials.gov. In case of a phase 1/2 or 2/3 trial, the trial has only been placed in the furthest developed stage.

There are six phase 1 trials and four candidates that have progressed to phase 2 trials. The National Insitute of Health and National Insitute of Allergy and Infectious Disease and others are developing LAV candidates with an NS2 deletion and temperature sensitivity mutation: RSV ΔNS2/Δ1313/I1314L (phase 2);[46] and RSV 6120/ΔNS2/1030s (phase 1).[47,48] MV-012–968 (altered NS1 and NS2 and G proteins, SH deletion, and ablation of secreted G protein)[49] has been shown to be safe and to generate a mucosal IgA response in seropositive adults and children.[50] A safety trial in seronegative children and a human challenge trial in healthy adults to show efficacy are being conducted for this vaccine candidate.[51–53] IT-RSV-ΔG (absent G protein) was safe in seropositive healthy adults. However, the serum neutralising antibody response was low, and nasal IgA antibodies were below the level of detection; immunogenicity needs to be further studied in children and eventually in seronegative infants.[54] Other candidates include LIDΔ M2–2/1030s,[55] 6120/ΔNS1 and 6120/F1/G2/ΔNS1 (NCT03596801) in phase 1 trials, and VAD00001 (NCT04491877) in phase 2 trial. RSV-MinL4·0 (altered polymerase gene) showed a humoral and cellular immune response similar to wildtype infection in non-human primates and is in phase 1 trials.[56,57]

Overall, LAVs provide an important needle-free tool for active intranasal immunisation of older infants who will not be sufficiently protected by a mAb or maternal vaccine. Moreover, a relatively small sample size (n=540) is needed for a phase 3 trial in this population.[45] Further clinical development using this vaccination approach might affect paediatric health directly by reducing paediatric infections and infections in older adults indirectly through herd immunity.

Chimeric

Chimeric live virus vaccine candidates express RSV proteins in related attenuated viruses with favourable safety profiles. In contrast to vectored vaccine candidates, chimeric vaccines show favourable antigen presentation

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See Online for appendix

For **Vaccines for the World Conference** see https://www.resvinet.org/rsvvw21.html

	Manufacturing process	Antigen	Adjuvant	Mechanism of action	Target population	Route of administration	Clinical phase	Animal models	Phase 1 trial	Phase 2 trial	Phase 3 trial	Results
Subunit vaccine												
VN-0200, Daiichi Sankyo	..	VAGA-9001a	MABH-9002b	..	O	IM	1	..	June, 2021, to January, 2022; NCT04914520; 48 participants	..	..	..
DS-Cav1, National Insitute of Health and National Insitute of Allergy and Infectious Disease	Structure-based vaccine design	Stabilised preF DS-Cav1	None or alum	RSV preF	O	IM	1	Cotton rats, calves, mice, macaques	February, 2017, to October, 2019; NCT03049488; 95 participants	..	..	Phase 1: safe and well-tolerated; vaccination elicited robust neutralising Ab response sustained at 44 weeks
DPX-RSV, Immunovaccine	DepovaxTM, a lipid-in-oil delivery system	SHe	DepoVax (DPX-RSV[A]), DPX, or aluminum hydroxide	SHe generate a non-neutralising Ab and CD4+ T-cell response	O	IM	1	Cotton rats, mice	May, 2015, to June, 2017; NCT02472548; 40 participants	..	..	Phase 1: safe and well-tolerated, no serious adverse events, antigen-specific Ab response durable >6 months
BARS13, Advaccine	..	G	None or cyclosporine A	RSV G; immuno–suppressant	P and O	IM	2	..	October, 2018, to August, 2019; NCT04851977 and ACTRN12618–000948291; 60 participants	May, 2021, to June, 2023; NCT04681833; 120 participants	..	Phase 1: safe and well tolerated, substantial Ab response (90% in low dose groups *vs* 100% in high-dose groups)
RSVPre-F3/ GSK3844766A, GlaxoSmithKline	..	PreF3	None	Induce immune response with stabilised preF	P and O	IM	3	..	January, 2019, to November, 2020; NCT04090658, NCT03814590, and NCT04657198; 1055 participants	January, 2019, to November, 2020; NCT04090658 and NCT04657198; 1055 participants	February, 2021, to May, 2024; NCT04886596; 25000 participants February, 2021, to May, 2024; NCT04732871; 1720 participants February, 2021, to May, 2024; NCT05059301; 750 participants	Phase 1 and 2: humoral and cellular immune responses in all vaccines; older adults had higher humoral response (mostly neutralising) with higher dosage and higher cellular response with adjuvant Phase 3: interim analysis showed efficacy and the effect was consistent among RSV A and B strains

(Table continues on next page)

	Manufacturing process	Antigen	Adjuvant	Mechanism of action	Target population	Route of administration	Clinical phase	Animal models	Phase 1 trial	Phase 2 trial	Phase 3 trial	Results
(Continued from previous page)												
RSVPreF3/ GSK3888550A, GlaxoSmithKline	..	PreF3	None	Induce immune response with stabilised preF	M	IM	3, halted	..	October, 2018, to September, 2019; NCT03674177; 502 participants	July, 2020, to May, 2021; NCT04126213; 534 participants	November, 2020, to February, 2024; NCT04605159; 20 000 participants September, 2021, to May, 2022; NCT05045144; 1541 participants	Phase 1 and 2: robust increase in maternal RSV-specific Ab responses and RSV-A and RSV-B neutralising Ab titres; successful Ab transfer to foetus until 6 months after birth
RSVPreF, Pfizer	Bivalent stabilised preF, sequence based on contemporary RSV A and B strains	pre-F	None or Al(OH)3 or alum adjuvanted	Induce immune response with stabilised preF	M	IM	3	Animal studies, specifics unknown		April, 2018, to December, 2020; NCT03529773; 1235 participants November, 2020, to August, 2021; NCT04785612 (controlled human infection model); 62 participants October, 2019, to December, 2019; NCT04071158; 713 participants; August, 2019, to September, 2021; NCT04032093; 1153 participants	June, 2020, to November, 2023; NCT04424316; 10000 participants	Phase 1 and 2: patients were aged 18–49 years; safe and well tolerated; immunisation elicited ten to 20 times increases in neutralising Ab titres
RSVPreF, Pfizer	Bivalent stabilised preF, sequence based on contemporary RSV A and B strains	preF	None	Induce immune response with stabilised preF	O	IM	3	Animal studies, specifics unknown		April, 2018, to December, 2020; NCT03529773; 1235 participants November, 2020, to August, 2021; NCT04785612; 62 participants October, 2019, to December, 2019; NCT04071158; 713 participants August, 2019, to September, 2021; NCT04032093; 1153 participants	August, 2021, to June, 2024; NCT05035212; 30000 participants	Phase 1 and 2: patients were aged 18–49 years; safe and well tolerated; immunisation elicited ten to 20 times increases in neutralising Ab titres

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	Manufacturing process	Antigen	Adjuvant	Mechanism of action	Target population	Route of administration	Clinical phase	Animal models	Phase 1 trial	Phase 2 trial	Phase 3 trial	Results
(Continued from previous page)												
Particle-based vaccine												
V306-SVLP, Virometix	Synthetic peptides conjugated to a synthetic nanoparticle made from self-assembling lipopeptides	V-306	Pam2Cys	Synthetic virus-like particle displays a universal T-helper epitope, lipid component (Pam2C) and mimetic of the Palivizumab epitope (FSIIm)	M	IM with skin patch boosters	1	Mice and rabbits	September, 2020, to March, 2022; NCT04519073; 60 participants	..	..	..
IVX-121, Icosavax	Self-assembling virus-like particle platform technology to deliver stabilized trimeric preF proteins	Stabilised Pre-F DS-Cav1	None or alum	Presentation of DS-Cav1 on compu–tationally designed virus-like particle generates a neutralising Ab response against preF	O	IM	1	Mice	2021; 90 participants	..	..	..
Nucleic acid vaccine												
mRNA-1345, Moderna	Lipid nanoparticle containing optimised protein and codon sequences	preF	None	mRNA encodes for a stabilised preF glycoprotein eliciting neutralising antibodies	O	IM	2 and 3	..	September, 2020, to September, 2023; NCT04528719; 100 healthy adults, 300 older adults, 180 women, and 40 children	Phase 2 and 3; November, 2021, to November, 2024; NCT05127434; 34000 participants	Phase 2 and 3; November, 2021, to November, 2024; NCT05127434; 34000 participants April, 2022, to January, 2023; NCT05330975; 1350 people	Phase 1: well tolerated at doses up to 200 µg; geometric mean concentration ≥9·8 times rise in neutralising Abs at 1 month for RSV A and ≥5·3 times rise in neutralising Abs at 1 month for RSV B; three doses helped maintain peak titers through month 5 in younger adults
mRNA-1345, Moderna	Lipid nanoparticle containing optimised protein and codon sequences	preF	None	mRNA encodes for a stabilised preF glycoprotein eliciting neutralising antibodies	P	IM	1	..	September, 2020, to September, 2023; NCT04528719; 40 children aged 12–59 months	..	..	..

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	Manufacturing process	Antigen	Adjuvant	Mechanism of action	Target population	Route of administration	Clinical phase	Animal models	Phase 1 trial	Phase 2 trial	Phase 3 trial	Results
(Continued from previous page)												
Recombinant vectors vaccine												
MVA-BN RSV, Bavarian Nordic	MVA-BN platform technology	F, G (A & B subtype), and N and M2	None	Simulate robust T-cell response against 5 RSV antigens and moderate humoral response against both RSV subtypes	O	IM	3	BALB, c mice, and cotton rats	August, 2015, to May, 2016; NCT02419391; 63 participants	September, 2016, to December, 2018; NCT02873286; 420 participants February, 2021, to June 2021; NCT04752644; 73 participants	To be announced end 2021	Phase 2: broad and durable Ab and T-cell response, substantial booster response after 1 year; phase 2: controlled human infection model; substantial reduction in viral load and no vaccine-related serious adverse events
AD26.RSV.PreF, Johnson & Johnson	Human cell line, PERC6 (Ad26) encoding RSV F from RSV-A2 strain	preF	None	Replication-incompetent Adenovirus 26 containing DNA for RSV-A2 F protein stabilised in preF conformation	O	IM	3	Neonatal and adult mice	November, 2016, to January, 2021; NCT02926430; 73 participants November, 2016, to January; NCT03795441; 24 participants November, 2016, to January; NCT04354480; 36 participants	October, 2017, to June, 2022; NCT04453202, 459 participants October, 2017, to June, 2022; NCT03502707; 669 participants October, 2017, to June, 2022NCT03–303625; 48 participants October, 2017, to June, 2022; NCT03334695; 64 participants October, 2017, to June, 2022; NCT03339713; 180 participants October, 2017, to June, 2022; NCT03982199; 5815 participants	July, 2021, to January, 2024; NCT04908683; 23000 participants	Phase 1: safe in older adults and sustained immune responses after 2 years Phase 2: 80% vaccine efficacy and robust cellular and humoral immune response; phase 2 controlled human infection model: lower viral load and lower infection rate and disease severity in intervention group
AD26.RSV.PreF, Johnson & Johnson	Human cell line, PERC6 (Ad26) encoding RSV F from RSV-A2 strain	PreF	None	Replication-incompetent adenovirus 26 containing DNA for RSV-A2 F protein stabilised in preF conformation	P	IM	1 and 2	Neonatal and adult mice	November, 2017, to April, 2020; NCT03303625; 48 participants January, 2019, to November, 2021; NCT03606512; 38 participants	November, 2017, to April, 2020; NCT03303625; 48 participants January, 2019, to November, 2021; NCT03606512; 48, and 38 participants	..	Phase 1 and 2: well-tolerated and elicited both humoral and cellular immune responses

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	Manufacturing process	Antigen	Adjuvant	Mechanism of action	Target population	Route of administration	Clinical phase	Animal models	Phase 1 trial	Phase 2 trial	Phase 3 trial	Results
(Continued from previous page)												
Immunoprophylaxis vaccines												
Narsyn, UMC Utrecht	Intranasal formulation of humanised mouse mAb	..	None	mAb targeting site 2 of the RSV F protein; neutralisation	P	IN	2	Balb/c mice	October, 2018, to November, 2018; NTR7378; 20 participants	November, 2018, to April, 2020; NTR7403; 408 participants	..	Phase 1: safe in healthy adults
Clesrovimab (MK 1654), Merck	In-vitro optimised human mAb with three YTE mutations in Fc-domain	..	None	mAb targeting site 4 of the RSV F protein with extended half-life; neutralisation	P	IM or IV	1, 2, and 3	Cotton rats	June, 2017, to February, 2019; 152 adults September, 2018, to September, 2022; NCT03524118; 180 infants	September, 2018, to September, 2022; NCT03524118; 180 infants March, 2020, to August, 2020; NCT04086472 (controlled human infection model); 80 participants	November, 2021, to August, 2025; NCT04938830; 1000 high-risk infants April 2021, to September, 2024; NCT04767373; 3300 healthy infants	Phase 1: safe in adults; controlled human infection model efficacy 0·62 (95% CI −0·05 to 0·86) for prevention of RSV lower tract respiratory infection
Nirsevimab, (MEDI8897), Astra Zeneca, MedImmune LLC	In-vitro optimised human mAb with YTE mutation in Fc	..	None	mAb targeting site Ø of the RSV F protein with an extended half-life; neutra–lisation	P	IM	3	Cotton rats, cynomolgus, and monkeys	April, 2015 to June, 2015; NCT0211426; 342 participants January, 2015 to September, 2016; NCT0229034; 151 participants	November, 2016, to July, 2018; NCT02878330; 1453 participants August, 2020, to January, 2023; NTC04484935; 100 immuno–compromised children	July, 2019, to May, 2022; NCT03959488 (MEDLEY); 925 high-risk children July, 2019, to March, 2023; NCT03979313 (MELODY); 3000 healthy children	Phase 2b: safety and tolerability similar to palivizumab in 25 countries worldwide; Phase 3 interim: 75% efficacy against medically attended RSV LRTI
RSM01, Gates Medical Research Institute	In-vitro optimised human mAb with YTE mutation in Fc	..	None	mAb targeting site x of the RSV F protein with an extended half-life; neutra–lisation	P	IM or IV	1	..	November, 2021, to February, 2022; NCT05118386; 56 participants	..	..	..
Chimeric vaccine												
SeV/RSV, National Insitute of Allergy and Infectious Disease	Modified mouse parainfluenza virus type 1	F	None	SeV expressing RSV F protein	P	IN	I	African green monkey	May, 2018, to February, 2019; NCT03473002; 21 participants	..	..	..
rBCG-N-hRSV, Pontificia Universidad Católica de Chile	Live-attenuated recombinant *Mycobacterium bovis* (rBCG) based on Danish strain 1331 that expresses N	N	None	Recombinant BCG used as a vector to deliver RSV N	P	IN	1	Mice and holstein calves	June, 2016, to June, 2018; NCT03213405; 24 participants	..	..	Phase 2: safe and well tolerated; no serious adverse events; humoral and cellular response against N and purified protein derivative

