

Equity-by-design in RSV immunisation development

Jonne Terstappen

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Equity-by-design in RSV immunisation development

Gelijkwaardige ontwikkeling van immunisatie tegen het RS-virus
(met een samenvatting in het Nederlands)

Proefschrift

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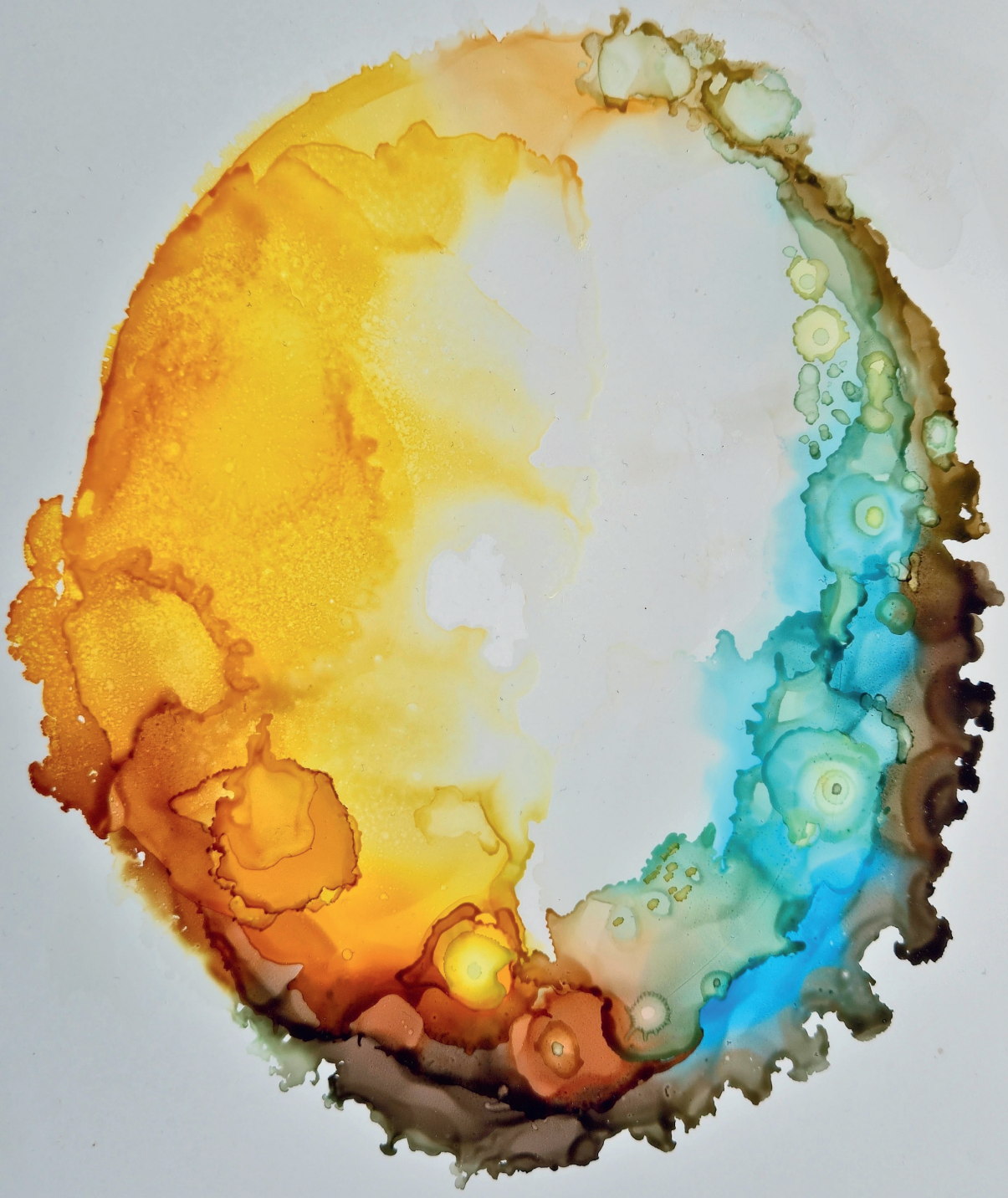
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Notes for Radical Living

Make friends with chaos
Hold a calm mind
Let things shake
Forgive human frailty
Champion second chances
Defy kindness
Reverence fellowship
Listen to the quiet
Respect the young
Seek growth
Trust in change
Treasure learning
Inspire faith in evolution
Reach beyond the binary
Be wary of the doubtless
Honor the bright headed
Grow plants
Attend to the weather
Be electric
Cherish language
Celebrate silence
Dance daily
Bless the handmade
Sing into pain
Challenge assumptions
Follow the wind
Look upwards
Swoon under clouds
Feel your courage
Face forward
Read history
Open your ears
Drop your shoulders
Bend your knees
Raise the roof
Keep breathing
Be trustworthy
Take care of yourself
Believe in goodness
Head for the light

Tilda Swinton, Ongoing



De cirkel symboliseert de wereld en het idee dat alles met elkaar verbonden is: elk begin draagt een einde in zich, elk verhaal is rond. Het symboliseert een hollistische benadering voor een werelds probleem. Binnen deze cirkel wordt ongelijkheid zichtbaar gemaakt middels contrasten: licht en donker, scherp en vaag, warm en koud, klein en groot, hard en zacht, fel en kalm, grillig en glad. RSV komt overal ter wereld voor, maar de lasten worden ongelijk gedragen — een spanning die in het beeld voelbaar is. De kleuren weerspiegelen ervaringen uit verschillende delen van de wereld en benadrukken dat dit een gedeeld, mondiaal probleem is.

Alle kunstwerken binnen dit proefschrift zijn geworteld in ditzelfde motief: wetenschap inzetten om gezondheidsongelijkheid bloot te leggen én te verkleinen.

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Part I

General introduction

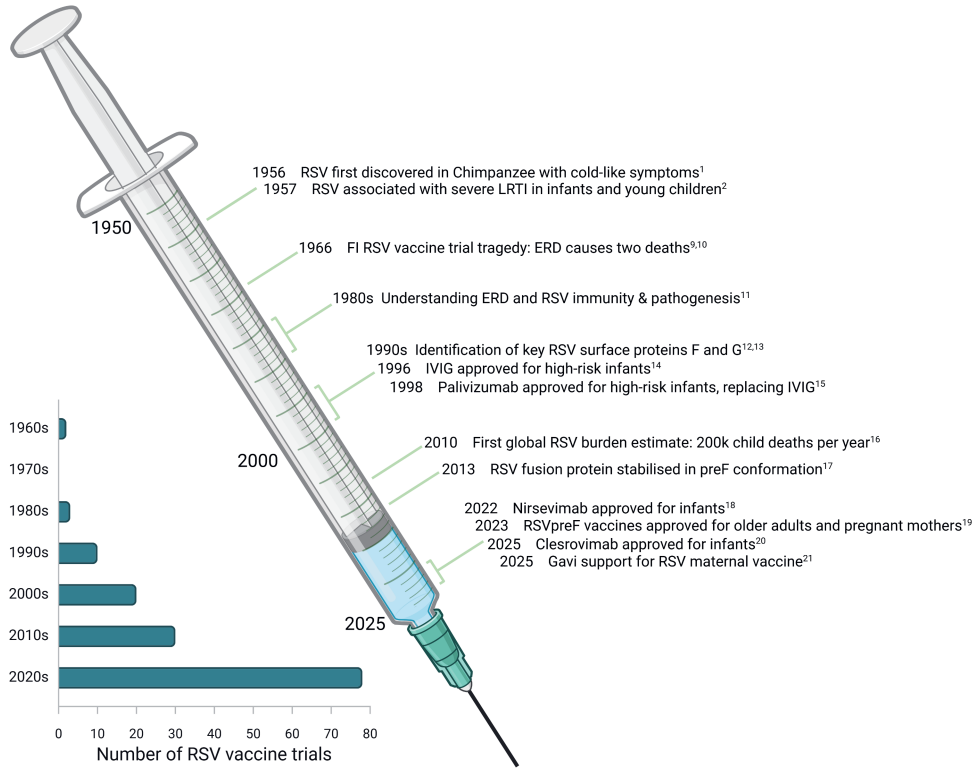




Respiratory syncytial virus

Respiratory syncytial virus (RSV) is a common respiratory pathogen, first isolated in 1956 from laboratory chimpanzees with upper respiratory tract infection, which was associated with a similar illness in a laboratory worker.[1] A year later, the virus was recognized to cause the clinical syndrome of bronchiolitis in children (**Figure 1**).[2] Since its discovery, our understanding of RSV has expanded rapidly. RSV is now recognized as the leading cause of lower respiratory tract infections (LRTI) in young children aged <5 years worldwide.[3] Nearly all children are infected with RSV at least once by the age of 2 years.[4,5] Reinfections occur throughout life,[6] but severe disease primarily affects infants and older adults (≥60 years) with comorbidities.[3,7,8]

Figure 1. RSV discovery, recognition, and prevention timeline



Since the discovery of RSV and its association with severe LRTI in infants,[1,2] research efforts immediately focused on prevention before understanding the virus and its pathogenesis. The tragic outcome of the first RSV vaccine trial[9,10] caused three decade delay in the development of RSV immunisation.[11-15] The first comprehensive global estimate of RSV disease estimated more than 3 million hospitalisations and 200 000 child deaths per year.[16] The stabilisation of the fusion protein in prefusion conformation[17] catapulted immunisation development, resulting in large amounts of clinical trials and the approval of three products for infants[18-20] Recently, Gavi has approved the support of RSV maternal vaccination.[21] ERD, enhanced respiratory disease; LRTI, low respiratory tract infection; mAb, monoclonal antibody; postF, post-fusion F-protein; preF, pre-fusion F protein; RSV, respiratory syncytial virus.

Virology

RSV is an enveloped negative-stranded RNA virus in the Pneumoviridae family.[22] Of its 11 proteins, the two surface proteins, fusion (F) and attachment (G) proteins, are the main targets for vaccine and monoclonal antibody (mAb) development because of their direct involvement with infection of the host. The G protein is responsible for attachment of the virus particle to the host cell. The F protein mediates viral entry and membrane fusion between the virus and the target cell. Importantly, during fusion, the F protein spontaneously undergoes an irreversible conformational change - it "flips" - from the less stable prefusion F (preF) to the stable post-fusion F (postF) conformation.[23] As blocking the preF protein prevents viral entry, it is a preferred target over postF or G proteins for vaccines and mAbs.[24]

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.[25] 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, while 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.[26]

RSV is often classified into an antigenic A and B subtype, distinguished primarily by differences in the G protein although surface exposed regions of preF protein can also differ between the subtypes.[27] The two subtypes diverged approximately 350 years ago from a common ancestor.[28] Despite genetic differences between the subtypes, there are no major differences in disease severity.[29] The two subtypes can co-circulate during annual RSV epidemic seasons.[27] RSV outbreaks typically occur during the winter months in temperate regions and with greater diversity in timing in tropical regions.[30] This seasonal pattern can be partially explained by meteorological factors (temperature and humidity) and the fact that transmission occurs when being indoors and in close contact with others.[30,31]

Transmission, pathogenesis, and host response

RSV is highly contagious and transmitted through inoculation of large-particle aerosols produced by infected individuals or by self-inoculation after touching contaminated surfaces.[32] Infection routes include the eyes and nose.[32] The virus can remain viable on hard surfaces for up to 6 hours.[33] The incubation period is usually between 3 to 6 days.

RSV primarily infects the ciliated airway epithelial cells of the respiratory tract causing damage and inflammation of the bronchioles[34] as well as forming characteristic syncytia (multinucleated cells). Counterintuitively, direct viral damage and cytopathology do not play the main role in the pathology of RSV infection but instead the host immune response largely precipitates airway damage.[34] The acute inflammatory responses, largely driven by neutrophils, can trigger capillary leakage, interstitial swelling, inhibited pulmonary surfactant

function, and excessive mucus production.[35,36] This may lead to severe respiratory symptoms due to airway obstruction and air trapping, especially within the still-narrow bronchioles of infants.

The first paediatric RSV vaccine candidate in the 1960s used formalin-inactivated RSV (FI-RSV), which was administered without informed consent to mostly Black and poor but otherwise healthy infants and children residing in Junior Village, a District of Columbia Welfare Institution for orphans and foster care.[10] Despite a surge in anti-RSV antibody titres, subsequent natural RSV infection led to hospital admission in 80% of the vaccinated children and the tragic deaths of two toddlers.[9,10] The mechanism of their deaths was identified as enhanced respiratory disease (ERD), in which RSV-naive recipients were primed to produce non-neutralising antibodies against postF presented on FI-RSV, which led to immune complex formation and deposition in small airways. Moreover, a T-helper 2 (Th2) biased immune response caused an allergic-like reaction to exposure to RSV, including eosinophilia, airway hyperreactivity, and mucus hypersecretion.[37,38] Interestingly, ERD never occurred in those infants who were seropositive for RSV at the time of FI-RSV administration and no child ever experienced ERD twice – suggesting an all-or-nothing mechanism.[11] For this reason, an RSV vaccine ideally elicits potent neutralising antibodies against preF without a Th2 bias. While a definitive correlate of protection against RSV infection remains elusive, cell-mediated immunity,[39] mucosal IgA,[40] and neutralising antibodies[41-44] have been associated with protection from RSV infection. Ultimately, large seasonal sampling trials will help to define a correlate of protection from clinical RSV disease.

Clinical presentation

Asymptomatic RSV infections are rare in young children.[45] Most RSV infections cause typical and mild upper respiratory tract infection (URTI) symptoms, commonly referred to as the "common cold", such as rhinitis and nasal congestion.[46] Lower respiratory tract infection (LRTI) occurs in 15-50% of cases,[47] with bronchiolitis being the most common manifestation in infants. Typically, symptoms begin with rhinitis and cough, which over the course of 2 to 5 days progress to wheeze, rales and breathing difficulty, often accompanied by feeding problems and low grade fever.[46,48] Severe disease is defined clinically by increased respiratory effort, hypoxemia and eventually respiratory failure. Hospitalisation should also be considered when the respiratory symptoms affect feeding ability, hydration, and mental status.[49] Usually, symptoms peak around day 5 of illness and improve by day 7.

Current management

Most RSV infections run a favourable natural course and can be self-managed at home.[50,51] Medical evaluation is primarily focused on evaluating adequate breathing and hydration. Evidence-based management is limited to supportive care as there is currently no treatment against RSV infection: hydration via the nasogastric or intravenous route, supplemental oxygen, and respiratory support in selected cases. Routine use of bronchodilators,

hypertonic saline, corticosteroids, ribavirin, antibiotics, chest physiotherapy, epinephrine, antitussives is not recommended.[52] Mechanical ventilation in the intensive-care unit can be life-saving, but evidence for a widely-used non-invasive respiratory support strategy (i.e. high flow nasal canula) remain insufficient[53] and is even associated with higher ICU admission rates.[52] The lack of access to mechanical ventilation likely explains higher RSV mortality in low and lower-middle income countries (LMICs): 100% versus 24% of RSV deaths had access to the intensive care unit in high-income countries (HICs) and LMICs, respectively.[54] Despite extensive development of antiviral agents, only ribavirin is licensed yet its use is highly limited to life-threatening RSV-LRTIs in immunocompromised patients due to safety, efficacy, and financial concerns.[55] As children often present relatively late in the course of illness, it is highly unlikely antivirals will be prescribed timely enough to be effective.

Burden of disease

Bronchiolitis is well recognized as the number one cause of hospitalisation for infants and young children globally. RSV is the most common pathogen in children hospitalised with LRTI (detected in 31% of cases)[56] and the most important cause of RTI-related child mortality and thus a major cause of global child mortality.[57]

Annually, RSV is associated with 3.6 million hospitalisations and 101 400 deaths in children younger than 5 years worldwide. More than 97% of these deaths occur in LMICs due to limited access to healthcare and resources.[3] Infant RSV mortality occurs inside the hospital but also in the community: it is estimated that for every in-hospital death, three additional children die at home.[58] Monitoring both in-hospital and in-community deaths is important to assess the full burden of RSV. Therefore, minimally-invasive tissue sampling and cause-of-death expert panels have been set up and used in several LMICs to ascertain the role of RSV in infant community deaths.[58] While mortality is low in HICs, RSV represents a substantial burden for health-care systems as 1 in 56 (1.8%) healthy term infants are hospitalised with RSV in their first year of life.[59]

Infants, and in particular those younger than 3 months, are at increased risk of RSV hospitalisation. Due to their low titres of maternally-acquired anti-RSV antibodies and their narrow airways that are easily obstructed by inflammatory infiltrates, young infants form an important risk group for severe RSV disease. Other risk factors for severe RSV disease include comorbidities such as congenital heart disease, chronic lung disease and malnutrition, Down's syndrome, prematurity, and low birth weight.[60,61] Despite these risk factors, the majority of children (80%) hospitalised with RSV infection are born healthy and at term.[62] Individual patient-level data come mainly from HICs because few trials have been conducted in LMICs and there is a general lack of RSV testing. Yet, global monitoring of RSV disease is important to identify target populations, to inform decision making once intervention products are available, and to monitor efficacy of intervention products after implementation.