(Table continues on next page)

	Manufacturing process	Antigen	Adjuvant	Mechanism of action	Target population	Route of administration	Clinical phase	Animal models	Phase 1 trial	Phase 2 trial	Phase 3 trial	Results
(Continued from previous page)												
Live-attenuated vaccine												
RSV-MinL4·0, Codagenix	Reverse engineering of 4 mutations in the L protein	All viral proteins	None	L altered for attenuation	O	IN	1	Non-human primates	July, 2020, to May, 2021; NCT04295070; 36 participants	..	..	..
RSV-MinL4·0, Codagenix	Reverse engineering of 4 mutations in the L protein	All viral proteins	None	L altered for attenuation	P	IN	1	Non-human primates	March, 2022, to February, 2023; NCT04919109; 36 participants	..	..	..
IT-RSVΔG, Intravacc	Reverse genetics to delete G protein from the RSV genome	All viral proteins	None	Severely impaired binding to host cells due to absent G-protein reducing infectivity	P	IN	1	Cotton rats	May, 2018, to March, 2019; NTR7173; 48 participants	..	..	Phase 1: safe and well tolerated; neutralising antibody response absent in seropositive adults
MV-012–968, Meissa	Codon deoptimisation of NS1/NS2/G, SH deletion and ablation of secreted G through AttenuBlock synthetic biology platform	All viral proteins	None	Reduced NS1 and NS2 expression for enhanced immuno–genicity, SH deletion and G de-optimisation for attenuation	P	IN	1 and 2	BALB/c mice; cotton rats	January, 2020, to August, 2020; NCT04227210; 20 participants June, 2020, to May, 2021; NCT04444284; 20 participants June, 2021, to October, 2022; NCT04909021; 45 participants	December, 202 to May, 2021; NCT04690335; 60 participants	..	Phase 1: well tolerated, heavily attenuated, and induces an RSV-specific mucosal IgA response in healthy seropositive O and P participants
RSV ΔNS2/ Δ1313 / I1314L, National Insitute of Allergy and Infectious Disease (Sanofi)	Reverse genetics: NS2 deletion, L stabilisation; I1314L stabilising mutation via reverse genetics	All viral proteins	None	NS2 deletion bolsters innate response, deletion at position 1313 of L protein, and I1314L stabilisation confers moderate temperature sensitivity	P	IN	1 and 2	Mice and chimpanzees	June, 2013, to April, 2023; NCT03227029; 65 participants June, 2013, to April, 2023; NCT03422237; 80 participants June, 2013, to April, 2023; NCT03916185; 160 participants June, 2013, to April, 2023; NCT01893554; 105 participants	May, 2019, to April, 2023; NCT03916185; 160 participants	..	Genetically stable; attenuated yet immunogenic in RSV seronegative P

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	Manufacturing process	Antigen	Adjuvant	Mechanism of action	Target population	Route of administration	Clinical phase	Animal models	Phase 1 trial	Phase 2 trial	Phase 3 trial	Results
(Continued from previous page)												
RSV LID/ΔM2–2/1030s, National Insitute of Allergy and Infectious Disease (Sanofi)	Reverse genetics: M2-2 deletion and L mutation at position 1030s	All viral proteins	Non-adjuvanted	Inefficient replication yet high immunogenicity due to ML2-2 deletion ; moderate temperature sensitivity due to L mutation	P	IN	1	Mice, African green monkeys	September, 2020, to April 2022; NCT04520659; 81 patients	..	..	85% of vaccinees shed LID/ΔM2–2/1030s vaccine and ≥4 rise in serum-neutralising Abs
RSV 6120/ΔNS2/1030s, National Insitute of Allergy and Infectious Disease (Sanofi)	NS2 gene deletion and temperature sensitivity mutation (1030s) of L	All viral proteins	Non-adjuvanted	NS2 deletion bolsters innate response, sensitivity mutation (1030s) of L	P	IN	1	..	May, 2019, to April, 2023; NCT03916185; 160 patients October, 2017, to May, 2021, NCT03387137; 45 patients		..	..
RSV 6120/F1/G2/ΔNS1, National Insitute of Allergy and Infectious Disease (Sanofi)	Optimised NS1 deletion	All viral proteins	Non-adjuvanted	NS1 gene-deletion; increased F and G expression by moving to first and second genome positions	P	IN	1	..	June, 2018, to December, 2023; NCT03596801; 75 patients	..	..	..
RSV 6120//ΔNS1, National Insitute of Allergy and Infectious Disease (Sanofi)	Optimised NS1 deletion	All viral proteins	Non-adjuvanted	NS1 gene-deletion	P	IN	1	..	June, 2018, to December, 2023; NCT03596801; 75 patients	..	..	..
VAD00001 (SP0125), National Insitute of Allergy and Infectious Disease (Sanofi)	..	..	Non-adjuvanted	..	P	IN	1 and 2	Mice	September, 2020, to April, 2023; NCT04491877; 300 patients	September, 2020, to April, 2023; NCT04491877; 300 patients	..	..

Ab=antibody. mAb=monoclonal antibody. preF=prefusion protein. RSV=respiratory syncytial virus. ..=not applicable or not available. ID=Intradermal. IM=Intramuscular. IN=Intranasal. M=Maternal. O=Adults and older adults (aged ≥65 years). P=Pediatric.

Table: **Overview of RSV vaccines and mAbs in clinical development**

which activates an adaptive immune response.[58,59] There are two chimeric RSV vaccine candidates in phase 1 trials. One of these candidates uses a replication-deficient Sendai virus modified to express RSV F protein (SeV/RSV)[58] and the other uses a live-attenuated recombinant BCG vector expressing RSV N protein (rBCG-N-hRSV)[60] administered via the intradermal route. The latter vaccine candidate was found to be safe in phase 1 trial.[60]

Subunit

Subunit vaccines are protein based; this approach has been avoided in RSV-naive children due to the formalin-inactivated-RSV experience with enhanced respiratory disease in which it became clear enhanced respiratory disease is a concern for people not primed with live virus infection.[61] In parallel to the phase 3 trail[39] failure of a post-F subunit vaccine candidate, five vaccine candidates have adopted pre-F as vaccine antigen. Eight subunit candidates are in development for two different target populations: pregnant women and older adults. We discuss vaccine candidates using fusion antigens first, followed by candidates employing nonfusion antigens.

The phase 1 results of DS-Cav1, a subunit vaccine using stabilised pre-F developed by the National Insitute of Health and National Insitute of Allergy and Infectious Disease, provide proof-of-concept of structure-based vaccine design. Vaccination resulted in more than ten times increase in serum neutralising activity[62] and is sustainable for an entire RSV season.[63] Two other candidates use a stabilised pre-F protein as vaccine antigen. RSVpre-F (PF-06928316) is a bivalent (subtype A and B) stabilised pre-F without adjuvants. A phase 2 trial[64] was done in non-pregnant women with RSVpreF. co-administered with diphtheria toxoid and acellular pertussis vaccine, which showed safety and noninferiority relative to RSV pre-F alone.[64] The anti-pertussis response was inferior (geometric mean concentration between 0·59 and 0·8 for pertussis antigens compared with diphtheria toxoid and acellular pertussis alone) yet the clinical significance of these findings is still unclear and did not differ when adjusted for age.[65] The phase 3 MATISSE trial[66] in women who were pregnant was started in 2020 and is expected to be unblinded in the fourth quarter of the trial in 2023.[67] A human challenge trial[66] showed 75% efficacy of RSV pre-F against RSV infection and informed dose and formulation selection for the maternal vaccine candidate.[68] The phase 3 RENOIR trial[60] using the same vaccine candidate has started in the fall of 2021, in 30 000 older healthy and high-risk adults.[69] Another pre-F subunit vaccine, RSVpreF3, is in phase 3 clinical trials without adjuvant (GSK3888550A) for RSV maternal immunisation to protect infants (GRACE trial)[70] and with AS01 adjuvant (GSK3844766A)[72] to protect the older adult population. Development was halted for the maternal vaccine candidate in Feb 18, 2022, due to a safety signal. The older adult candidate was safe and induced approximately a ten times increase in pre-F IgG and IgA antibodies (48 adults aged 18–40 years; 1005 adults aged 60–80 years) in phase 1 to 2 clinical trials.[72–74] Interim analysis of the phase 3 trial for the older adult candidate showed efficacy against RSV lower respiratory tract infection (NCT04886596). For the maternal candidate, phase 1 and 2 studies showed a 14 times increase in RSV A and B neutralising antibody titers 1 week after vaccination and maintained a six times increase or more after 91 days in healthy women who were not pregnant (n=502).[74] In the phase 3 study[75] of the maternal vaccine candidate, the immune response was durable as antibody levels for vaccinees remained elevated against RSV A and RSV B for 6 months after birth. Registration of RSVpreF maternal vaccine is expected in 2024, and RSVpreF older adult and both RSV pre-F3 vaccine candidates in 2025, assuming registration is obtained within 1 year after phase 3 completion date according to the clinical trial registry.

There are three protein-based vaccines in clinical development that use non-F viral antigens. First, BARS13 uses RSV G protein as an antigen and cyclosporine A (CSA) immunosuppressant to induce regulatory T cells. BARS13 was safe and immunogenic in phase 1[76] and is now in phase 2[77] trials. Second, DPX-RSV, uses the ectodomain of RSV-A-SHe protein as a vaccine antigen formulated in depot-based lipid-in-oil delivery platform to allow for prolonged antigen and adjuvant exposure. The proposed mechanism of action against this antigen is generation of SHe-specific antibodies, which promote clearance of RSV-infected cells by alveolar macrophage phagocytosis. DPX-RSV showed safety and immunogenicity in a phase 1 first-in-human trial in adults aged 50–64 years.[78,79] Finally, VN-0200, uses VAGA-9001a as antigen and an MABH-9002b adjuvant (phase 1).[80] We were not able to define the biological background of VAGA-9001a.

Particle-based

Particle-based vaccines harness the immunogenical potential of displaying multiple antigens via particle assembly. IVX-121 uses a self-assembling synthetic virus-like particle platform technology to deliver 20 copies of stabilised trimeric pre-F proteins (DsCav-1). The computationally designed nanoparticle allows for stabilisation of the pre-F protein and in-vitro adjustment of antigen density. IVX-121 showed 10 times higher neutralising antibody responses than did DSCav1 alone in preclinical studies.[81] A phase 1 trial[82] started in 2021, with first results expected in 2022. After completion of the monovalent RSV candidate trial, the company plans to shift to development of a bivalent virus-like particle vaccine with both RSV and human metapneumovirus antigens.

A second particle-based vaccine candidate, V306-VLP, uses a synthetic virus-like particle to display a site 2 F

protein epitope. The vaccine platform uses conformationally constrained synthetic peptides conjugated to a synthetic nanoparticle made from self-assembling lipopeptides containing a T-helper epitope and toll-like receptor ligand.[83] The vaccine candidate aims to boost pre-existing immunity in pregnant women or older adults.[84] A phase 1 trial is being done in healthy women.[85] The needle-free intradermal delivery route via an epicutaneous patch is being explored for boosters and has shown similar antibody titers for pertussis as a commercial vaccine in a phase 1 trial[84] but might require a delivery enhancement procedure to optimise vaccine delivery. Overall, particle-based vaccines are still in early development but have the potential to elicit a powerful immune response for pregnant women and the elderly.

Nucleic acid

mRNA vaccines have shown safety and high efficacy against SARS-CoV-2 infection and were developed based on previous work for RSV.[86] Both mRNA COVID-19 vaccines express stabilised versions of SARS-CoV-2 pre-F spike protein patterned after the success of RSV pre-F as a vaccine antigen. The extensive work on RSV vaccine-associated enhanced respiratory disease was also important for the rapid development of COVID-19 vaccines and provided regulatory guidelines for vaccine safety. Because of the successful scale-up and establishment of a robust supply chain, mRNA will be a new vaccine modality available for other purposes including RSV vaccines. An mRNA vaccine (mRNA-1345) encodes stabilised RSV pre-F and uses the same lipid nanoparticle formulation as for the SARS-CoV-2 vaccine SpikeVax (Moderna) that is known to induce and boost antibody and T-cell responses, including CD8+ T cells, Th1 cells, and T follicular helper cells. The interim results of the phase 1 trial in younger and older adults showed favourable safety and potent boosting of neutralising activity.[87] A phase 2/3 trial (ConquerRSV) started on Nov 17, 2021, with 34 000 adults older than 60 years.[88] The company intends to combine mRNA-1345 with mRNA-1653 (an mRNA vaccine against two other pediatric viruses, hMPV and parainfluenza virus type 3 intended for use in the pediatric population). A phase 1 trial is ongoing (NCT04144348) in women of childbearing age and seropositive children.

Recombinant vectors

Recombinant vector vaccines use a modified replication-defective virus to induce humoral and cellular immunity by delivering genes for RSV antigens. Three such candidates are in clinical development for the pediatric and older adult population.