The burden of RSV is not limited to young infants: in older adults (age ≥60 years) and adults with underlying comorbidities, RSV is an important cause of severe respiratory illness, comparable with seasonal influenza.[63]

RSV prevention strategies

Given the major burden and limited treatment options, RSV prevention is considered an urgent global health priority.[64] RSV immunisation strategies can be broadly categorized into three categories: passive immunisation through (1) maternal immunisation or (2) monoclonal antibodies (mAbs), and active immunisation through (3) vaccination. Active immunisation is the preferred strategy for adults and children aged >1 year, while passive immunisation is favoured for infants (<1 year), due to their immature immune system,[65] which limits their ability to mount an effective vaccine response in the first months of life. Vaccinating a pregnant mother leads to transplacental transfer of increased levels of maternal vaccine-induced RSV antibodies that provide protection in infants immediately after birth and during the first months of life.[66] This approach is being used successfully for pertussis and tetanus globally.[66] When maternally-acquired antibodies naturally decline, passive immunisation with antibodies will provide protection during the "immunity gap" until the child develops natural immunity.[67] Children with high risk for severe RSV disease could benefit from boosting RSV immunity through paediatric vaccination. Theoretically, paediatric vaccination could also provide protection of infants through herd immunity, although more data on RSV transmission are needed.

At the start of this PhD in 2021, the short-acting mAb palivizumab had been the only licensed RSV immunisation for 25 years.[15] Due to high costs and monthly administration, it was only available for high-risk groups in HICs. A maternal vaccine based on RSV preF-protein (RSVpreF)[19] and two mAbs (nirsevimab and clesrovimab)[18,20] have received regulatory approval in various HICs and UMICs to protect infants in the last few years. First-year experience with nirsevimab showed greater than 80% effectiveness against RSV-associated hospitalisation. The RSV vaccine and mAb landscape is still active with dozens of agents in development for infants, toddlers, and at-risk (older) adults.

Vaccine trials: safety, efficacy, and immunogenicity

Vaccine trials are required to bring new products to market. Phase 1 trials assess dosage and safety, while phase 2 and 3 trials also assess efficacy against clinical endpoints, side-effects, and immunogenicity. Safety outcomes include adverse events (such as local and systemic adverse reactions), and severe adverse events (e.g. ERD, premature birth, low birthweight, or death). Clinical endpoints used to assess vaccine efficacy typically include RSV-associated hospitalisation or medically-attended infections. Immunogenicity is commonly tested in serum: antibody geometric mean titres (GMT) and/or neutralising antibodies function as a correlate of protection. In post-market safety surveillance and effectiveness studies, similar endpoints are tested in larger cohorts that represent the real population rather than those adhering to strict in- and exclusion criteria of trials.

Global access to immunisation

The world health organisation (WHO) and the Strategic Advisory Group of Experts on Immunisation (SAGE) recommend that every country should adopt measures to prevent severe RSV in infants.[64] When deciding to use maternal vaccination and/or monoclonal antibodies, factors such as cost, cost-effectiveness, financing, supply, expected coverage and the practicality of integrating these measures into the current health-care system should be taken into account.

To address global inequity in child health, equitable access to immunisation is required. From an ethical standpoint, it is essential that RSV prevention products are made available first where the disease burden is highest. Addressing RSV is essential to achieve the United Nations (UN) Sustainable Development Goal (SDG) 3.2: ending preventable deaths in new-borns and children under five.[68] In reality, no form of RSV vaccine or antibody is currently available in the public sector in LMICs. Financial support to ensure access to future RSV vaccines in LMICs can be provided by Gavi, the Vaccine Alliance (formerly: Global Alliance for Vaccines and Immunisation). This international organisation seeks to increase equitable and sustainable use of vaccines through public-private partnerships and a variety of donors. Depending on Gross National Income, countries may be eligible for vaccine support programs that are based on a co-financing model.[69] In 2018, Gavi decided to provide support for future RSV immunisation products for the 2021-2025 strategic funding period once a suitable product would be WHO prequalified, recommended by the WHO, and meet specified financial assumptions. Following the prequalification of the single-dose vial of the maternal RSVpreF vaccine in March 2025, Gavi approved the RSV maternal vaccine investment case and its introduction into Gavi's portfolio in July,[21] bringing the introduction to LMICs one step closer.

Aim and outline of this thesis

The principle aim of this thesis is to use a multidisciplinary approach to reduce inequitable access to RSV immunisation for infants in low- and lower-middle income countries (LMICs). Five research types on the translational science spectrum were used in the pursuit of global RSV equity:

1. Public health research: to provide an overview of the RSV vaccine pipeline and outline the road to global access
2. Basic research: to develop an assay to measure RSV neutralising antibodies in dried blood
3. Preclinical research: to evaluate the development of resistance to RSV mAbs
4. Clinical research: to evaluate novel affordable protective strategies against RSV infection
5. Implementation research: to assess readiness for implementation of maternal immunisation

Over the course of this PhD research (2021–2025), the field of RSV prevention witnessed major advancements with the market approval of multiple RSV immunisations and their implementation in several countries. This thesis begins with the end in mind, namely global access to RSV immunisation [**part II**]. **Chapter 2** reviews the RSV vaccine and mAb landscape

(as of 2024) highlighting different target populations, antigens, and trial results. As RSV vaccines have not yet reached LMICs, we outline next steps to minimize the vaccine delay to the area with the highest disease burden. As affordability is a major barrier to access to RSV mAb in LMICs, we propose in **chapter 3** that public-health-oriented access-to-medicine mechanisms (such as voluntary licensing and technology transfers to manufacturers) have been successful in ensuring at-scale access to affordable therapies in LMICs. The Maternal Immunisation Readiness Network in Africa and Asia (MIRNA) provides a holistic understanding of vaccine acceptance and implementation readiness. In **chapter 4**, we establish the barriers and facilitators to maternal immunisation in Soweto, South Africa, as experienced by local health care professionals (HCPs) and community representatives as part of the MIRNA study. During co-creation workshops, stakeholders recommend solutions to the perceived barriers to locally improve maternal immunisation supply and demand.

Part III exemplifies how simplicity can serve as a powerful enabler of inclusivity in RSV research, benefiting both participant populations and the global research community. **Chapters 5** and **6** present findings of a phase 1 trial with a novel RSV mAb, RSM01, developed by the Gates Medical Research Institute. In this trial, we compare the serum and dried blood samples of immunised adults head-to-head. In **chapter 5** we develop and validate a neutralisation assay to measure RSV neutralising antibodies in the participants' dried blood. This assay produced data for **chapter 6**, where the dried blood samples replace serum for pharmacokinetic, neutralisation, and immunogenicity bioanalysis. We transfer the RSV neutralisation assay to a Gavi-eligible country, thereby strengthening the laboratory research capacity in **chapter 7**. Reliable measurement of RSV neutralising antibodies in a Gavi-eligible country and the use of dried blood can contribute to inclusion of LMICs in RSV vaccine development and ultimately access.

In **part IV** we develop affordable RSV mAbs. **Chapters 8** and **9** describe the industry-independent development of palivizumab nose-drops for infants. First, we investigate the safety and efficacy of daily intranasal mAb prophylaxis with palivizumab on RSV infection in a phase 1 (healthy adults) and phase 2b (healthy preterm infants) randomised controlled trial in **chapter** 8. We then evaluate the lack of efficacy of intranasal mAb to prevent respiratory infections in the first out-patient RSV controlled human infection model in healthy adults in **chapter 9**. In **chapter 10**, we assess the potential risk for emergence of mAb resistant mutants (MARMs) after global RSV mAb introduction by simulating RSV replication under mAb pressure in a laboratory setting. MARMs are developed for three RSV mAbs: palivizumab, nirsevimab, and RSM01. We characterise in-vitro cultured MARMs, evaluate naturally occurring RSM01 resistance-associated polymorphisms, and the susceptibility of global contemporary RSV strains to RSM01.

Finally, **chapter 11** presents the general discussion of this thesis, in which we review and contextualize the key findings. We emphasize that RSV immunisation equity requires a paradigm shift in how translational science is conducted from a trickle-down innovation pattern to equity-by-design principles.