Firstly, MVA-BN-RSV uses a poxvirus vector, modified vaccinia Ankara virus, to express RSV surface antigens (F and G) and intracellcular proteins (M2 and N).[89] In a phase 1 trial cellular and humoral immune responses were similar in younger and older adults.[89] Results of a phase 2a human challenge study (n=61) showed 79% reduction in symptomatic RSV infection and a reduction in viral load.[90] The phase 2 trial[91] in older adults showed elevated antibody responses for 6 months which can be safely boosted at 12 months. After dose selection from the phase 2 trial, preparations for a phase 3 trial are ongoing.[92]

Ad26.RSV.pre-F is being developed for two different target populations: paediatric (phase 2 trial; NCT03303625 and NCT03606512) and elderly (phase 3 trial; NCT04908683). Ad26.RSV.pre-F vaccine candidate uses an adenoviral vector to express the RSV F protein in the pre-F conformation.[93] The vaccine candidate showed improved immunogenicity in comparison to the previous vaccine candidate with post-F RSV protein (Ad26.RSV.FA2). In neonatal mice, the vaccine candidate showed a biased reponse to a Th1-biased response cells.[94] A durable humoral and cellular immune response was shown for at least 2 years after immunisation in the first-in-human study in adults aged 60–81 years.[95] Proof-of-concept was obtained in the first RSV vaccine human challenge study.[96] In the primary efficacy results from the proof-of-concept CYPRESS study,[97] Ad26.RSV.pre-F showed 80% (95% CI 52 to 93) efficacy against RSV LRTI through the first RSV season in the older adult population. Ad26.RSV.pre-F was found to be safe and well-tolerated[98] and showed efficacy in adults aged 65 years or older with or without risk factors (68% [–27 to 95] *vs* 85% [50 to 97]).[99] Furthermore, there was no interference when an RSV vaccine was co-administered with seasonal influenza vaccine in older adults in a phase 2 trial,[100] and the vaccine candidate was shown to have an acceptable safety profile although showing increased reactogenicity compared to influenza vaccination. In 2019, this candidate was granted US Food and Drug Administration breakthrough therapy designation. Subsequently, the phase 3 EVERGREEN trial[101] was started on July 21, 2021 and will examine efficacy across two RSV seasons in 23 000 adults aged 60 years and above. For the paediatric vaccine candidate a phase 1/2b trial in seropositive infants aged 12–24 months showed Ad26.RSV.Pre-F was well-tolerated and elicited both humoral and cellular immune responses.[102] Of note, a SARS-CoV-2 adenovirus vector vaccine candidate uncovered new safety concerns with adenoviral vector vaccines, including vaccine-induced immune thrombotic thrombocytopenia which was observed for at least two of the COVID-19 adenoviral vector vaccines.[103]

MAbs

MAbs have been labelled as the magic bullet against infection because of their high pathogen specificity.[104] For RSV, increased knowledge of the structure and immunogenicity of the RSV fusion protein has resulted in next generation antibodies targeting highly neutralisation-sensitive epitopes located on the RSV pre-F protein. Furthermore, next generation RSV antibodies have been

engineered with Fc mutations to extended half-life and enable protection of all infants against lower respiratory tract disease for an entire RSV season.

The leading candidate is nirsevimab (formerly MEDI-8897), a human mAb targeting site Ø of the F protein with a YTE mutation in the Fc portion to allow for an extended half-life. In phase 2 trial[105] results (n=1453) nirsevimab showed 70% (95% CI 52 to 81) efficacy against medically-attended RSV LRTI and 78% (52 to 90) against RSV hospitalisation in preterm infants,[105] which is similar to the phase 3 trial interim results: 75% (50 to 87) against RSV lower tract respiratory infection and 62% (–9 to 87) against RSV hospitalisation among healthy late preterm and full-term infants (n=1490).[106] The safety profile of nirsevimab is similar to that of the current standard of care, monthly palivizumab, administered to infants with congenital heart or lung disease (n=310) and preterm infants between 29 and 35 weeks gestational age (n=615).[106] RSV monoclonal antibody resistant mutants were generated and were shown not to have an effect on viral replication and had a low natural frequency amongst circulating strains.[107] The most prominent advantages of nirsevimab in contrast to the approved palivizumab are that a single intramuscular injection protects infants for an entire season compared with monthly doses, and reduced costs (vaccine-like pricing expected) allowing for administration to all infants compared with only high-risk children.

Clesrovimab (MK-1654), an extended half-life mAb with the same YTE mutation as nirsevimab, targets site IV (although preferentially binding pre-F due to partial targeting of site V of the RSV F protein). This mAb is in phase 2b/3 trials (NCT04767373) and phase 3 trials (NCT04938830) in infants. This mAb has shown high potency against RSV clinical isolates in vitro and is equipotent against RSV subgroup A and B strains.[108] A human challenge trial[109] (n=80) showed reduced viral load after viral challenge and reduced RSV symptomatic infection rates. A meta-analysis[110] was done to assess the relationship between serum neutralising antibodies and clinical endpoints; the study estimated a single 75 mg dose would have more than 75% efficacy lasting 5 months in term infants. The company developing this agent has committed to helping navigate uncertainty and improving issues of access through ongoing research and innovation to help address the burden of potentially preventable childhood diseases (Andrew W Lee, Merck, personal communication).

Affordability remains a key consideration for mAb development as it is a potential barrier to global access. There are three different clinical development efforts underway to circumvent this problem: (1) an affordable extended half-life site Ø mAb, (2) local administration, and (3) a biosimilar. First, a phase 1 trial of RSM01,[111] a site Ø mAb, intended for LMICs has a target price of less than US$5 per dose.[112] Second, local needle-free administration of palivizumab, a market-approved site II mAb, via nose drops might significantly reduce costs by reducing the drug dose needed.[113] Results of a phase 1 and 2b trial will soon be published for intranasal palivizumab administration to prevent RSV infection. Finally, a biosimilar for palivizumab is being developed in a public-private partnership with the Utrecht Center for Affordable Biotherapeutics, Utrecht, the Netherlands, for which a human challenge trial was done in 2020 (n=56), but the results of this trial have not yet been made publicly available.[114]

Important considerations for the development of next-generation mAbs are affordability, which can be achieved by investing in higher efficiency production or developing biosimilars or potentially through local administration. Likewise, viral resistance needs to be monitored and might be prevented through administration of a cocktail of mAbs targeting multiple epitopes. Monthly administration of intranasal mAbs or a palivizumab biosimilar might have a programmatic limitation in most LMICs. Potentially, a combination of mAbs targeted to different epitopes might provide a solution to loss of efficacy due to viral resistance. However, practical barriers exist to this solution as the combination would have to consist of separately registered antibodies. Thus far, the epitopes for mAbs being developed are highly conserved with minimal naturally occurring antibody-resistant strains, which have shown similar or lower viral fitness when compared with non-resistant viral strains in vitro.

Discussion

In the last decade, the RSV vaccine landscape has had a major transition from empirical to rational vaccine design. In two previous reviews[33,38] we characterised the dynamics of the RSV vaccine landscape, which included multiple late-phase failures. These failures have laid the foundation for future success by guiding development of vaccines: supporting pre-F as a vaccine antigen, highlighting the importance of conducting vaccine trials over more than one RSV season, providing knowledge of a protective immune response, emphasising the importance of monitoring viral resistance to mAbs, and highlighting the value of controlled human infection model to decrease the risk of RSV vaccine development. The number of candidates in late-phase development is expanding: only one mAb and one maternal vaccine candidate were in phase 3 development as of 2015 and 2018, respectively. Development was halted for both after failure to meet the primary endpoints of the trials, but important lessons learned have been incorporated into current trials. A better understanding of RSV neutralising epitopes has resulted in rapid expansion to the nine vaccine candidates in phase 3 trials (figure 4).

RSV prevention appears to be on the horizon with market access expected for nirsevimab within the next 12 to 24 months as of July, 2022. This approval might be followed shortly by approval of a maternal vaccine and

a vaccine for older adults (subunit, vector-based, and nucleic vaccines in late phase trials). In this case a situation will emerge in which multiple RSV vaccine candidates are approved. If all the ongoing phase 3 trials generate positive results, relative efficacy and safety trial data, delivery strategies, and costs might determine vaccine uptake for different maternal and older adult candidates. Despite the approval of next-generation antibodies, palivizumab might remain on the market until there is global market access of extended half-life mAbs and because mAb supply might not meet global demand. RSV has shown negligible viral resistance against palivizumab after 20 years on the market.[115] For this reason, despite multidose schedule and costs, palivizumab might act as a back-up prophylaxis strategy while waiting for global real-time viral resistance data upon mAb implementation in HICs.

With both infant immunoprophylaxis and maternal vaccines on the market, it is important to consider how these two prevention strategies aiming to protect young infants will coexist. Maternal vaccines and infant immunoprophylaxis might have a complementary role in the prevention of severe RSV infection during infancy. There is a clear use-case for mAbs even if a maternal vaccine is approved: mAbs are expected to protect premature and full term infants, might be able to provide a longer duration of protection than maternal vaccines,[116,117] can be applied flexibly where RSV seasonality is variable, and can be implemented in cases where maternal immunisation did not occur. There is also a use case for a maternal vaccine in coexistence with an approved mAb. Active maternal vaccination provides broad protection; however, it is still unclear if maternal vaccination persists until a subsequent pregnancy and whether booster vaccination provides even stronger protection. Maternal vaccination might also provide an alternative for parents preferring not to vaccinate their babies. Finally, maternal vaccines serve as a backup in the case of viral resistance to mAbs or prohibitively high costs related to the production of biologicals that limit administration only to select populations.

In LMICs, there is a use case for mAbs to protect young infants: if there is a substantial time gap until a vaccine becomes available, if their mothers are not immunised, and if there is insufficient time for an immune response after vaccination (ie, premature infants).[112] LMICs might even prefer mAbs over maternal immunisation due to potential higher coverage and ease of implementation into the Expanded Programme on Immunisation. However, there are several challenges to LMIC implementation, including (1) year-round RSV circulation (mAb administration at birth might be more cost-effective than seasonal), (2) scalability dependent upon use of multi-dose vials and stability at ambient temperature, (3) scarcity of data on both RSV burden and health-care use in LMICs to have a case for implementation, (4) trial design in the case nirsevimab reaches the market (requiring a large sample size), and (5) potential limitations of surveillance of viral resistance in LMICs (though potentially enhanced since COVID-19). Thus, mAbs could have a high impact in LMICs, but implementation challenges remain.

Panel: **Future RSV vaccine and monoclonal antibodies landscape research priorities**

- Generating knowledge of RSV awareness
- Understanding cost-effectiveness of RSV prevention in different parts of the world
- Defining a correlate of protection for RSV
- Defining efficacy of RSV prevention in low-income to middle-income countries
- RSV genetic surveillance

Passively acquired antibodies from maternal immunisation or mAb immunoprophylaxis will wane overtime, at which point paediatric vaccines for active immunisation might serve an important role. Live-attenuated or replicating vector vaccines do not prime for enhanced respiratory disease; delivered via the intranasal route, they are immunogenic in presence of maternal antibodies. Thus, these active immunisation approaches might be safe and effective for the older paediatric age group and will be complementary to infant immunoprophylaxis and maternal vaccines.

Although the approval of multiple RSV vaccines is within reach, several obstacles to worldwide access remain. Globally representative trials are needed and vaccine trials need to be done in countries with the highest disease burden as efficacy can differ between LMICs and HICs as observed for vaccines against RSV[39] and other pathogens.[118] Access in LMICs might be delayed due to a scarcity of trial data in populations with a high incidence of HIV and malaria and regulatory drug lag between time of regulatory submission and approval in sub-Saharan Africa.[119] GAVI's (the Vaccine Alliance's) vaccine investment strategy includes RSV and will be important in making RSV prevention available in countries eligible for GAVI support. Differential pricing might be an important consideration for countries not eligible for GAVI support. Other challenges to global access include measuring protection in the case maternal vaccine boosters are indicated. A correlate of protection for RSV is absent as well as a simple tool to measure protection after RSV vaccination. It will be important to assess the sustainability of RSV prevention via ongoing genetic surveillance. The development of viral resistance is most relevant for infant immunoprophylaxis with mAbs. A cocktail of mAbs targeting different epitopes might help prevent the emergence of viral resistance. However, there are currently no ongoing clinical trials with multiple mAbs which might hamper approval of a drug cocktail by regulatory bodies such as the approved mAb cocktail to prevent SARS-CoV-2. Finally, awareness

of RSV is low amongst patients, policy makers and health-care providers and further cost-effectiveness studies of products are needed.[7] These knowledge gaps related to global vaccine implementation are important future research priorities (panel).

Overall, we are at an exciting phase of vaccine and mAb development in which RSV prevention is within reach. It is likely that multiple immunisation strategies with complementary value, unique advantages and use-case scenarios will shape the RSV prevention landscape. To guarantee worldwide access, urgent steps are required to surmount challenges of measuring protection, monitoring viral resistance, and prioritising LMIC access and affordability.

Contributors

LB and NIM contributed to the review design and plan. NIM, JT, and YL contributed to the data collection, extraction, and quality assessment. JT created the figures for the manuscript with Biorender.com. All authors contributed to the writing of the manuscript. The manuscript was written in collaboration with the ReSViNET Foundation.

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Part 5

AWARENESS AND ETHICS OF POST-TRIAL ACCESS

11

PROMOTING GLOBAL AWARENESS OF RSV MORTALITY: 3 MILLION VIEWS WITHIN 24 HOURS

www.rsvgold.com/awareness, 2019

Many an object is not seen, though it falls within the range of our visual ray, because it does not come within the range of our intellectual ray, i.e. we are not looking for it. So, in the largest sense, we find only the world we look for.
Henry David Thoreau (1817-1862)

Promoting Global Awareness of RSV Mortality: 3 million views within 24 hours
European Society for Pediatric Infectious Diseases (ESPID) 2020 Abstract 1803

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Title of Case

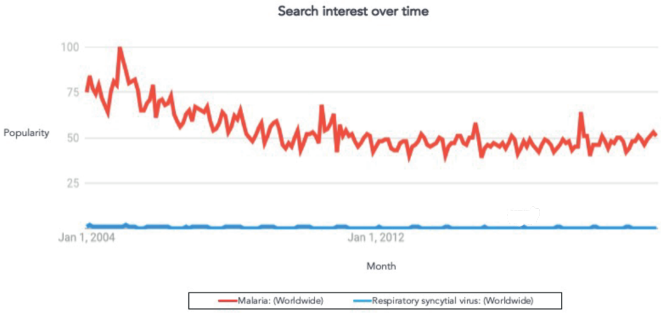

Figure 1. Worldwide search interested in malaria and RSV

RSV is the second cause of death in young children after malaria and 700 children die every day from RSV. More than 99% of these deaths occur in lower-income countries. However, in contrast to malaria these numbers are not enough for general RSV awareness (Figure 1). Several vaccines are in clinical development for RSV, but lack of awareness may hamper future vaccine uptake.