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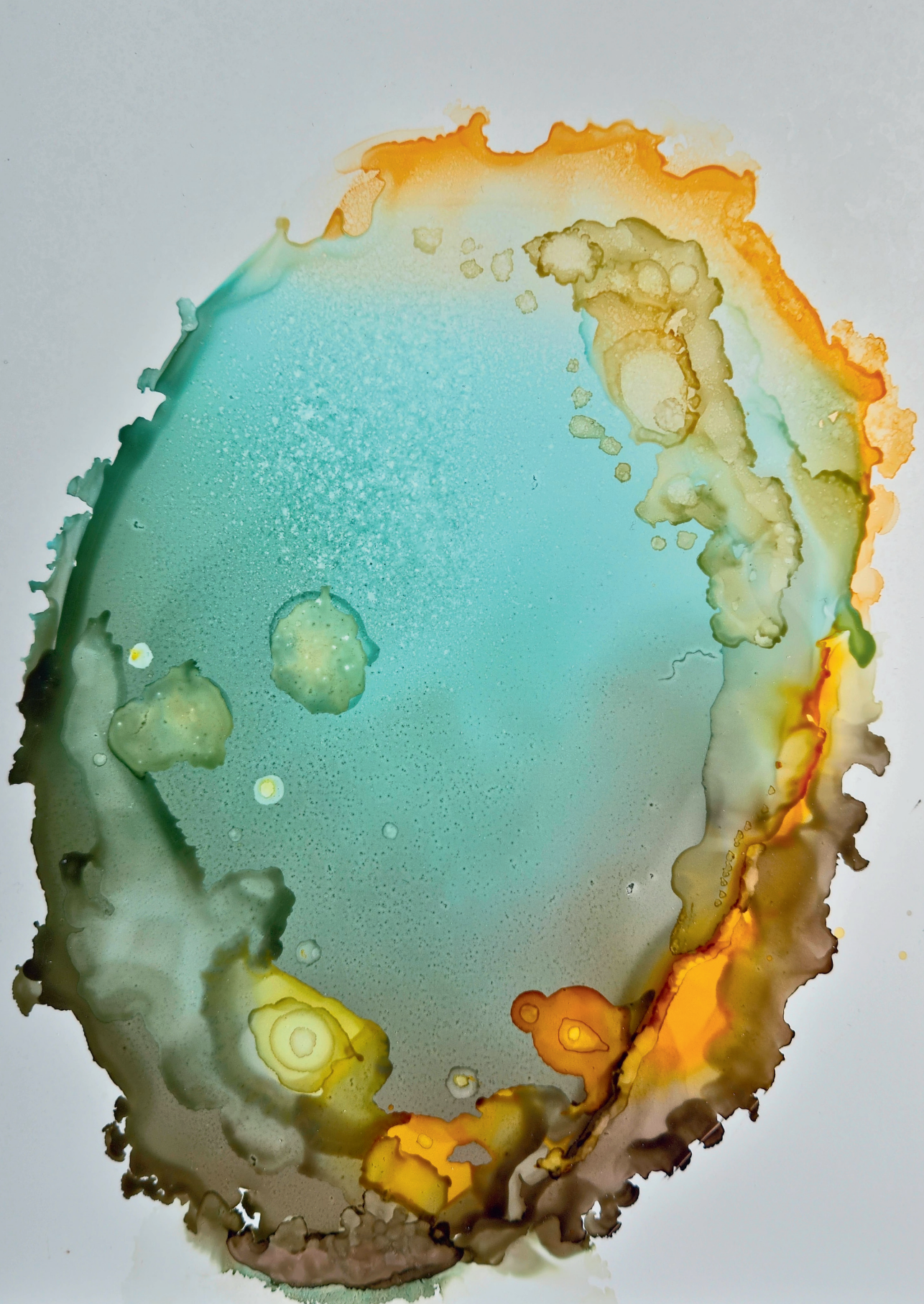
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Part II

Begin with the end in mind: global access to RSV immunisation





The RSV vaccine and monoclonal antibody landscape: the road to global access

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The Lancet Infectious Diseases, 2024

Summary

Respiratory syncytial virus (RSV) is the second most common pathogen causing infant mortality. Additionally, RSV is a major cause of morbidity and mortality in older adults (age ≥60 years) similar to influenza. A protein-based maternal vaccine and monoclonal antibody (mAb) are now market-approved to protect infants, while an mRNA and two protein-based vaccines are approved for older adults. First-year experience protecting infants with nirsevimab in high-income countries shows a major public health benefit. It is expected that the RSV vaccine landscape will continue to develop in the coming years to protect all people globally. The vaccine and mAb landscape remain active with 30 candidates in clinical development using four approaches: protein-based, live-attenuated and chimeric vector, mRNA, and mAbs. Candidates in late-phase trials aim to protect young infants using mAbs, older infants and toddlers with live-attenuated vaccines, and children and adults using protein-based and mRNA vaccines. This Review provides an overview of RSV vaccines highlighting different target populations, antigens, and trial results. As RSV vaccines have not yet reached low-income and middle-income countries, we outline urgent next steps to minimise the vaccine delay.

Key messages

- A maternal vaccine (based on respiratory syncytial virus [RSV] prefusion F protein, RSVpreF) and monoclonal antibody (mAb, nirsevimab) have received regulatory approval in various high-income and upper-middle income countries to protect infants. First-year experience with nirsevimab showed greater than 80% effectiveness against RSV-associated hospitalisation.
- Vaccines based on the RSVpreF are available to protect older adults and an RSV mRNA vaccine is approved. Co-administration with vaccines against other pathogens and revaccination schedules are being studied in late-phase trials.
- An alternative mAb to nirsevimab (clesrovimab) is currently being studied in a phase 3 trial with possible market approval in 2026. One mAb (RSM01), specifically developed for low-income and lower-middle-income countries (LMICs) has completed a phase 1 trial. Additional mAbs on the market could affect supply and demand issues, LMIC access, and viral resistance.
- A live-attenuated RSV vaccine to reduce medically attended RSV infection among older infants and toddlers is currently in a phase 3 trial. It is unclear whether paediatric vaccines will have an effect on RSV transmission and could indirectly protect young infants and older adults (age >60 years).
- Access to infant RSV prevention in LMICs is at high risk of a minimum of 3 years delay for introduction compared with high-income countries. Closing the global vaccine gap is an urgent priority shared by multiple stakeholders.

Introduction

Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract infections (LRTIs) globally and the second leading pathogen to cause infant mortality.[1] It is estimated that RSV accounted for more than 100 000 deaths among children younger than 5 years in 2019 of which more than 97% occurred in low-income and middle-income countries (LMICs).[2] In high-income countries (HICs), 1.8% of healthy term infants are hospitalised for RSV during the first year of life.[3] Underlying medical conditions, prematurity, and socioeconomic vulnerabilities increase the risk of RSV hospitalisation,[4–7] although three-quarters of hospitalised infants were previously healthy.[8] While RSV-LRTI incidence and hospital admission rates are highest in young infants, older infants (age 6–12 months), and toddlers (age 1–2 years) also bear a substantial burden.[2]

In older adults (age ≥60 years) and adults with underlying comorbidities, RSV is an important cause of severe respiratory illness, comparable with seasonal influenza.9 The incidence of RSV-associated hospitalisation in HICs is estimated at 0.15–0.18%,[10,11] but adjusting for under-ascertainment due to diagnostic limitations in adults suggests the true burden might be twice as high.[10,12] The in-hospital case fatality rate might be as high as 7.1% in the general older adult population, and up to 11.7% in adults with comorbidities.[11]

Currently, there is no treatment for RSV infection, underscoring the need for preventive measures. The first paediatric RSV vaccine in the 1960s used formalin-inactivated RSV. Despite a surge in anti-RSV antibody titres, subsequent natural RSV infection led to hospital admission in 80% of the vaccinated children and the tragic deaths of two toddlers.[13,14] The mechanism of their deaths was identified as enhanced respiratory disease in which RSV-naive recipients were primed to produce non-neutralising antibodies and a T-helper 2-biased immune response causing airway hyper-reactivity and mucus hypersecretion.[15,16] Consequently, the trial was stopped and RSV vaccine research was stalled for decades. In 1998, the development of palivizumab—a short-acting monoclonal antibody (mAb) against RSV fusion (F) protein—served as proof-of-principle of infant protection against RSV-LRTI via passively acquired antibodies.[17] However, due to its high costs and need for monthly injections, palivizumab use is reserved for high-risk infant and paediatric populations. The breakthrough stabilisation of F-protein in prefusion (preF) morphology was the tipping point in RSV vaccine development.[18,19] The preF antigen now serves as the basis of all five licensed vaccines and 11 of 30 vaccines and mAbs in clinical development.

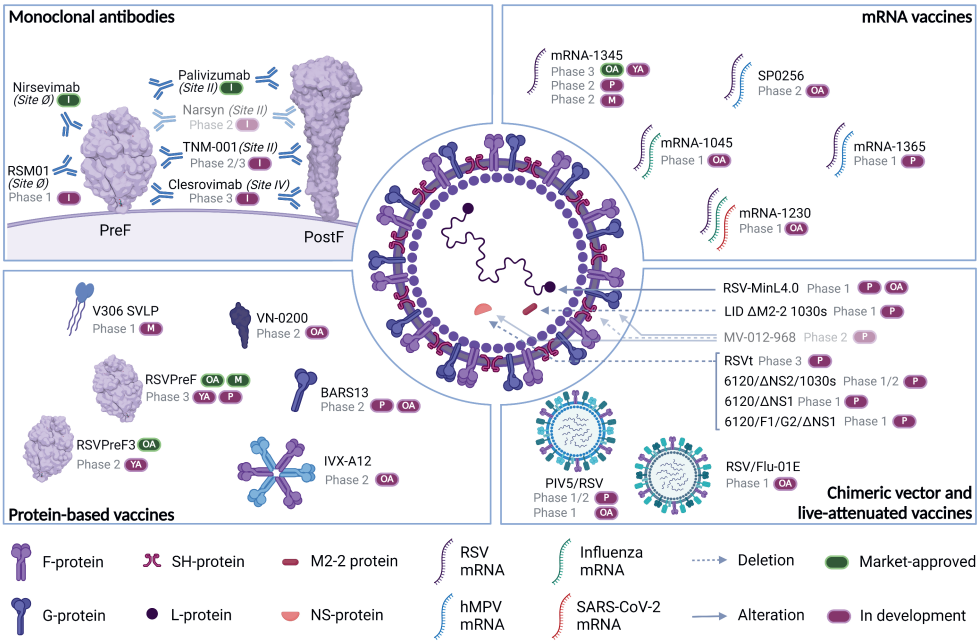
In 2023, we conducted a review of the RSV vaccine and mAb landscape,[20] distilling the lessons learned from late-phase vaccine failures and identifying 33 candidates in clinical trials. This updated review provides an overview of the drastic developments in the field over the past year. Of the 33 candidates, five have reached the market, 17 have continued development, 12 are no longer in development,[21–30] while 13 new candidates have expanded the field. Currently, RSV prevention is only available in HICs and upper-middle-income countries (UMICs) while the burden of life-threatening disease predominantly exists in LMICs. This

inequitable distribution delays vaccine access for those who need a vaccine most. In this Review, we outline urgent next steps to reduce this delay.

Methods

Vaccine and mAb candidates that are market-approved or in clinical phases of development were identified using the PATH (centre for vaccine innovation and access) RSV Vaccine and mAb snapshot resource (last updated Jan 5, 2024).[31] Based on the PATH clinical trial tracker, trial registrations, and personal communications, 13 more candidates were identified and two are considered no longer in development.[32] A data collection template[20] was updated and filled out with targeted searches of PubMed, clinical trial registries, regulatory bodies, and pharmaceutical websites using the candidate names with no date or language restrictions (**Supplement**). 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. Information was selected by date and relevance. When available, peer-reviewed publications were preferred to information from trial registries or pharmaceutical websites. Additional data were collected from the RSV Vaccines for the World Conference organised by the Respiratory Syncytial Virus Network (ReSViNET) Foundation from Feb 13–16, 2024. During the meeting, information was systematically collected from scientific presentations, posters, and discussions using the data collection template. Preventive interventions were divided into four categories: protein-based, chimeric vector and live-attenuated, mRNA, and mAbs (**Figure 1**). The **table** provides an overview of recently approved vaccines and mAbs, while **Figure 2** presents all candidates in clinical development. Detailed vaccine characteristics, such as mechanism of action, route of administration, and summary of trials have been compiled in the **Supplement.**

Figure 1. Overview of RSV vaccine candidates by preventive approach



Target population per vaccine candidate is indicated by green and purple ovals (I, infants age <12 months; P, paediatric age 1–18 years; M, maternal; OA, older adults age ≥60 years; YA, younger adults age 18–60 years). Narsyn and MV-012-968 are depicted in light grey as their development is halted. PreF protein was created with RCSB Protein Data Bank 4MMU[19,33] and postF protein was created with 3RRT.[34,35] F, fusion; G, glycoprotein; hMPV, human metapneumovirus; L, large polymerase; NS, non-structural; PIV5, parainfluenza virus 5; postF, post-fusion protein; preF, prefusion protein; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SVLP, synthetic virus-like particle.

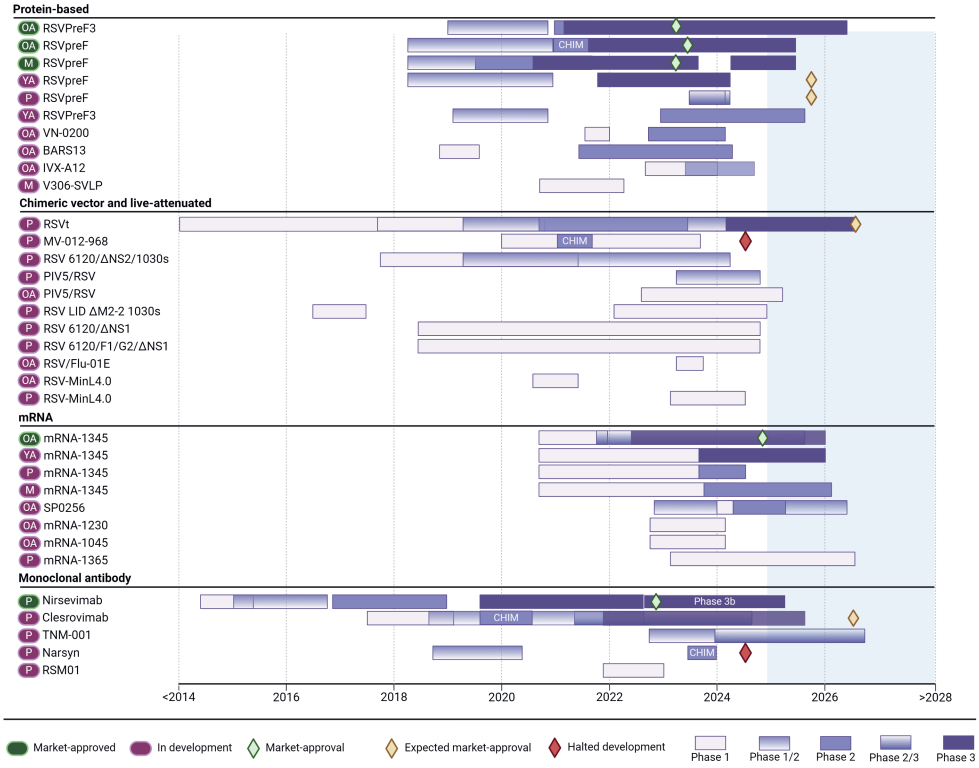
Table. Overview of recently market-approved RSV vaccines and mAbs

	Antigen	Adjuvant	Mechanism of action	Registered population	Route of administration
RSVpreF3	PreF	$AS01_E$	Stabilised monovalent preF induces anti-preF IgG with neutralising activity and CD4+ T cells	Older adults (≥ 60 years)	Single dose, intramuscular
RSVpreF	PreF	None	Stabilised bivalent preF induces anti-preF IgG with neutralising activity and CD4+ T cells	Older adults (≥ 60 years)	Single dose, intramuscular
RSVpreF	PreF	None	Stabilised bivalent preF induces anti-preF IgG with neutralising activity and CD4+ T cells; antibodies are transferred to the fetus across the placenta	Maternal; WGA varies based on registration bodies§	Single dose, intramuscular
mRNA-1345	PreF	None	Lipid nanoparticle contains mRNA encoding for stabilised RAV-A preF eliciting nAbs	Older adults (≥ 60 years)	Single dose, intramuscular
Nirsevimab	..	None	Passive neutralisation by mAb targeting RSV preF site Ø with YTE mutation in Fc for extended half-life	Infants (≤ 12 months)	Single dose of 50 or 100 mg, intramuscular

*It is important to note that these data only cover the first months after administration when effectiveness is expected to be at its highets. Real-world effectiveness data from prospective cohort studies covering the entire first year of life are necessary. †Non-exhaustive examples that are subject to change. ‡Although available year-round, a seasonal approach is recommended. §For example, 24-36 WGA (European Medicines Agency, Pharmaceuticawls and Medical Devices, and Anvisa), 28-36 WGA (Medicines and Healthcare Products Regulatory Agency), or 32-36 WGA (US Food and Drug Administration. .., not applicable; mAb, monoclonal antibody; MA-LRTI, medically-attended lower respiratory tract infection; PICU, paediatric intensive care unit; preF, prefusion protein; RSV, respiratory syncytial virus; WGA, weeks gestational age; YTE, Met252Tyr, Ser254The, and Thr256Glu mutation.