Case Presentation Summary

The aim of the mortality awareness campaign was to increase awareness on the global burden of RSV by having a short film viewed more than 100.000 times within 24 hours.Together with the independent RSV patient advisory board, we produced a video to put a face to the global health threat of RSV by sharing the story of a mother who lost her child to fatal RSV infection. Ethical approval was obtained, the consent process was reviewed by the legal department and a participant was recruited via the Child Health and Mortality Prevention Surveillance study (CHAMPS). The film was produced by a communication agency, Beyond Borders Media, with experience in health development aid. A communication plan was developed to identify the target population and create an action plan with content planning for the video launch.

Learning Points/ Discussion

Social media analysis software registered 3,229,637 views and 353 messages within 24 hours. The video was shared by public health organizations such as the Bill & Melinda Gates Foundation as well as national radio, newspapers and television. A local viewing in Soweto was organized to reach approximately 450 people of the local population.

The RSV mortality awareness campaign exceeded its goal with more than 3 million views within 24 hours. Sharing a personal story can be used to increase vaccine impact for diseases where awareness is lacking.

12

AN ETHICS FRAMEWORK AND PRACTICAL GUIDANCE FOR POST-TRIAL ACCESS TO AN RSV MATERNAL VACCINE

The Lancet Respiratory Medicine, 2019

And those who were seen dancing were thought to be insane by those who could not hear the music.
Friedrich Nietzsche (1844-1900)

An ethics framework and practical guidance for post-trial access to an RSV maternal vaccine

iStockphoto/jovanmandic

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Respiratory syncytial virus (RSV) is the second most frequent cause of death after malaria in the post-neonatal period,[1] yet there is no vaccine or treatment besides supportive care.[2] Prevention of RSV will be an important step in achieving Sustainable Development Goal target 3.2: to end preventable deaths of newborns and children younger than 5 years of age. Maternal vaccination is a powerful tool to prevent infection in the first months of life; neonatal tetanus mortality has been successfully reduced by 92% through maternal tetanus toxoid vaccination.[3] Of 21 RSV vaccine candidates, two are maternal vaccine candidates that were in late-phase clinical trials at time of writing.[4] A historic milestone was reached this year when Novavax released the results from its phase 3 nanoparticle-based maternal vaccination trial. At the time of writing of this Comment, the Novavax trial results were not yet known, but they have since been published. The trial was done in 4636 pregnant women in 11 countries around the world. The primary endpoint was just missed with 39% (97·5% CI −1 to 64) vaccine efficacy against RSV lower respiratory tract infection with hypoxia or tachypnoea in the first 90 days of life. Despite the trial not meeting its prespecified efficacy endpoint, 39% vaccine efficacy is not trivial. Moreover, the trial showed a higher efficacy against more severe infection, with 60% efficacy against severe hypoxaemia due to RSV lower respiratory tract infection. The fate of this vaccine is uncertain at the time of publication.

If any RSV maternal vaccine shows efficacy, it will also create a need to ensure post-trial access. We therefore aim to define the populations entitled to post-trial access to such a vaccine, identify the stakeholders responsible for ensuring this access, and issue practical guidance on the mechanisms needed to establish access (table).

Since 2000, major international ethics guidelines, including the guidelines of the Council for International

Organizations of Medical Sciences and the Declarations of Helsinki, have added clauses requiring the provision of post-trial access to be included as part of the trial design to ensure continued access to a proven beneficial intervention. The first WHO meeting about RSV vaccine development concluded that post-trial availability of the vaccine in low-income or lower-middle-income countries (LMICs) should be a requirement before doing RSV vaccine trials.

Beneficence and justice are the key drivers of a commitment to post-trial access for an RSV maternal vaccine. Beneficence—balancing benefits against risks—can be used as justification for providing continued access to an intervention after a study population bears the risks of research. The risks are greater for maternal vaccination than in trials of RSV treatment because two lives are concerned (mother and infant) and both are previously healthy. Justice concerns the equitable distribution of benefits and burdens in society. In the context of maternal vaccination, considerations for justice include distribution of an intervention proportional to the RSV disease burden and a just distribution of the responsibility to provide post-trial access among stakeholders.

A global commitment to post-trial access for an RSV maternal vaccine has been expressed in multiple WHO expert meetings with a focus on LMICs, as well as investment from international stakeholders. The development pathway of the lead maternal vaccine candidate, RSV F, was facilitated by investment from PATH and the Bill & Melinda Gates Foundation;[5] PATH invested a total of US$6·9 million for the phase 2 trial and phase 3 trial preparations, whereas the Bill & Melinda Gates Foundation invested $89 million for the phase 3 trial, regulatory licensing efforts, and WHO prequalification. Subsequently, Novavax has publicly agreed to make its vaccine affordable and accessible in developing countries.[5]

Post-trial access hinges upon the efficacy of an intervention. The pivotal Prepare trial[6] was done in 11 countries of which three were lower-middle income (Bangladesh, the Philippines, and India) and none were low income. Sufficient efficacy data are needed from LMICs because they can differ from higher-income countries. In post-licensure trials of rotavirus vaccines, efficacy was more than 90% in high-income and upper-middle-income settings, and 50–64% in high-mortality settings in LMICs.[7] Due to impaired RSV maternal antibody transfer or reduced maternal immune response due to hypergammaglobulinaemia,[8] malaria,[9] and HIV,[10] there could be reason to expect differential efficacy in LMICs. If the generalisability of maternal vaccine efficacy to LMICs remains uncertain, there will be a dilemma between pursuing further scientific evaluation of the vaccine and ensuring quick access to a potentially life-saving intervention. Phase 4 trials will be an urgent next step to evaluate efficacy in low-resource, high-mortality settings.

Regarding post-trial access, there are three populations to consider (table). First, the trial participants who have carried the risks of trial participation for whom such access might mean a booster vaccination or revaccination for subsequent pregnancies. The second population is the larger community from which the trial participants are selected. If a trial for an RSV maternal vaccine has positive results, vaccine introduction in the communities and countries from which the participants were selected should be prioritised for vaccine introduction. Finally, the third population is the larger global community. Because more than 99% of RSV-related mortality occurs in LMICs, they are the most important population for post-trial access in terms of distributive justice. In LMIC populations, there is high potential impact of a vaccine, as well as expected difficulties in gaining access to it.

Post-trial access is the shared responsibility of different stakeholders and the justification for such access can help to define the responsible parties for

	Stakeholders responsible	Practical guidance
Trial participants	Researchers and sponsors	Crossover extension studies; open-label extension studies (protocol addendum or new protocol); booster vaccination or revaccination for subsequent pregnancies
Community participants are selected from	Governments, national regulatory agencies, community representatives	Discussions between vaccine manufacturers, UN procurement agencies, national governments, and regulatory agencies
Community participants are selected from	Researchers and sponsors	Open-label extension studies (protocol addendum or new protocol)
Global community	Public health stakeholders (WHO, Gavi)	Inclusion of vaccine in Gavi VIS; WHO prequalification
Global community	Governments, national regulatory agencies, and community representatives	Increase RSV awareness and diagnostic capacity in LMICs to increase acceptability and uptake
Global community	Researchers and sponsors	Tiered pricing scheme to ensure affordability in LMICs

RSV=respiratory syncytial virus. VIS=vaccine investment strategy. LMICs=low-income or lower-middle-income countries.

Table: **Practical guidance for post-trial access for trial participants, the communities they are selected from, and the global community for an RSV maternal vaccine, by stakeholders responsible**

different populations.[11] Based on reciprocity, a fair share of the benefits from research should be provided to trial participants and ensured by those who facilitated the research—namely, the researchers and sponsors. Based on the principle of justice, the responsibility for post-trial access includes stakeholders responsible for national vaccine introduction (such as governments and national regulatory agencies). Finally, based on the principle of distributive justice, the benefits of research must also be aligned with the global burden of disease and responsible stakeholders here include international public health organisations such as WHO and Gavi, the Vaccine Alliance.

Practical solutions are needed to guarantee post-trial access in a timely manner. HIV prevention trials provide a useful example of post-trial access mechanisms for a proven beneficial life-saving intervention for an infectious disease with the largest burden in LMICs. In 28 phase 2b/3 HIV prevention trials, five interventions were shown to be effective.The mechanisms of post-trial access included immediate crossover from placebo to intervention arm,[12] a plan to transition trial participants from trial-supplied antiretroviral therapy to local antiretroviral therapy,[12] transition to a rollover study for trial participants,[13] extension studies through a protocol amendment allowing randomisation of participants in the placebo group to the intervention arm,[13] and open-label extension studies (with no delay as trial addendum or substantial delay as a new protocol). For an RSV maternal vaccine, open-label extension trials could provide a temporary solution until national access can be guaranteed in countries that participated in the trial but are not guaranteed immediate access.

In conclusion, we advocate for a framework to ensure post-trial access for an RSV maternal vaccine in which low-resource settings are prioritised and the responsibility is shared among international stakeholders through a sustained global commitment and, finally, through active engagement of local stakeholders in low-resource settings.

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LJB has regular interaction with pharmaceutical and other industrial partners and has not received personal fees nor personal benefits; he is also the founding chairman of the ReSViNET Foundation. All remaining authors declare no competing interests.

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POST-TRIAL ACCESS IN MATERNAL VACCINE TRIALS

American Journal of Perinatology, 2019

Only when it is dark enough you can see the stars.
Martin Luther King, Jr. (1929-1968)

Post-trial Access in Maternal Vaccine Trials

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Abstract

Provisions for post-trial access (PTA) of the experimental intervention are required before the start of a clinical trial. Although there has been ample attention for PTA in the context of preventive vaccine research, discussions on PTA barely include maternal vaccine trials in which mother–infant pairs are exposed to the intervention. In maternal vaccination trials, specific PTA arrangements are required because pregnancy is transient and PTA may apply to the next pregnancy or the child. In this article, we examine the application and adherence to PTA in the context of maternal vaccine trials. We focused on differences between publications before and after 2000 when international ethical guidance documents formalized PTA requirements. Randomized maternal vaccine trials were included after a systematic search for clinical trials in phases II and III with a maternal vaccine as intervention. We used PTA as defined at the time of publication in the World Medical Association's Declaration of Helsinki (DoH) or in the ethical guidelines of the Council for International Organizations of Medical Sciences (CIOMS). In addition, we investigated whether PTA was included in the trial design. Therefore, we contacted principal investigators (PI's) of the publications found in the review to fill out a questionnaire regarding provisions for PTA. Before and after 2000, no trial articles examined in the systematic review described PTA in their trial publication (0/7, 0% and 0/17, 0%, respectively). In addition, more than half of the PI's of the trials found were not familiar with PTA recommendations in international ethical guidelines. Most cases of PTA included making knowledge available by publishing the results of the trial. The revision of the DoH in 2002 and the CIOMS ethical guidelines in 2002 has not resulted in increased PTA provisions for maternal vaccination trials. PTA is a shared responsibility of various stakeholders including sponsors, Institutional Review Boards, regulators, political entities, and researchers. Inclusion of PTA provisions in trial protocols and publications on maternal vaccination trials is essential to increase transparency on the form and content of these provisions.

Keywords
- post-trial access
- maternal vaccination
- vaccine trials
- research
- ethical

Maternal vaccination is an intervention to protect newborns from life-threatening infectious disease in the first month of life. Maternal immunization can protect newborns via an immunoglobulin G (IgG) antibody response to an (in)activated micro-organism. IgG antibodies are transported actively across the placenta to the fetus and thereby provide passive immunity in the newborn which lasts for the first 6 months of life. After this period, the immune system of the

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child can generate active antibody responses via infant vaccination.[1] Maternal vaccines for several pathogens are already approved and recommended for pregnant women in various countries: influenza, tetanus, and pertussis, while meningo-/pneumococcus, group B *Streptococcus*, and *Haemophilus influenzae* type B are still in clinical development and not yet recommended. More vaccines are in the pipeline: cytomegalovirus, herpes simplex virus, and respiratory syncytial virus (RSV).[2,3] Despite maternal vaccination as a rapidly growing field, there is still hesitancy to vaccinate pregnant women.[4] However, various RSV trials are now moving forward from early to late phase clinical trials.[5] This development requires reflection on post-trial access (PTA) provisions.

International ethical guidelines for research involving human subjects support the value of PTA requirement for clinical trials. In 2000, PTA was added to the Declaration of Helsinki (DoH) paragraph 20. The DoH stated that "At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study." Various other international ethical frameworks, including the National Bioethics Advisory Commission, the Nuffield Council on Bioethics, and the Council for International Organizations of Medical Sciences (CIOMS) have adopted PTA requirements in their guidance documents (►**Fig. 1**).[6–9] Despite agreement about the importance of the requirement, there is extensive discussion about the underlying rationales for PTA, about its content, the length, and to whom it applies. In general, according to small interpretations fulfilling the PTA requirement implies making provisions for continued access to interventions identified as beneficial, but broader interpretations also include provisions for transitioning participants who continue to need care or preventive measures to appropriate health services when the study has ended. The responsibility to fulfill PTA requirements is typically shared among several stakeholders including sponsors, regulators, political entities, and researchers. The shared responsibility makes providing PTA a complex issue. Investigators of a study cannot provide PTA alone and are dependent on the government, pharmaceuticals, and sponsors.[10]

Although there is ample literature on PTA in the context of human immunodeficiency virus (HIV) prevention research, scholars have barely reported on PTA for maternal vaccination studies. Poor attention is remarkable since it is reasonable to assume that in the case of maternal vaccination, PTA could be conceived as access to the vaccine in future pregnancies not only for women receiving placebo.

To understand why PTA requirements receive limited attention in discussions about maternal vaccination studies, we performed an in-depth study whether and how PTA requirements as formulated in the CIOMS guidelines and the DoH are included in publications on late phase maternal vaccine trials and contacted principle investigators of these publications about provisions made. We compared PTA provisions made before and after 2000, when the guidelines were not in place yet. Furthermore, this study identifies best practices for implementation of PTA provisions.

Materials and Methods

Systematic Review

Randomized maternal vaccine trials were included after a systematic search (►**Supplementary Appendix A**, available in the online version) in PubMed for clinical trials in phases II and III with a maternal vaccine or prophylaxis as intervention. All articles were screened for eligibility by two people independently, using Rayyan.[11] The World Health Organization (WHO) clinical trial registry and ClinicalTrials.gov were searched for phase II/III maternal vaccine trials, using the same in- and exclusion criteria as for PubMed. Relevant completed or ongoing trials were included, and withdrawn trials were excluded. Trials with no article available were also excluded for the systematic review (►**Table 1**). Trials before 2000 and after 2000 were compared since PTA was first included in the ethical guidelines in 2000.

Guideline	Section	Obligation
CIOMS 2016	Guideline 6: Caring for participants' health needs	Addressing participants' health needs requires at least that researchers and sponsors make plans for: providing continued access to study interventions that have demonstrated significant benefit; and consulting with other relevant stakeholders, if any, to determine everyone's responsibilities and the conditions under which participants will receive continued access to a study intervention, such as an investigational drug, that has demonstrated significant benefit in the study.'
NBAC 2001	Section 4.1	'..make reasonable, good faith efforts before the initiation of a trial to secure, at its conclusion, continued access for all participants to needed experimental interventions that have been proven effective for the participants. ....research protocols should typically describe the duration, extent, and financing of such continued access. When no arrangements have been negotiated, the researcher should justify to the ethics review committee why this is the case.'
	Section 4.2	'Research proposals submitted to ethics review committees should include an explanation of how new interventions that are proven to be effective from the research will become available to some or all of the host country population beyond the research participants themselves...'
	Section 4.3	'Whenever possible, preceding the start of research, agreements should be negotiated by the relevant parties to make the effective intervention or other research benefits available to the host country after the study is completed.'
Nuffield Council on Bioethics Group report 2002	Section 9.48	'.. we recommend that the following issues are clearly considered by researchers, sponsors, national healthcare authorities, international agencies and research ethics committees as part of any research protocol before research relating to healthcare involving the testing of new interventions is undertaken:....the possibility of providing participants with the intervention shown to be best (if they are still able to benefit from it), for an agreed period of time and the possibility of introducing and maintaining the availability to the wider community of treatment shown to be successful.'
DoH 2013	Guideline 22	'...In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.'
	Guideline 34	In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post- trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Fig. 1 Overview posttrial access in various ethical guidelines.[8–11]

Table 1 In- and exclusion criteria systematic review

Inclusion	Exclusion
Pregnant women	Women of childbearing age/ nonpregnant women
	Animals
Passive or active immunization	No vaccination
Maternal vaccine trials	HPV 16/18 trials (because goal of vaccination is not child protection)
	HIV PMTCT trials
Phases I/II, II, and III trials	Phases I and IV trials
Positive and negative outcomes	
Prospective randomized controlled trials	Editorial
Secondary analysis (NB: if duplicate, only primary article was included)	Review
	No PDF available
	No author information provided
	Language barrier
	Duplicates or secondary analysis while primary article was already included

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; PMTCT, prevention of mother-to-child transmission.

Definitions

A vaccine was defined according to the WHO's definition: an intervention that augments immunity to a particular disease, which contains an agent that resembles a disease-causing microorganism.[12] Human intravenous immunoglobulin (HIVIG) and intravenous immunoglobulin (IVIG) trials were included as relevant interventions. HIV prevention of mother-to-child transmission trials, HBV tenofovir trials, and antibiotic prophylaxis were not included since they do not enhance immunity or contain a part of a microbe, but only prevent mother-to-child transmission by reducing the viral load. Full-text articles were screened for description of PTA as defined at the time of publication in the DoH or CIOMS (►**Fig. 1**).

Data Collection

The principal investigator (PI) of each trial was contacted and asked to fill out a short questionnaire regarding provisions for PTA in the trial design. Contact with the PI was still attempted for trials that were excluded from the systematic review if there was no article available. All ongoing and completed trials from the WHO clinical trial registry and ClinicalTrials.gov were included. PI's were contacted first by telephone, then e-mail for a maximum of three times of follow-up. They were also asked to inform us if they were not willing to participate. The questionnaire was shared using Qualtrics software, version 2017. Where questionnaire data are factual, facts were verified against other sources such as trial protocols. The methods were modeled after the methodology of Haire and Jordens, *Developing World Bioethics*, 2015 in which PI's of phase IIB/III HIV efficacy trials were contacted in an empirical study of PTA.[12]

Results

Systematic Review

Twenty-four maternal vaccine trials were identified for this systematic review (►**Fig. 2**, ►**Table 2**). Before and after 2000, no trial articles examined in this systematic review described PTA in their trial report (0/7, 0% and 0/17, 0%, respectively); 6/17 (35.3%) trials mentioned that they were conducted in accordance with the DoH in their trial report but did not specify PTA provisions.

Questionnaires

Thirty trials were identified as the PI was contacted to collect data on PTA (►**Tables 2** and **3**). Thirty trials were eligible for the qualitative analyses. Out of 30 PI's, 17 responded to the questionnaire. One PI was not willing to participate, and 12 investigators did not respond after follow-up. Eighty-two per cent (14/17) of PI's from trials conducted after 2000 described provisions regarding PTA.

Awareness

The majority (59%, 10/17) of the PI's for maternal vaccine trials were not aware of post-trial recommendations in international ethical guidelines. In several cases, the PI was not aware of PTA, but the PI indicated that he or she had made provisions for PTA. Half of the PI's who were aware of posttrial provisions still did not describe them for their trial.

Best Practice

From the PTA provisions that have been made by investigators in phase II/III maternal vaccine trials, most of them included making knowledge available for the population and transition to care when the research is concluded (79% [11/14] and 64% [9/14]). Researchers who described PTA provisions shared their protocol. Some only described making knowledge available through publication of the article as PTA provision in their protocol. Researchers indicated that the best way to incorporate obligation of PTA in the future would be to state intentions to local Institutional Review Board and Research Ethics Committee. Several PI's indicated incorporating obligations in trial protocols and informed consent to be a best practice to conform to PTA obligations.

Challenges

Researchers reported different reasons to not address PTA. One reason was that PTA was felt to be the responsibility of the local government rather than that of the researcher. Other challenges included the lack of proven benefit, awaiting WHO recommendation or national approval, a delay caused by lack of funding, consulting with other relevant stakeholders, and determining the responsibilities of different stakeholders. Finally, the PI's indicated that there was no

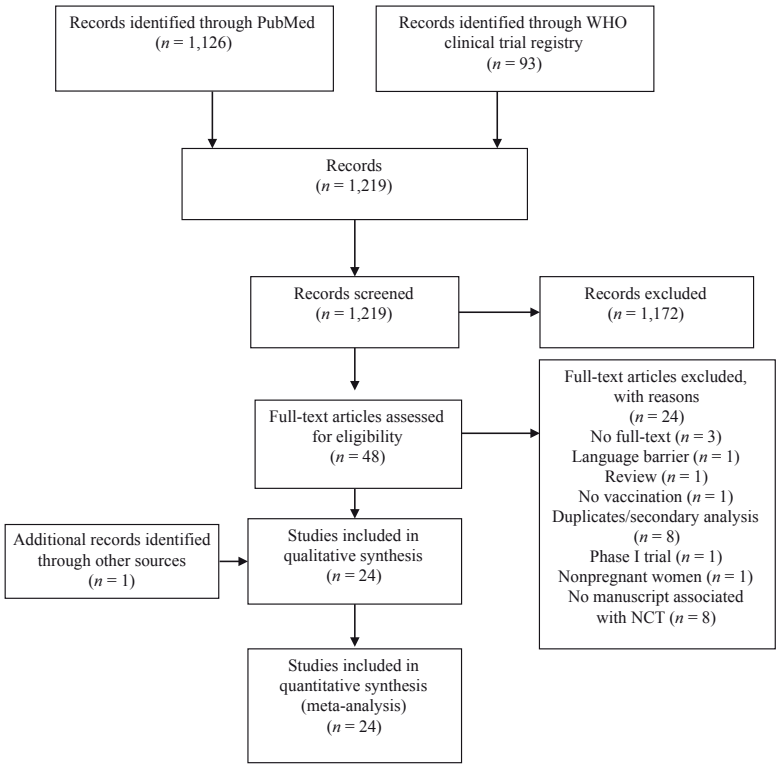

Fig. 2 Flow chart systematic review maternal vaccine trials.[13]

practical guidance available, or at least none that the investigators were aware of.

Discussion

No trial article, before and after 2000, examined in this systematic review described specifically PTA in their publication. However, 82% of the trials after 2000 described provisions regarding PTA elsewhere, for example, in trial protocols. Most of them included making knowledge available for the population. The percentage of 82% may be relatively high but should be critically examined. Several researchers published the article and did not address other aspects of PTA that are important for the community, such as providing continued access for study participants and making the vaccine available for the population.

The majority of the PI's was not aware of the concept of PTA. Remarkably, there were several cases in which the PI was not aware of PTA, but still indicated that he or she had made provisions for PTA. Lack of awareness of the concept PTA despite inclusion of provisions may indicate that provisions were included in the research without knowledge of the underlying concept of PTA such as publication of results without awareness of PTA obligations. Half of the PI's who were aware of posttrial provisions still did not describe them for their trial. This finding demonstrates a gap in implementation of PTA guidelines and not only awareness.

Best practices and obstacles in the process of PTA were identified. According to researchers, the best way to incorporate PTA obligations for trial planning in the future would be including PTA provisions in the protocol submitted to the ethical committees. Obstacles to including PTA in the trial planning were shared responsibilities, lack of funding, and awaiting proven benefit and recommendation. Lack of practical guidance available for PTA provisions in prevention trials remains an important obstacle and the creation of such guidance may also enhance awareness.

This study provides the first data on whether researchers implement provisions in the planning of published maternal vaccine trials. Thorough methodology was used including a systematic search with an extensive search term and careful examination of trials by two independent researchers. Furthermore, PI's of the trial were contacted to verify whether PTA provisions were included in the trial planning process. Where possible, facts have been verified against other sources, such as trial protocols. A limitation of this

Table 2 Maternal vaccine trials overview systematic review

	Pathogen	Article	Year	Country	Study size
Before 2000	Pneumococcal	Quiambao et al[14]	1994–1995	Philippines	160
		Munoz et al[15]	1995–1996	United States	60
	Meningococcal	Shahid et al[16]	1995–1998	Bangladesh	157
	Hib	Mulholland et al[17]	1993–1995	The Gambia	451
	Tetanus	Newell et al[18]	1961–1966	Colombia	1,618
	Varicella zoster	Koren et al[19]	1999–2000	United States	60
	RSV	Munoz et al[20]	1999–2002	United States	35
After 2000[b]	Influenza	Jackson et al[21]	2009	United States	120
		Tielsch et al[22]	2010–2018	Nepal	3,000[a]
		Omer et al[23]	2011–2013	Nepal	3,700
			2011–2013	Mali	4,193
			2012	South Africa	2,108
		Tsatsaris et al[24]	2009	France	107
		Abzug et al[25]	2009	United States	127
		Madhi et al[26]	2011–2012	South Africa	2,310
		Zaman et al[27]	2004–2005	Bangladesh	340
	Tetanus	Salama et al[28]	2002–2003	Egypt	131
	GBS	Donders et al[29]	2011–2013	Belgium Canada	86
		Madhi et al[30]	2010–2011	South Africa	417
		Heyderman et al[31]	2011–2012	Malawi South Africa	270
	Pneumococcal	Binks et al[32]	2006–2011	Australia	227
		Daly et al[33]	2000–2003	United States	153
		Lopes et al[34]	2005–2006	Brazil	139
	Tdap	Hoang et al[35]	2012–2014	Vietnam	103
		Villarreal Perez et al[36]	2011–2014	Mexico	204
		Munoz et al[37]	2009–2012	United States	80

Abbreviations: GBS, group B *Streptococcus*; Hib, *Haemophilus influenzae* type b; RSV, respiratory syncytial virus; Tdap, tetanus, diphtheria, pertussis.
[a]Ongoing trial.
[b]All trials after 2000 were included for contact with PI's.

Table 3 Additional trials contact PI's

Pathogen	NCT	Year	Country	Study size	Phase
Influenza	NCT00992719[38]	2009–2011	United States	84	II
	NCT01173211[39]	2010–2011	United States	183	II
	NCT00905125[40]	2009–2010	United States	102	II
	NCT01577316[41]	2012–2013	Mexico	240	II/III
	NCT01527825[42]	2012–2014	South Africa	800	III
GBS	NCT02046148[43]	2014–2016	United States	75	II
Pneumococcal	NCT02628886[44]	2016–2019[a]	Gambia	600	III
	NCT02717494[45]	2016–2019[a]	Brazil	345	II
RSV	NCT02624947[46]	2015–2020[a]	United States	8,618	III
	NCT02247726[47]	2014–2016	United States	50	II
Pertussis	NCT00553228[48]	2007–2016	Canada	440	II/III
Tdap	NCT02301702[49]	2016–2018[a]	Guatemala	376	II
HIV	NCT00000751[50]	2001–2007	United States	1,600	III

Abbreviations: GBS, group B *Streptococcus*; HIV, human immunodeficiency virus; PI, principal investigator; RSV, respiratory syncytial virus; Tdap, tetanus, diphtheria, pertussis.
[a]Ongoing trial.

study is the small group of maternal vaccine trials. Unfortunately, 12/30 investigators did not respond to our questionnaire. There may be selection bias. PI's who were aware of PTA and made provisions for PTA may have been more likely to respond to our questionnaire than PI's who did not make them. PI's with PTA provisions in place could be more likely to respond to our questionnaire, and therefore, the proportion of PTA provisions may be an overestimation of the actual provisions implemented.