	Registration trial results	Real-world coverage and effectivenss results*	Examples of implementation†
	Efficacy of 94.1% (95% CI 62.4–99.9) against severe RSV-LRTI over one season; acceptable safety profile[36] (NCT04886596)	USA: effectiveness of both RSVpreF3 and RSVpreF of 73–82% against RSV hospitalisations in four studies[37] with general coverage of 24.4% in adults older than 60 years[38]	Year-round in general population ≥ 60 years (Austria) or ≥ 75 years with shared decision making (USA, Australia, and UK);‡ additional use in high-risk population ≥ 60 years (USA and Australia)
	Efficacy of 85.7% (96.66% CI 32.0–98.7%) against RSV-LRTI with three or more symptoms over one season;[39] acceptable safety profile[40] (NCT05035212)		
	Efficacy of 82.4% (95% CI 57.5–93.9) against severe MA-LRTI within 3 months after birth and 70.0% (95% CI 59.6–82.5) within 6 months after birth;[41] numerical imbalance in premature births, not statistically significant[42] (NCT04424316)	USA: 17.8% coverage	Year-round at 28-36 WGA (UK and Australia); year-round at 32-36 WGA (Argentina); year-round at 32-36 (USA)‡
	Efficacy of 83.7% (95% CI 66.0–92.2) against RSV-LRTI with at least two symptoms in primary analysis (NCT05127434)[40]	..	..
	Efficacy of 78.6% (95% CI 48.8–91.0) against very severe MA-LRTI and 76.8% (95% CI 49.4–89.4) against RSV hospitalisation;[43] no safety concerns within 6 months after immunisation[44] (NCT03979313)	Spain: effectiveness of 82.0-88.7% against RSV hospitalisation in four studies with reported coverage of 91.7-92.0%[45-48] and effectiveness of 81.0% against RSV-LRTI;[49] France: effectiveness of 83.5% against RSV bronchiolitis hospitalisation[50] and 75.9% against RSV PICU hospitalisation;[51] Luxembourg: 62% decrease in RSV hospitalisation compared with previous year in population with 84% coverage;[52] Italy: 0% RSV hospitalisations in nirsevimab group compared with 8.3% in no-nirsevimab group in a population with 69% coverage;[53] USA: effectiveness of 90% against RSV-associated hospitalisation and coverage by jursidiction between 2.4 and 22.8%[38]	Seasonally at birth and follow-up in children about to enter their first RSV season (Italy, Australia, Chile, Luxembourg, and France), children ≤ 6 months (Spain), ≤ 8 months (USA) additional use in high-risk children ≤24 months (Spain, Australia, and Luxembourg) or 8-19 months (USA); use in high-risk population and if available also seasonally in children ≤3 months (Finland)

Figure 2. RSV vaccine and monoclonal antibody clinical development over the last 10 years and expected market access



All respiratory syncytial virus (RSV) vaccines and monoclonal antibodies that have recently been market-approved (green oval) or are still in clinical development (purple oval). As of May 2024, trials and market approval are projections based on the available information (blue background) and current development can be found on the PATH RSV vaccine and monoclonal antibody snapshot.[31] Trials of the same phase overlapping in time are compressed to one box for ease of reading. Detailed information on clinical trial timing can be found in the **supplement** (pp 3–9). Market approval is depicted as the first registration date and expected marketapproval is projected as 1 year after (expected) interim phase 3 results or as publicly available intention of submission to regulatory bodies. CHIM, controlled human infection model; I, infants younger than 12 months; P, paediatric age 1–18 years; M, maternal; OA, older adults age ≥60 years; YA, younger adults age 18–60 years.

RSV vaccine and mAb landscape

Protein-based vaccines

Protein-based vaccines include subunits, such as RSV preF protein or glycoprotein (G) as single antigens and particle-based vaccines that might achieve increased immunogenicity by displaying multiple antigens. These proteins facilitate rational vaccine design by using structural information and protein engineering to preserve and optimally display antigenic sites most susceptible to neutralisation. Three subunit vaccines were market-approved in 2023 and seven other candidates are still in clinical development.

RSVPreF3 (Arexvy), a stabilised monovalent preF vaccine based on RSV-A F-protein adjuvanted with AS01E,[55] is approved for older adults (age ≥60 years) in the European Union (EU) and major HICs including the USA, Japan, the UK, Canada, and Australia (May, 2023–January, 2024). In clinical trials, RSVPreF3 showed a vaccine efficacy of 67.2% against RSV-LRTI and 78.8% against severe RSV-LRTI across two RSV seasons (ie, for 18 months).[36,56,57] Peak vaccine efficacy against RSV-LRTI was estimated at 88% with a monthly waning rate of –2.1%. Extrapolating these data, a single dose of RSVPreF3 might provide some protection after two seasons (efficacy 39.7% at 24 months follow-up).[58] Preliminary results show that revaccination after 2 years provides higher RSV-A and RSV-B neutralising antibody titres than annual revaccination.[59] Results from a phase 3 trial (NCT05590403) in younger adults (50–59 years) at increased risk of RSV disease due to comorbidities indicate non-inferior humoral responses compared with older adults (>60 years),[60] prompting label expansion to adults aged 50–59 years at increased risk.[59] Co-administration with quadrivalent influenza vaccine elicits non-inferior immune response.[61] Ongoing studies in older adults evaluate the safety and immunogenicity of co-administration with several influenza (NCT05568797 and NCT05559476) and herpes zoster vaccines (NCT05966090) and optimal revaccination schedules (NCT04732871).

Special attention for safety concerns in maternal RSV vaccination have centred on the potential for increased occurrence of preterm births and low birthweight, which arose from the halted RSVPreF3 maternal vaccine trial (NCT04605159) conducted during the COVID-19 pandemic. The statistically higher risk of preterm birth in the total RSVPreF3 group compared with placebo was driven by the increased risk in LMICs (relative risk [RR] 1.56, 95% CI 1.17–2.09), which was not found in HICs (RR 1.04, 95% CI 0.68–1.57).[62] Most premature births occurred 4 weeks or more after vaccination, were predominantly late preterm births, and were mostly coinciding with peaks of COVID-19, most strikingly during the Delta wave and remain unexplained.[30]

RSVpreF (Abrysvo), a non-adjuvanted bivalent preF vaccine based on currently predominant RSV-A and RSV-B strains, obtained market-approval for administration in older adults (August, 2023) and pregnant women (March–August, 2023) in the USA, Canada, Argentina, Japan, and the EU. The RENOIR phase 3 trial conducted in older adults showed a robust vaccine efficacy over two RSV seasons of 84.4% against RSV-LRTI with at least three symptoms and 56.8% efficacy against RSV-LRTI with at least two symptoms.[63] Co-administration studies of RSVpreF with inactivated influenza vaccine showed immunogenic non-inferiority without safety concerns.[64] Results from a phase 2 study on safety and efficacy of a combined RSV-COVID-19 vaccine when given with or without influenza vaccine are pending (NCT05886777). Efficacy in older people who are immunocompromised and those with major comorbidities remains uncertain as these populations were excluded from the trials.

RSVpreF for pregnant women induces polyclonal vaccine-specific antibodies that cross the placenta to protect infants up to 6 months after birth. The pivotal MATISSE phase 3 trial

enrolled 7396 pregnant participants between 24 and 36 weeks of gestational age during two seasons across both the northern and southern hemispheres.[42] Final analysis revealed high efficacy against severe medically attended LRTI (MA-LRTI) of 82·4% within 3 months after birth and 70·0% for the first 6 months of life. Against less severe MA-LRTI, the vaccine showed an efficacy of 57·6% within the initial 3 months and 49·2% for the first 6 months after birth.[41] Efficacy wanes after 6 months for both severe and less-severe MA-LRTI.[41] There were no safety concerns as slight numerical imbalances in birth outcome lacked statistical significance (prematurity of 5·7% in the RSVpreF group *vs* 4·7% in the placebo group and low birthweight 5·1% in the RSVpreF group *vs* 4·3% in the placebo group).[41] The mean duration between vaccination and delivery was similar between groups (57·6 days for RSVpreF group and 57.9 days for placebo) and the mean gestational age at delivery was 39.1 weeks in both groups. The numerical imbalance was only seen in UMICs[65] and some maternal risk factors (COVID-19, previous history of preterm births, maternal adverse events, or hypertensive conditions) were reported more commonly in the RSVpreF group and among those with preterm births. The strict exclusion criteria for the RSVpreF MATISSE trial, which were similar to the RSVPreF3 maternal vaccine trial, resulted in lower prematurity rates in South Africa in the study population (5%) compared with the general population (15%).[66] Therefore, there is a need to assess risk of prematurity after vaccination in a population with high prematurity rates. A systematic review and meta-analysis of safety results of six randomised controlled trials describing three RSV maternal vaccines (n=17 991 pregnant women immunised with RSVPreF3, RSVpreF, or RSV-F nanoparticle compared with placebo) observed a potential higher risk of preterm births in the vaccine group and further investigation of the preterm safety signal is warranted.[67] For this reason, the Pan-American Health Organization and the US Food and Drug Administration have recommended the vaccine for use later in pregnancy (32–36 weeks gestational age) than the European Medicines Agency (24–36 weeks gestational age), and the regulatory bodies monitor outcomes in both maternal and infant populations in post-marketing safety studies. The WHO Strategic Advisory Group of Experts on immunisation (SAGE) will make policy recommendations on maternal vaccine use in LMICs in September, 2024. A phase 3 trial is ongoing in South Africa to establish the safety and immunogenicity in pregnant women living with HIV (NCT06325657). A phase 2 trial showed that co-administration of RSVPreF with the Tdap (tetanus, diphtheria, and polio) vaccine during pregnancy induced statistically non-inferior immune responses to those elicited by individual vaccinations, except for the pertussis component.[68] The clinical significance of the reduced antibody response to the pertussis component is uncertain. While 31% of data from the MATISSE study originated from LMICs, the vaccine has so far received market-approval solely in the EU and HICs and UMICs (Argentina, Canada, Japan, the UK, and the USA). The USA introduced the maternal vaccine in the previous RSV season and Argentina has introduced it for the coming season. No early experience data are available yet.