In conclusion, the publication of international ethical guidelines in 2000 has not resulted in increased publication of ethical provisions in maternal vaccine trial literature. PTA provisions were described in trial protocols, but often the only PTA provision described was publication of the article to make knowledge available instead of providing continued access to interventions that have been proven significant benefit. Future studies should include PTA in their trial protocols, which will increase transparency on the form and content of these provisions. In theory, it can be stated that trials adhere to ethical guidelines and have PTA provisions in place, but in reality, studies do not incorporate all important aspects of PTA provisions into trial planning.

Conflict of Interest
None declared.

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14

RESPIRATORY SYNCYTIAL VIRUS TRIALS AND BEYOND

The Lancet Infectious Diseases, 2015

The least I can do is speak out for those who cannot speak for themselves.
Jane Goodall (1934)

Respiratory syncytial virus trials and beyond

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See Articles page 1398

Katherine O'Brien and colleagues[1] lucidly present the results of a phase 3 anti-respiratory syncytial virus (RSV) monoclonal antibody trial in healthy term infants for the prevention of medically-attended RSV acute lower respiratory tract infection. The study is unique because efficacy and safety of RSV immunoprophylaxis were tested in healthy term infants, who constitute 79% of all children experiencing severe RSV disease.[2] The study was meticulously done in 2127 Native American infants, known to have an exceptionally high risk of severe RSV infection.[3]

Motavizumab reduced the incidence of hospital admission for RSV infection from 11·3% in the control group to 1·5% in the treatment group. Additionally, a similar decrease in absolute incidence was recorded in outpatient RSV infection. No significant differences in adverse events occurred between motavizumab and placebo. The researchers were equally thorough in their follow-up, which included the measurement of medically attended wheezing until children reached 3 years of age. Motavizumab did not decrease the development of medically attended wheezing in children aged between 1 and 3 years. This finding is in apparent contrast with the results from previous palivizumab trials,[4-6] which might be explained by differences in patient populations, medication use, or definitions of wheezing.

The study by O'Brien and colleagues has shown a significant beneficial effect of motavizumab to prevent severe RSV infection in a high-risk population for whom there is no alternative intervention at present.[7] This raises the question of why it has not been registered for this patient population and why further development was interrupted.

After US Food and Drug Administration (FDA) approval of palivizumab in 1998, a second-generation antibody, motavizumab, has been developed with 70-fold higher binding affinity to the RSV F protein than palivizumab. Motavizumab showed higher neutralising capacity in vitro than palivizumab,[8] as well as greater reduction of viral load in the cotton rat model.[9] Further clinical development finally led to three phase 3 immunoprophylaxis trials. In addition to the placebo-controlled trial by O'Brien, two trials[10,11] compared efficacy and safety between palivizumab and motavizumab, one in preterm infants and one in infants with congenital heart disease. In these two head-to-head comparative trials, motavizumab was not better than palivizumab and was associated with a 2% increased risk of mild adverse skin reactions compared with palivizumab.

In June 2010, the FDA held a meeting to decide on the licensure for motavizumab. Based on data of the three trials, the FDA did not grant licensure with a 14–3 vote. The FDA concluded that motavizumab was not proven to be more efficacious than palivizumab in preterm infants and infants with congenital heart disease and had safety concerns about a higher rate of non-fatal hypersensitivity reactions. In December 2010, the manufacturer decided to discontinue development for motavizumab: this resulted in a loss of US$445 million[12] and the potential to distribute a new and more potent preventive intervention.[7]

The decision to discontinue development of motavizumab for RSV prevention raises the ethical question of whether post-trial obligations were sufficiently met. Native American parents participated in this trial under the reasonable assumption that if the drug were effective it would become available to Native American babies. Despite the 9·8% reduction in RSV admissions to hospital using motavizumab, Native American babies were left without RSV immunoprophylaxis because palivizumab is not an option for term children, resulting in ongoing severe RSV infections in this susceptible population.

What can we learn from the story of this drug candidate for the 21 RSV therapeutics that are currently in clinical development?[7] For future studies of RSV therapeutics it is clear that post-trial obligations should be considered and agreed upon during early phases of trial design. According to the 2013 declaration of Helsinki[13] and the new version of WHO guidelines of the Council of International Organisations of Medical Sciences (CIOMS),[14] post-trial obligations include providing access to successful drugs to the participating populations after the end of a study. Sponsors, researchers, and relevant public health authorities should make every effort to ensure that populations participating in trials stand to benefit from the knowledge, practices, or interventions that result from the research.

O'Brien and colleagues offer compelling evidence that severe RSV disease in healthy term infants is preventable using immunoprophylaxis. The fate of motavizumab has also confronted us with the ethical dilemma of our post-trial obligations in the development of upcoming RSV therapeutics, which promise better health for many infants worldwide.

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LJB received grants from MedImmune and AbbVie and acted as consultant for Janssen, Gilead, Okairos, Mabxience, Alios, and AIT. JJMvD is currently the president of CIOMS and the chair of the guideline revision work group. NIM declares no competing interests.

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Part 6

GENERAL DISCUSSION

15

SUSTAINABLE DRUG DEVELOPMENT

Live in each season as it passes; breathe the air, drink the drink, taste the fruit, and resign yourself to the influence of the earth.
Henry David Thoreau (1817-1862)

Chapter 15 — Sustainable Drug Development

Evidence before this thesis

Over the past ten years, respiratory syncytial virus (RSV) is increasingly recognized as a global health problem by organizations such as the World Health Organization (WHO) and Bill & Melinda Gates Foundation (BMGF). RSV is the second most frequent cause of death after malaria in the post-neonatal infant period. The burden of life-threatening RSV is not equitably distributed globally: 99% of mortality occurs in the developing world[1]. The burden is likely underestimated as burden estimates are largely based on passive, in-hospital surveillance and more than 50% of RSV deaths occur out of hospital[2].

Despite the RSV disease burden, there is still no vaccine and no treatment for RSV. RSV prevention is important in achieving sustainable development goal 3.2 that focuses on ending preventable deaths of newborns and children under 5 by 2030[3]. In 2015 a WHO convention on RSV was held for the first time in more than a decade to provide guidance on RSV vaccine development for lower and lower-middle income countries (LMICs) urging pivotal trials to include populations and endpoints relevant to LMICs[4]. The WHO convention resulted in guidance documents including the creation of RSV preferred product characteristics[5,6] and a vaccine roadmap[7]. The WHO articulated a strategic goal "to develop and license high-quality, safe, effective RSV vaccines that prevent severe disease and death… and to ensure that they are available and affordable for global use including in LMICs"[7]. Challenges of novel vaccines with global health importance include conducting research in settings with limited clinical trial and laboratory infrastructure, setting up simple regional manufacturing, and implementation in areas with limited burden or economic data and limited disease awareness.

RSV drug development is unique as unlike vaccines for HIV, TB and malaria; the target population for an RSV vaccine is not limited to LMICs. Due to the high disease burden in the high-income countries, an RSV vaccine is perceived by industry as the next potential "blockbuster drug" even when the LMIC market is excluded. There is one approved immunoprophylaxis, palivizumab, which has been approved for 20 years with a yearly revenue of 1,5 billion United States dollars (USD). However, due to high costs this drug is still not available in developing settings and has not been registered in a single low-income setting. Despite an active RSV vaccine development pipeline with more than 20 vaccine candidates in clinical development, there is a persistent mismatch between the RSV vaccine development pipeline and global disease burden. Overall, more than 80% of post-neonatal deaths under 5 years of age due to pneumonia occur in South Asia and Sub-Saharan Africa[8]. Except for RSM01 (an RSV monoclonal antibody (mAb) in early clinical development), nearly all candidates in clinical development have a rich-world first approach (96%, 24/25). Despite WHO efforts, affordability, and access in LMICs are seldom a hallmark of the clinical development program [Figure 1, next page].

Added Value of this thesis

In 2000, international ethical guidance documents formalized the concept of post-trial access to ensure continued access to a proven beneficial intervention after a clinical trial is over. Post-

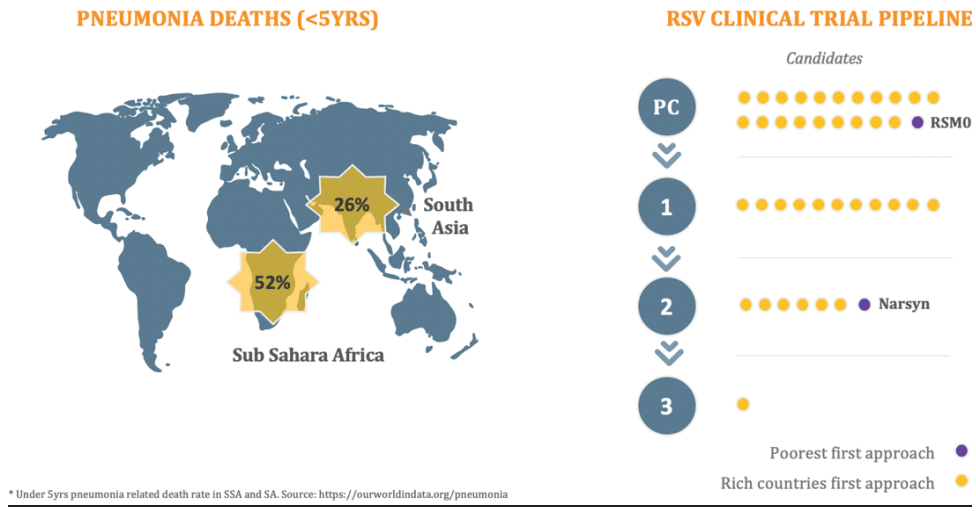

Figure 1. Mismatch in global RSV pipeline and geographical focus in 2020. Reproduced with permission of Daan Sanders.

trial access (PTA) became a requirement for medical research in humans, highly relevant in vulnerable populations or trials conducted in a low-resource settings. The Japanese concept of Ikigai (purpose) can be used to frame the importance of post-trial access for RSV [Figure 2]. These ethical principles can be found at the intersection of (1) passion (2) global need (3) skills and (4) financial considerations of drug development. As such, post-trial obligations may be the core driving force for RSV drug development. Although supported by international ethical regulations, post-trial access principles are not sufficient to guarantee access to drugs after development [this thesis]. In the case of maternal vaccine trials, formalization of PTA in international ethical guidelines (CIOMS[9] and Declaration of Helsinki[10]) did not result in increased PTA provisions [this thesis]. To increase transparency and evaluate adherence, trial protocols and publications should include PTA in peer-reviewed publications [this thesis]. The RSV vaccine landscape provides two recent case studies where the PTA principles failed to ensure that the population exposed to the risks of clinical research gained access to RSV drug candidates which showed efficacy in vulnerable populations.

The first case study concerns the clinical development of motavizumab, a highly potent second-generation anti-RSV mAb. A phase 3 trial was conducted in more than 2000 Native American infants, a vulnerable population at high risk for severe RSV disease. Although motavizumab

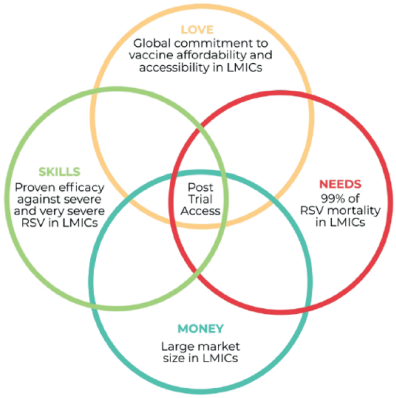

Figure 2. Finding purpose in post-trial access reflected via the Japanese concept of Ikigai (translated as purpose). SDG: Sustainable development goal. RSV: Respiratory Syncytial Virus. LMICs: Lower and lower-middle income countries.

showed a significant reduction in RSV hospital admissions (11.3 *vs*. 1.5), drug development was discontinued after an investment of 445 million USD due to a higher rate of mild hypersensitivity reactions and non-superiority to palivizumab[11]. Thus, Native American parents participated in this trial under the reasonable assumption that if the drug were effective, it would become available to Native American babies. However, these children were left without prophylaxis resulting in ongoing severe RSV infections. Lessons learned from this trial include considering and agreeing upon post-trial obligations during early phases of trial design for future RSV therapeutics in development [this thesis].

The second case study concerns the phase 3 milestone PREPARE trial of an RSV F nanoparticle vaccine in more than 4000 pregnant women. The candidate showed the first proof-of-concept for efficacy of RSV maternal immunization against severe RSV infection in infants. Although the drug candidate failed to meet its primary endpoint in the overall study population, there was superior efficacy in the South African population: 54% (95% CI 30-70%) efficacy against medically significant RSV lower respiratory tract infection (LRTI) and 74% (50-86%) efficacy against RSV LRTI with severe hypoxemia. Moreover, there was 49% efficacy against all-cause infant pneumonia through one year after vaccination. Approximately 60% of pregnant women were from LMICs and consented under the assumption that if the vaccine were effective it would be made available[12]. The BMGF invested $89 million to ensure affordability and accessibility to this intervention in LMICs. Unfortunately, the vaccine program has been terminated. What would be the fate of this vaccine if it were efficacious in HICs but not LMICs? Despite the reduction in severe RSV lower respiratory tract infection (LRTI), South African babies were left without RSV prevention, resulting in ongoing severe infections in this population.

To plan for and ensure post-trial access for an RSV vaccine there are practical solutions which vary per target population (trial participants, the community trial participants are selected from and the global community) [this thesis]. Practical solutions for trial participants include open label extension trials as a trial addendum and temporary solution. For the larger community from which trial participants are selected, prioritization of local government and national regulatory authority engagement may be effective. Finally, for the global community important mechanisms include increasing the awareness of disease burden, tiered pricing schemes and inclusion of public health stakeholders. To prioritize low-resource settings, an ethics framework for PTA to an RSV vaccine shows shared responsibility amongst international stakeholders and local stakeholders.