There are five protein-based vaccines in clinical development that use the F-protein as antigens. The first is RSVPreF3 in phase 2 development for immunocompromised younger

adults (≥18 years, NCT05921903). Second and third are RSVpreF vaacines in late-phase development for both children (age 2–18 years, phase 2/3, PICASSO trial, NCT05900154) and younger adults at high risk of severe RSV disease (age 18–60 years, phase 3, MONET trial, NCT05842967) with results expected at the end of 2024. The fourth is V306-SVLP, a particle-based vaccine designed with an F-protein antigenic site II epitope-focused approach rather than the entire fusion protein. The specific IgG response elicited was not translated to the expected increase in neutralising antibody (nAb) titres against RSV-A or RSV-B in the phase 1 trial. Future modifications of the vaccine (eg, generation of F-protein sites Ø and IV peptide mimetics) might improve the immunogenicity.[69] The fifth is the particle-based vaccine IVX-A12 that combines stabilised RSVpreF with human metapneumovirus (hMPV)-preF to induce an immune response against both pathogens.

The particle-based vaccine was well tolerated and immunogenic against RSV-A and RSV-B and hMPV-A and hMPV-B without immune interference in older adults.[70] Two subunit vaccines target other viral antigens: BARS13 and VN-0200. BARS13 is based on the RSV G-protein with cyclosporine A adjuvant to induce regulatory T cells. BARS13 was safe in a phase 1 trial and interim results of the phase 2 trial showed a dose-dependent and frequency-dependent antibody response.[71,72] VN-0200 uses VAGA-9001a as an antigen with MABH-9002b adjuvant, both of which have an undefined biological background. Phase 1 trial results are unpublished while the phase 2 trial is expected to be completed in February, 2024.

Chimeric vectors and live-attenuated vaccines

The paediatric RSV burden is highest in infants younger than 6 months, but substantial burden persists in older infants and toddlers, aged 6–24 months. There is an unmet need for protection, which can be fulfilled by chimeric live virus constructs and live-attenuated RSV vaccines (LAVs). Chimeric live virus vectors can replicate efficiently in the presence of pre-existing RSV immunity, while LAVs cannot. Such vectors are ideal for boosting RSV immunity after RSV vaccination or infection, but typically only express a single RSV protein. Both strategies are administered intranasally and provide a child-friendly solution for mucosal immune priming for future infections.

In the last three decades, the National Institute of Allergy and Infectious Diseases and National Institutes of Health have been dedicated to striking a delicate equilibrium between attenuation and immunogenicity within LAV formulations (**Supplement**). LAVs should be sufficiently attenuated to avert upper respiratory tract infections upon vaccination, while remaining resilient against reversion to wild type and yet immunogenic enough to confer protection upon future RSV exposure. Rational vaccine design informed by basic research has coalesced into three LAV families, each targeting distinct non-essential viral proteins namely M2-2, NS1, and NS2. The lead candidate RSVt (also known as SP0125, VAD0001, or RSV ΔNS2/Δ1313/I1314L) belongs to the ΔNS2 family. The phase 2 trial of two doses of RSVt in children aged 6–18 months showed a favourable safety profile, with transient

rhinorrhoea and nasal congestion observed more frequently in vaccinees.[73] A robust four-fold rise in nAb titres was observed in 62–68% of toddlers,[73] which is associated with protection from disease.[74] Vaccine viral shedding decreased markedly following the second vaccination suggesting immune system priming. A global phase 3 efficacy trial is underway (NCT06252285) and submission for regulatory approval is expected in 2026.[73] In the meantime, the lead candidate is also in a head-to-head comparison with another candidate from the ΔNS2 family (RSV 6120/ΔNS2/1030s, NCT03916185). The two candidates of the ΔNS1 family (RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1) are in a phase 1 head-to-head comparison trial (NCT03596801).

Five other candidates are in early phases of clinical development. PIV5/RSV, a chimeric vaccine based on live parainfluenza 5 (PIV5) encoding the RSV F antigen, is in phase 1/2a development for children aged 18–59 months. Preliminary results show no safety signals and 3.6-fold to 57-fold rise in nAbs over baseline levels in 80% of participants.[75] A phase 1 trial in older adults showed an acceptable safety profile and increased nasal RSV-specific IgA in 48% of participants.[76] RSV-MinL4.0, an LAV with four mutations in the L polymerase gene, is in phase 1 clinical development for both children and older adults (NCT04919108 and NCT04295070). RSV/Flu-01E, using an attenuated influenza vector displaying the RSV F-protein, is in phase 1 clinical development for older adults (NCT05970744). Little information about this candidate is available in the public domain. The development of MV-012-968, an LAV with attenuated NS1, NS2, and G proteins and SH-protein deletion, was recently halted to review the company›s strategic options despite positive results from a phase 1c trial in seronegative toddlers.[77]

mRNA vaccines

mRNA-1345 (mRESVIA), leveraging the same lipid nanoparticle technology as the SARS-Cov-2 SpikeVax, aims to enhance immunogenicity by encoding for a stabilised RSV-A preF protein. The pivotal phase 2/3 trial among older adults revealed efficacy of 63.3% against RSV-LRTI with at least two symptoms and 63.0% against RSV-LRTI with at least three symptoms after extended follow-up (median 8.6 months).[78] The efficacy was consistently higher against RSV-A compared with RSV-B, potentially due to fewer cases observed in the RSV-B subtype or due to the mRNA coding for RSV-A preF.[78] FDA approval was obtained for older adults on May 31, 2024, and regulatory approval is anticipated for the EU and various other countries (Switzerland, Australia, Canada, and the UK).[1–3,79–81] Ongoing trials seek to elucidate the durability of protection, revaccination strategies at both 1 and 2-year intervals, and co-formulation with seasonal influenza or SARS-CoV-2 vaccines (mRNA-1045 and mRNA-1230) to streamline older adult vaccination regimens (NCT05330975 and NCT06060457). mRNA-1345 is also in phase 3 clinical development for younger adults at high risk (age 18–60 years, NCT06067230), phase 2 for pregnant women (NCT06143046) and seropositive children (NCT06097299), and phase 1 as a co-formulation with mRNA encoding hMPV F-protein for seropositive children (mRNA-1365, NCT05743881).

Building upon the promising outcomes of a phase 1/2b trial with SP0256, an RSV mRNA vaccine with unknown viral antigens targeted at older adults, plans were announced for a combination mRNA vaccine targeting up to three respiratory pathogens (RSV, hMPV, and PIV).[82] Presently, SP0256 is undergoing evaluation in a phase 2b trial for older adults as a standalone RSV vaccine (NCT06251024) and phase 1 trials for its combination with hMPV (NCT06134648 and NCT06237296). The company announced a target regulatory submission in 2026.

Immunoprophylaxis with mAbs

Next generation mAbs targeting highly neutralisation-sensitive epitopes located on the RSV preF protein have been engineered with fragment crystallisable region mutations to extend the half-life for an entire RSV season (typically 4–5 months). Palivizumab, with limited potency and without extended half-life mutations,[83] was the only market-approved protection against RSV for infants for nearly 25 years. At present, nirsevimab is also in use, and four more mAbs are in clinical development.