Lack of access to RSV drugs has also been recognized on a local level in low-resource settings. In Soweto, one of the largest townships in the world, the dilemma of the inequity of drug development was clearly expressed during the RSV Mortality Awareness Campaign. Local healthcare workers expressed a clear need for drug access despite low awareness among the general population:

We know there are antibodies to prevent RSV, but children need to meet specific criteria to receive them. They are only available in the private sector and at very high cost. If there was a way to fund that and expand to the public sectors, then we would have a cure or treatment.

All children should get monoclonal antibodies. This is very important in our setting. There is an antibody, but we can't give it to the high-risk population because it is too costly. But if we can work out a cost/benefit ratio surely, we can make it available.

Although best practices may help to implement PTA, a new perspective that aligns drug development with impact may be more important to guarantee access to an RSV vaccine in LMICs. Global health equity requires a novel drug development paradigm.

Implications of all the available evidence

Context: the 10/90 Gap. Vaccines are a simple but highly effective solution for global health problems: (1) the context to deliver them is limited, (2) prices can be kept relatively low, and (3) the health benefits are large of a young child protected against life-threatening disease. Clinical development of life-saving vaccines for LMICs is of key importance to address global health threats yet is limited by inequity in global health research. The 10/90 gap is a well-known concept used to describe the misalignment between global health research and global health burden. Namely, less than 10% of the global health research occurs for health problems in developing countries which represent more than 90% of the world's preventable mortality burden [Figure 3]. Equitable drug development includes addressing both diseases with a clearly unmet need geographically limited to low-resource settings (*i.e.*, malaria, TB) as well as diseases for which health research is ongoing and drugs exists but remain unaffordable in most of the world (*i.e.*, RSV). The Narsyn trial [this thesis] was an effort to address the 10/90 gap for an existing but unaffordable drug.

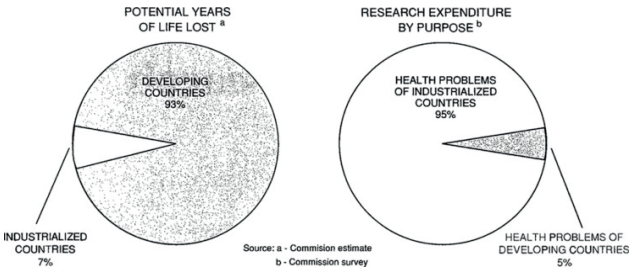

Figure 3. Contrast between global distribution of death and health research funding. 93% of preventable mortality occurs in the developing world; only 5% of global investment in health research is used for developing countries [Commission on Health Research for Development, Oxford University Press 1990].

Lessons Learned from a poorest-first approach. The Narsyn drug development program piggybacks on an existing approved drug with the goal of making RSV prevention affordable, acceptable, and more effective through local mucosal administration. There are several lessons learned from this drug development program which may bring a novel perspective to RSV vaccine development to ensure a poorest-first approach.

(1) Simple concepts may help improve drug efficiency such as the bouncer hypothesis, blocking RSV at the port of entry. Biologics such as mAbs remain expensive drugs and high costs limit potential global access. Local drug administration to the site of infection may be more efficient and limit side effects for drug administration.
(2) The notorious valley of death occurs after the preclinical phase of drug development and translational step from proof-of-principle to proof-of-concept in human clinical trials. A shift in which the first translational steps are taken in the academic setting allows for a fail-fast approach, limiting the long-term chance of failure and increasing drug affordability. We have shown that the translational step to clinical trials can be taken in an academic setting without external funding with involvement of an interdisciplinary team including expertise from pharmacology, immunology, clinical trial design, statistics and relevant medical specialties who helped with investigational new drug (IND) application, trial design and ethics approval. Controlled human challenge trials (CHIM) may be used for first-in-human trials and affordable and rapid proof-of-concept. CHIM may be key for fast failure in clinical development.

(3) A business model that is driven by impact-maximalization instead of profit-maximalization is key to a poorest-first approach. As advocated by the Bill & Melinda Gates Research Institute "lives-saved" may be a fresh metric for market access.
(4) Understanding the age distribution and characteristics of children with life-threatening RSV disease will be essential to drive vaccine policy and make a case for vaccine uptake. Global data sharing to study severe life-threatening disease in low-resource settings is key to obtaining more information on RSV disease burden in low-resource settings.
(5) Local capacity building to raise awareness and diagnostic capacity is key in creating vaccine acceptance in areas where relatively little is known about disease burden.

Recommendations for sustainable drug development. Drug development is in a global crisis. Money invested into developing new drugs has diverged from successful drugs developed: expenditures for research and development have doubled while the average number of new drugs approved has declined[13]. Approximately one in ten drug candidates succeeds from phase I trials to registration[13] and the cost of development is estimated at $2.6 billion[14]. The notoriously high-risk pharmaceutical industry is compensated by high profits. Namely, the pharmaceutical industry is the highest profit industry with nearly double the average return on invested capital across all industries[15]. Overall, the combination of high upfront investment, minimal innovation, high profit, and high costs for the end-user underline the clear need for drug development reform.

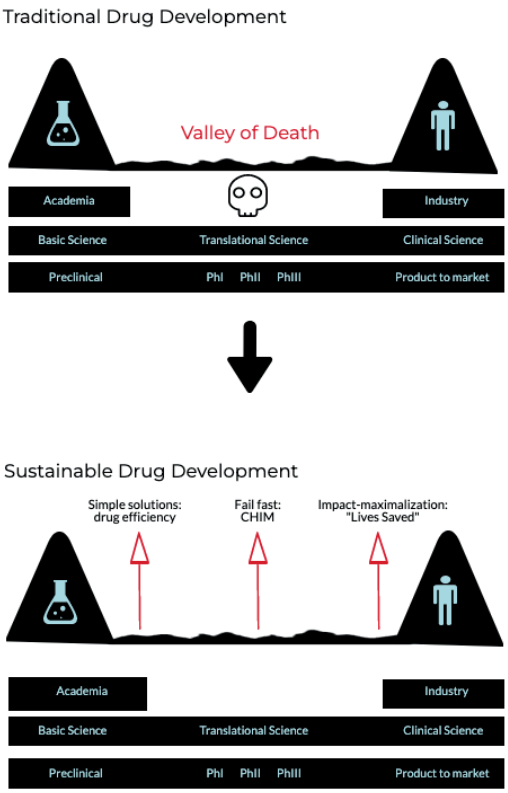

Figure 4. Key recommendations to move from traditional to sustainable drug development.

Looking toward the future, it may be desirable to replace traditional drug develop-ment with sustainable drug development, an approach aligned with the global agenda of the sustainable development goals. Below, we make three key recommendations for a transition to sustainable drug development [Figure 4].

(1) Do more with less: efficiency-based clinical development:

Question-based clinical development (QBCD) has been suggested as a rational and efficient replacement of the traditional stage-oriented clinical development[16]. This approach has been

developed to better integrate scientific considerations with financial considerations in the research and development process. The suggested approach allows biomedical researchers to identify the most critical scientific questions to be answered during development and identify the ideal order to address these questions. A development program often includes 5-6 key questions such as:

- Does the drug reach the site of action?
- Does the drug act on the intended target?
- Does the drug have the intended clinical effect?
- Does the effective dose have an acceptable safety profile?
- Does variability of the target population affect response to drug?

In this thesis, drug efficiency is added as an additional consideration for the research and development process. In this new drug development paradigm, the optimal path for drug development includes one that minimizes drug waste and incorporates efficiency. Key considerations for drug efficiency include:

- Can the drug be administered directly to the site of action?
- What is the minimal effective dose needed?
- Does the target population selected have the highest need for the drug?

(2) Fail fast:

In an industry where investments costs are high and the trajectory to drug approval is long (about 10 years) failing fast is key to limiting development costs. Controlled human infection studies (CHIM) are a useful tool to accelerate drug development while minimizing costs[17]. Although CHIM is not a novel tool (in use since the 17th century), the utility is being increasingly recognized with more than 120 publications in the past decade.[18] CHIM has been pivotal in Food & Drug Administration (FDA) licensure of live oral cholera vaccine, European Medicine Agency's (EMA) approval of the world's first malaria vaccine as well as phase 3 evaluation of dengue vaccine candidates. For sustainable drug development rapid safety, dosing and proof-of-concept trials can be done in the academic setting using CHIM. Adequate transmission control and home quarantine measures may allow RSV CHIM in the outpatient setting and reduce costs of proof-of-concept trials even further.

(3) Impact-maximalization:

Traditionally drug development choices are driven by profit-maximalization, to ensure maximum return on investment for investing partners. Investors that are able to provide adequate sums of money for drug development (venture capital, private funds) most often have minimum requirement for return on investment and profit margins. In the sustainable drug development model, we advocate for drug development choices that maximize drug impact as opposed to maximum profit. A business model that does not maximize profit can still be self-sustaining and generate profit, however development decisions will ultimately be guided by decisions that lead to maximal drug impact. Alternative investments which allow for more freedom regarding profit margins may need to be sought out such as public funding (Eurostars Eureka grant, Wellcome Trust) or private funding (impact investing, venture philanthropy). Furthermore, university technology transfer offices should also transparently describe their

policies on profit-maximalization. Maximal impact has a different metric than "return on investment," namely, "lives saved." This metric is already being employed to measure performance by the Gates Medical Research Institute (MRI). Impact maximalization as a successful drug development approach already exits: the Ravidasvir (a Hepatitis C drug) development pathway was based on impact over profit and showed that it is possible to place affordability and public health priorities at the core of development[19]. Thus, proof-of-concept for LMIC-based drug development already exits as a tool to promote global equity.

(4) Other considerations:

Our key recommendations mainly focus on choices made during clinical development from an academic perspective and are therefore not exhaustive. We focus on the early phases of drug development in which academia play a role. Other important considerations include the average drug lag of 4–7 years between regulatory submission and approval in Sub-Saharan Africa leading to delayed access to essential vaccines. Potential solutions to minimize delay include focusing on value-added activities (WHO-PQ), harmonizing regulatory standards across countries, and improving manufacturer inputs. Local engagement is key to increasing disease awareness, including the end-user perspective in drug design and understanding local health-economic considerations. Drug pricing schemes can be implemented to further reduce drug costs in LMICs in the long run by introducing tiered-pricing or using income from the HIC market to subsidize the LMIC market. Additionally, patent extensions allow long-term protection and high pricing from drugs and make the biosimilar market less attractive. Furthermore, high costs of patent application and maintenance drive up costs of drug development. The goal of intellectual property law is to protect knowledge in order to be able to commercialize it. Exceptions to patent pricing and patent law are needed for business models with a mission for impact-maximalization instead of profit-maximalization.

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APPENDICES

PhD Portfolio

Natalie Isabelle Mazur, PhD period 2017 – 2022
Promotor: Louis J Bont

Courses

2022	EUREKA International Certificate Course in Translational Medicine (Siracusa, Sicily)
2019-2021	TULIPS Postdoc Curriculum
2017-2019	TULIPS PhD Curriculum
2019	Paul Janssen FutureLabs Question-Based Clinical Development Course
2018	FOCIS Advanced course in basic and clinical immunology (Scottsdale, Arizona)

Fixed Milestones

2018	Basic Course for Clinical Investigators (BROK)
2017	Infection & Immunology PhD Retreat (CTI)
2017	Research Data Management
2017	Research Ethics

Conferences

2021	RSV Vaccines for the World; virtual (poster)
2021	Infectieziekten Symposium Amsterdam (IDSA); Amsterdam (oral)
2019	RSV Vaccines for the World; Accra, Ghana (conference chair, oral)
2019	Pediatric Infectious Diseases Conference (ESPID); virtual (poster)
2019	Japan Global Expert Meeting; Tokyo, Japan (oral)
2019	RSV Global Expert Meeting; Venice, Italy (oral)
2019	International Society for Vaccines (ISV); Ghent, Belgium (oral)
2018	International RSV Symposium; Ashville, USA (oral)
2018	Ethics in Global Health; Amsterdam (oral)
2018	World Vaccine Conference; Washington, USA (oral)
2018	Annual IMPRINT Meeting; London, UK
2017	RSV Vaccines for the World; Malaga, Spain (oral)
2017	RSV High Level Expert Meeting; Amsterdam
2016	International RSV Symposium; Patagonia, Argentina (poster)
2015	International RSV Symposium; Stellenbosch, South Africa

Seminars

2019	UREKA Megachallenge Finale (pitch)
2017	BMGF Global RSV Burden Estimates Symposium; Seattle, WA USA (oral)
2015	RSV Live Case Young Leaders League; Doctors Without Borders, Amsterdam
2018	Independent Scientific Advisory Board Meeting RSV GOLD; Utrecht

Teaching

2018-2021 Global Child Health Summer School (UMCU-AMC); Utrecht
2019 Young Researchers Program RSVVW; Malaga, Spain
2018 Global Health Writing Retreat; Utrecht
2018 Global Health Research Night; Utrecht
2017-2021 Global Health and Tropical Medicine Course; Utrecht

Supervision

2021 Master thesis, Jonne Terstappen (MSc MD, SUMMA Utrecht University)
Saal van Zwanenburg prize for best Master's Thesis
2019 BMGF Pilot Research, Maria Garba (MD, ABUTH; Zaria, Nigeria)
Grant recipient for African Research Excellence Fund (BMGF)
2018 Research Internship, Nicole Horsley (BSc, Univ of Washington)
Stanford University Stem Cell PhD Program
2018 Honors Program Medicine, Ichelle van Roessel (MD, Universiteit Utrecht)
KNAW van Walree Grant

Grants/ Awards

2019 NVK Young Investigator Award 2nd Place: 1k
2018 KNAW Ter Meulen Grant: 8,8k
2021 Dutch Lung Foundation Junior Investigator Grant: 200k

Peer review

Pediatrics
PIDJ
Lancet Public Health
Lancet Child & Adolescent Health
Journal of Infectious Diseases
Pediatric Pulmonology

Curriculum Vitae

All good things are wild and free - Henry David Thoreau (1817-1862)

Natalie Isabelle Mazur, born December 3rd, 1988 in Malden (USA), grew up in Concord, Massachusetts with her mother (Angela), father (Eric), brother (Marc) and sister (Sophie). She graduated from Concord Carlisle High School in 2006 during which time she was an enthusiastic alpine and cross-country ski-racer. She delayed her undergraduate studies to work and travel: learning Argentine Tango, becoming a certified ski instructor in Switzerland, working on an organic farm, and researching family history. During her undergraduate studies at Harvard University, Natalie spent a semester abroad in Paris, France studying public health at Sciences Po. Additionally, she volunteered in a malaria eradication program in the Amazon jungle of Ecuador and working for an NGO in Bambous, Mauritius. In her free time, she danced salsa in the Candela Salsa dance group. In 2011 Natalie graduated from Harvard University *cum laude* with a BA in Biology, a minor in politics and Spanish. After graduating, Natalie worked as a researcher in pediatric respiratory emergencies at the Emergency Medical Network (EMNet) at Massachusetts General Hospital, Boston (USA). Natalie spent her free time dancing salsa and travelled to Cuba with Metamovements Dance Company for a salsa dance exchange.

In 2012 Natalie moved to the Netherlands and enrolled at Utrecht University for the Research Master MD/MSc Clinical Scientist Training Program (Selective Utrecht Medical Master, SUMMA), from which she graduated in 2016. During this time, Natalie researched life-threatening RSV at the Respiratory & Meningeal Pathogens Unit in the township of Soweto (Johannesburg), South Africa, did her elective medical internship in tropical medicine in Ekwendeni, Malawi, and completed a social medicine course in Porte-au-Prince, Haiti.

In 2017 Natalie started graduate studies (PhD candidate) in the RSV Research Group at University Medical Center Utrecht with support from by the Bill & Melinda Gates Foundation. Natalie completed the Training Upcoming Leaders in Pediatrics (TULIPS) PhD and Postdoc programs. In 2018 Natalie obtained the KNAW ter Meulen grant to research RSV antibodies in breast milk in the Chu lab in Seattle, WA (USA). Natalie was actively involved in the RSV Vaccines for the World conference series. In 2019 Natalie put her PhD on hold and enjoyed working as a non-resident doctor in pediatrics in Spaarne Gasthuis, Haarlem before resuming her PhD from 2020 to 2022.

Since 2022, Natalie is a pediatric resident at UMC Utrecht and has started her training at St Antonius Hospital in Utrecht. She also works as a postgraduate researcher in the department of pediatric infectious diseases at the University of Utrecht Medical Center. She has been awarded the Junior Investigator Grant from the Dutch Lung Foundation to set up the first RSV controlled human infection model in the Netherlands and is supervising the development of a low-tech diagnostic for LMICs to measure RSV neutralization, financed by the Gates Medical Research Institute. Finally, she is involved in the RSV GOLD Mortality Registry and Intensive Care Unit network, a global real-time surveillance platform of pediatric deaths in LMICs, financed by the Bill & Melinda Gates Foundation. She has been selected to participate in the UMCU Research Career Development Talent Program.

English Summary

Aims of this thesis

The principle aim of this thesis was to use a multidisciplinary approach to establish the foundations for an RSV vaccine in lower and lower-middle income countries (LMICs). Four different approaches were used:

(1) Global epidemiology: to understand the age distribution for life-threatening RSV
(2) Translational medicine: to understand protection through mucosal antibody transfer
(3) Clinical development: to provide an overview of the RSV vaccine pipeline
(4) Ethics: to analyze the ethical framework of post-trial access for RSV vaccine trials

Outline of this thesis

Part 1. General Introduction: In **Chapter 1** we discuss background information on the virology, epidemiology, pathogenesis and host response, clinical presentation, current management, transmission and prevention, and ethics of RSV and RSV vaccine development.

Part 2. Global Life-Threatening Lower Respiratory Tract Infections: RSV is an important cause of infant death, yet the full burden of life-threatening RSV is poorly understood, especially in LMICs. More than half of RSV-related deaths occur outside of the hospital and this group of children remains poorly characterized. Thanks to global research efforts and data sharing, we compare the age distribution and clinical characteristics of infant deaths occurring in the community to those occurring in-hospital in 38 developing countries using the RSV GOLD Database (RSV Global Online Mortality Database) in **Chapter 2**.

Real-time data collection of RSV mortality is important to promote awareness of RSV and show the impact of a vaccine once implemented. We set up GOLD-III, a study with a network of Intensive Care Units worldwide acting as sentinel sites for real-time RSV mortality surveillance in 10 GAVI-eligible countries. We describe the study design in **Chapter 3**.

Although multiple pathogens can easily be detected in the pediatric respiratory tract, it is unclear whether viral coinfections are associated with life-threatening disease. In a large cohort of South African children, we describe whether RSV coinfection is associated with more severe disease in a low-resource setting in **Chapter 4**.

Finally, maternal vaccination against influenza has been prioritized by the World Health Organization (WHO). In **Chapter 5** we set up a global database of pediatric influenza deaths to estimate the impact of maternal vaccination on pediatric mortality and compare this to RSV-related mortality.

Part 3. Mucosal Antibody Transfer and Local Protection: Mucosal antibody transfer is a key mechanism of protection of maternal vaccination. For RSV little is known about the protective effect of RSV-specific antibodies in breast milk. In **Chapter 6** we describe prefusion (preF) IgA and IgG antibodies collected from mother-infant pairs in Nepal in infants who did or did not develop RSV ARI.

Finally, we investigate the safety and efficacy of daily intranasal monoclonal prophylaxis with palivizumab on RSV infection in a phase 1 (healthy adults) and phase 2b (healthy preterm infants) randomized controlled trial in **Chapter 7**.

Part 4. Global RSV Vaccine Pipeline: Due to vaccine-enhanced disease in a 1960's trial, vaccine development for RSV has been slower than expected. However, the RSV vaccine development landscape has rapidly expanded in the past decades. In **Chapter 8** we analyze global management guidelines and summarize evidence-based interventions for bronchiolitis and different vaccine approaches in clinical development in 2015.

Subsequently, we summarize lessons learned from landmark late-phase vaccine trial failures and update the global RSV vaccine landscape which was revolutionized by advances in knowledge of the structural biology of the RSV surface fusion (F) protein in **Chapter 9**.

Finally, in **Chapter 10** we show that market access for an extended half-life RSV monoclonal antibody (mAb) is within reach to prevent RSV in all infants by 2023 and may be followed by other approaches. However, no vaccine or mAb is within reach for LMICs with the highest pediatric mortality burden.

Part 5. Awareness and Ethics of Post-Trial Access: Clinical trials are increasingly being conducted in low-resource settings which has led to ethical dilemmas about lack of access to a drug after the conclusion of a trial. Since 2000, major international ethics guidelines require provisions of post-trial access during trial design to ensure access to a proven beneficial intervention. In **Chapter 11** we share the personal story of a mother who has lost her child to RSV in a low-resource setting to advocate for disease awareness for future vaccine implementation.

In **Chapter 12** we propose an ethics framework with practical guidance to implement post-trial access for a maternal RSV vaccine.

In **Chapter 13** we review adherence to post-trial access in the context of global maternal vaccine trials before and after 2000.

Finally, in **Chapter 14** we review the ethical dilemma of lack of post-trial access in the motavizumab phase 3 trial failure and urge future RSV therapeutics to consider post-trial obligations early in clinical trial design.

Part 6. General Discussion: In **Chapter 15** we review lessons learned from the work in this thesis. There is a mismatch between RSV vaccine development and burden and post-trial access insufficiently addresses this ethical dilemma. We propose three key recommendations for sustainable drug development.

Nederlandse Samenvatting (Dutch Summary)

Doelstellingen van dit proefschrift

Het voornaamste doel van deze dissertatie was een multidisciplinaire benadering te gebruiken om de grondslag voor een RSV-vaccin in lagere en lager-middenklasse inkomenslanden te leggen. Vier verschillende benaderingen zijn gebruikt:

1) Wereldwijde epidemiologie: om de leeftijdsverdeling van kinderen met levensbedreigende RSV infecties te onderzoeken
2) Translationele geneeskunde: om bescherming door mucosale antistof overdracht te begrijpen
3) Klinische vaccinontwikkeling: om een overzicht van de RSV-vaccin pijplijn te geven
4) Ethiek: om het ethische kader te analyseren bij post-trial toegang voor RSV-vaccin trials

Indeling van dit proefschrift

Deel 1. Algemene inleiding: In **Hoofdstuk 1** beschrijven we achtergrondinformatie over virologie, epidemiologie, pathogenese en host response, klinische presentatie, behandeling, transmissie en preventie en ethiek van RSV en RSV-vaccin ontwikkeling.

Deel 2. Wereldwijde levensbedreigende lagere luchtweginfecties: RSV is een belangrijke oorzaak van kindersterfte, maar de volledige omvang van levensbedreigende RSV-infecties is onvoldoende bekend, met name in laag-middel inkomens landen (LMICs). Meer dan de helft van sterfgevallen door RSV vindt plaats buiten het ziekenhuis en deze groep kinderen is nog nauwelijks beschreven. Dankzij wereldwijde onderzoeksinspanningen en het delen van gegevens hebben wij de leeftijdsverdeling en de klinische kenmerken van kinderen die buiten het ziekenhuis met RSV-infectie zijn overleden kunnen vergelijken met die van kinderen die in het ziekenhuis met RSV infectie zijn overleden in 38 LMICs, door gebruik te maken van de RSV Gold Database (RSV Global Online Mortality Database) in **Hoofdstuk 2.**

Real-time dataverzameling van RSV-sterfte is belangrijk om het bewustzijn over RSV te bevorderen en het toont de impact van vaccinimplementatie. In **Hoofdstuk 3** bespreken wij de GOLD-III studie, bestaande uit een wereldwijd netwerk van Intensive Care Units welke fungeren als surveillance locaties voor het monitoren van real-time RSV-sterfte in 10 LMICs.

Hoewel meerdere pathogenen makkelijk gedetecteerd kunnen worden in de luchtwegen van kinderen, is het niet duidelijk of virale co-infecties samenhangen met levensbedreigende ziekte. Bij een groot cohort van Zuid-Afrikaanse kinderen beschreven we of RSV co-infectie samenhangt met ziekte ernst in een low-resource setting in **Hoofdstuk 4**.

Tenslotte heeft de Wereld Gezondheids Organisatie (WHO) prioriteit gegeven aan het vaccineren van zwangere vrouwen. In **Hoofdstuk 5** hebben we een wereldwijde database opgezet met informatie over kindersterfgevallen door influenza om de mogelijk impact van maternale vaccinatie op kindersterfte te kunnen analyseren en te vergelijken met de impact van RSV maternale vaccinatie.

Deel 3. Mucosale antistof overdracht en lokale bescherming: Mucosale antistof overdracht is een belangrijk mechanisme van bescherming van maternale vaccinatie. Er is weinig bekend over het beschermende effect van RSV-specifieke antistoffen in moedermelk. In **Hoofdstuk 6** beschrijven we prefusie (preF) IgA en IgG antistoffen van moeder-kind koppels in Nepal, waarbij het kind wel of geen RSV-luchtweginfectie heeft ontwikkeld.

Tenslotte onderzochten we de veiligheid en effectiviteit van dagelijkse intranasale monoclonale immunoprofylaxe met palivizumab tegen RSV-infectie in een fase I en fase IIb gerandomiseerde klinische trial in **Hoofdstuk 7**.

Deel 4. Wereldwijde RSV-vaccin pijplijn: De vaccinontwikkeling voor RSV verliep langzamer dan verwacht vanwege vaccine-enhanced disease tijdens een klinische trial in de jaren '60. Het landschap van RSV-vaccinontwikkeling is echter snel uitgebreid in de laatste decennia. In **Hoofdstuk 8** analyseren we de wereldwijde behandelrichtlijnen en vatten we de evidence-based interventies voor RSV infecties samen en bespreken we verschillende vaccins in klinische ontwikkeling in 2015.

We vatten de lessen van de gefaalde mijlpaal RSV-vaccin trials samen en geven een update over het wereldwijde RSV vaccin landschap, dat drastisch is veranderd door de toename in kennis over de structurele biologie van het RSV oppervlakte fusie (F) eiwit in **Hoofdstuk 9.**

Tenslotte laten we in **Hoofdstuk 10** zien dat markttoegang voor een monoklonale RSV antistof (mAb) met verlengde halfwaardetijd binnen bereik is om RSV infecties te voorkomen bij alle kinderen in 2023. Dit kan worden opgevolgd door andere vaccin benaderingen. Er is echter geen vaccin of mAb binnen bereik voor LMICs waar kindersterfte het hoogst is.

Deel 5. Awareness en ethiek van post-trial beschikbaarheid: Klinische onderzoeken worden steeds meer uitgevoerd in low-resource settings, iets wat heeft geleid tot ethische dilemma's door een gebrek aan toegang tot onderzochte medicijnen nadat een onderzoek is afgerond. Sinds 2000 vereisen grote internationale ethiekrichtlijnen voorzieningen voor post-trial toegang om toegang te garanderen voor een bewezen gunstige interventie nadat een klinische trial is afgerond. In **Hoofdstuk 11** delen we het persoonlijke verhaal van een moeder die haar kind heeft verloren aan RSV in een low-resource setting om te pleiten voor het belang van awareness over dit ziektebeeld voor toekomstige vaccinimplementatie.

In **Hoofdstuk 12** stellen we een ethisch kader voor met praktische richtlijnen om post-trial toegang voor een maternaal RSV-vaccin te kunnen implementeren.

In **Hoofdstuk 13** herzien we de naleving van post-trial toegang in de context van wereldwijde maternale vaccinatie trials voor en na het jaar 2000.

Tenslotte bekijken we in **Hoofdstuk 14** het ethische dilemma van het gebrek aan post-trial toegang in de fase 3 gefaalde motavizumab trial, en verzoeken we toekomstige RSV-therapieën dringend om post-trial verplichtingen al vroeg in het klinische onderzoeksontwerp te overwegen.

Deel 6. Algemene discussie: In **Hoofdstuk 15** bekijken we de lessen geleerd uit dit proefschrift. Er is een discrepantie tussen RSV-vaccin ontwikkeling en ziektelast welke onvoldoende geadresseerd kan worden met post-trial toegang. We bespreken drie voorstellen voor duurzame medicijn ontwikkeling.

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