Nirsevimab (Beyfortus) targets site Ø of the RSV preF protein and has a mean half-life of 68.7 days.[44] The MELODY registration trial showed an efficacy of 76·4% for MA-LRTI, 78·6% for very severe MA-LRTI, and 76·8% for RSV hospitalisations for 150 days post-dose.[43] In this trial, children followed into their second RSV season were found to remain unexposed to the theoretical risk of enhanced respiratory disease despite suboptimal nAb levels during this period.[84] The burden of disease did not shift to the second year of life as RSV events (MA-LRTI or hospitalisation) were balanced between treatment groups (1·8% in nirsevimab and 2·1% in placebo group).[84] Seroresponse rates to postF were similar between nirsevimab recipients (68–69%) compared with the placebo group (63–70%), suggesting that the presence of nirsevimab still allows an active immune responses upon RSV exposure.[85] In the HARMONIE pragmatic trial, efficacy against RSV hospitalisation was 83·2%.[86] More than 99% of RSV isolates from two clinical trials retained susceptibility against nirsevimab, escape variants were rare, and the binding site was highly conserved over the last 65 years.[87,88] Nirsevimab has been market-approved in the EU and in Argentina, Australia, Brazil, Canada, Chile, China, Saudi Arabia, Switzerland, the United Arabic Emirates, the UK, and the USA (October, 2022 to December, 2023) and is under review in several other countries. The demand was higher than anticipated and exceeded manufacturing capacity,[89] therefore some regions had to limit the use to children at high risk and other regions could not introduce at all.[90] The USA, France, Spain, Portugal, and Luxembourg had a rapid response in integrating nirsevimab into routine immunisation schedules for all children within one season of approval. Nirsevimab real-world early-effectiveness was 82·0% against RSV hospitalisation, 86·9% against severe RSV-LRTI requiring oxygen support, and 69·2% against all-cause LRTI hospitalisations in Galicia, Spain.[45,91] The universal prophylaxis programme showed notable coverage of 91·7% for combined in-seasonal and catch-up immunisation, with no severe adverse events reported among the 9408 administered doses.[45,92] RSV hospitalisations decreased

by 89·8% compared with previous seasons (2016–23).[45,93] A population-based cohort study in Navarre, Spain, with 92·0% coverage showed early-effectiveness of 88·7% against RSV hospitalisations.[46] In line with this, a post-licensure, prospective case-control study in France found real-world effectiveness against RSV hospitalisations to be 83·5%.[50] Finally, Luxembourg had a coverage of 84%, with a 62% decrease in RSV hospitalisations compared with the same period the year before.[52] It is important to note that these data are from the first months after administration when effectiveness is at its highest. Notably, the reduction in hospitalisations primarily affected infants under 6 months (30% of admissions in 2023 *vs* 60% in 2022). As the number of hospitalisations among older infants remained similar, most hospitalisations are in older infants leading to an increased mean age at hospitalisation from 7·8 months to 14·4 months. The increase in age at RSV hospitalisation was also seen in France with a 75% coverage.[94] The real-world experiences recommend flexible appointments for parents, early negotiations to ensure supply, extensive parent education, and simple logistics for successful implementation and high coverage.[95]

Clesrovimab (MK-1654) primarily targets site IV of the RSV F-protein, featuring the same YTE mutation as nirsevimab to extend its half-life to 44.9 days in both preterm and term infants.[96] The site IV epitope exhibits high conservation, with more than 99·9% identity observed among over 3058 reported complete RSV F-protein sequences.[97] Two phase 3 studies are ongoing: the results of a large phase 2b/3 trial are expected in 2024 (NCT04767373) alongside a head-to-head comparison with palivizumab (NCT04938830). Clesrovimab is characterised by improved penetration into the nasal epithelial lining fluid and neutralisation ex vivo in a controlled human infection model compared with other mAbs (with nasal concentrations ranging 1.4–3.3% of serum concentrations *vs* 1–2% in three other mAbs, including nirsevimab).[98] These data support the potential for improved local protection at the site of infection. The differences in pharmacokinetics between clesrovimab and nirsevimab regarding the potential of protection during the second season should be further clarified when the phase 3 trial efficacy results are available.

Three additional mAbs are currently in earlier stages of development. RSM01, a mAb against RSVpreF site Ø with YTE mutations, is developed by the Bill & Melinda Gates Medical Research Institute with a primary focus on addressing the needs of LMICs with a vaccine target price.[99] Preliminary findings from their phase 1 trial in healthy adults revealed an extended half-life of 79 days and a safety profile similar to placebo.[100] Modelling projections estimated that African infants aged 0–12 months might attain serum concentrations four-times higher than the protective threshold 150 days after intramuscular immunisation.[101] Narsyn (palivizumab administered intranasally) exhibits potential for increased accessibility and affordability due to child-friendly administration by parents, but encountered futility in the interim analysis of the phase 2b trial.[102] Timely interim analysis prevented further waste of resources and capital. Lastly, TNM-001 is an extended half-life mAb against RSVpreF with unknown target site. Little information is available in the public domain besides the neutralising capacity of at least 30-fold higher than palivizumab.[103] A phase 2/3 trial is planned but not yet recruiting (NCT06083623).

The alignment of multiple mAbs within the landscape of RSV prevention remains uncertain. Clesrovimab's anticipated registration in 2025, alongside the already approved nirsevimab, begs the question how the two mAbs will coexist. Given the supply challenges encountered in the inaugural season,[89] there could be a need for two mAb producers to meet the increased global demand. Furthermore, having several options with distinct viral epitopes could prove advantageous if resistant mutants become predominant circulating strains under drug pressure. Global introduction of mAbs should be monitored with global genomic surveillance, such as the inclusion of RSV in the WHO Global Influenza Surveillance and Response System sentinel surveillance, to allow immediate action upon the occurrence of substantial resistance.

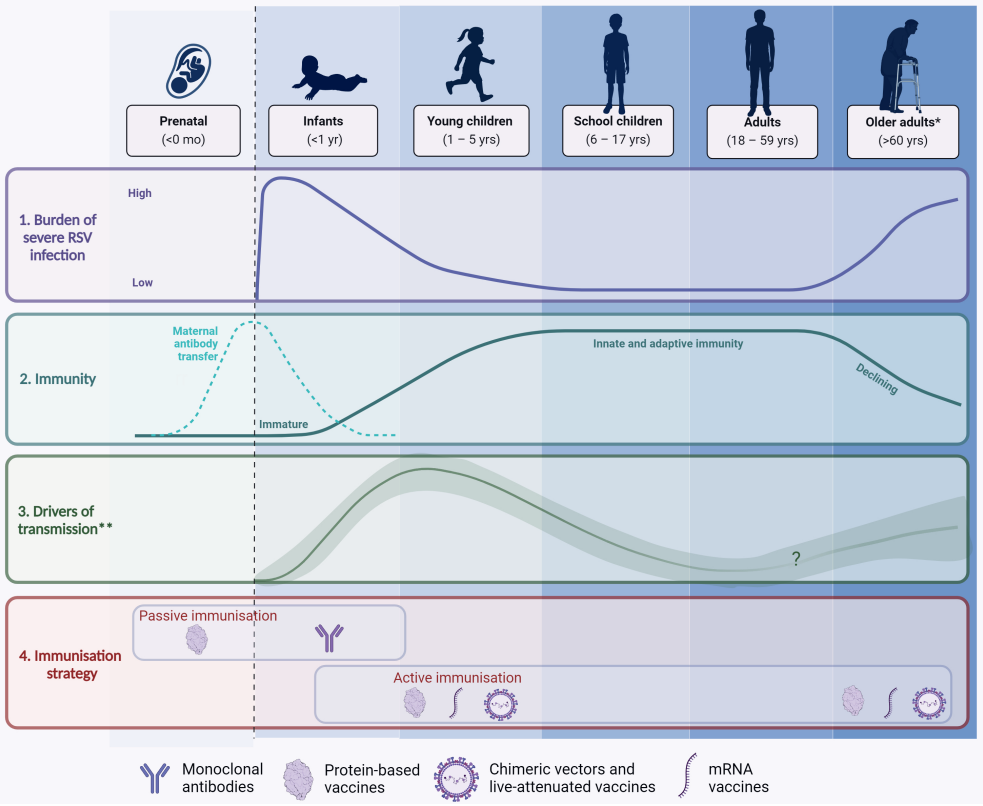
Vaccine strategy and implementation per target population

RSV immunisation strategies, their rationale, and implementation considerations vary among different target populations (**Figure 3**; **Supplement)**. For infants, passive immunisation either by maternal vaccines or long-acting mAbs offers protection against severe RSV infections during the first months of life. With both strategies now available, it is important to consider their comparative advantages and implementation challenges, which are discussed in detail in the **Supplement**. The preferable immunisation strategy (ie, mAbs, maternal vaccine, or combined) and implementation approach (seasonal or year-round) is country-specific, depending on RSV seasonality, costs, sociocultural preferences, and health-care infrastructure. Toddlers and primary school children could be important drivers of RSV transmission,[104,105] but it is unclear whether paediatric RSV vaccines could indirectly protect infants and older adults. Adult RSV vaccination shows promise for protecting susceptible individuals, yet determining the specific target group requires careful consideration with current national guidelines ranging from vaccinating all adults older than 60 years (age-based strategy) to targeting specific groups based on comorbidities (high-risk strategy) and higher age thresholds.[106–108]

Public health effects

Cost-effectiveness of RSV immunisation is essential for country-level decision making, especially in lower-resource settings. RSV vaccination in older adults is estimated to be cost-effective in HICs.[109,110] The two strategies available for infants can be cost-effective in HICs,[111–113] but in LMICs affordability remains a barrier[114] although the clinical effect is expected to be substantially higher than in HICs. Reduction in RSV-LRTI is also expected to reduce all-cause LRTI, RSV and all-cause deaths, hospital bed occupancy, oxygen and antibiotic use, recurrent LRTIs and wheezing episodes in toddlers, and generally improve lung health.[46,115,116] The **Supplement** provides a more detailed discussion on public health and cost-effectiveness considerations.

Figure 3. Conceptual overview of RSV prevention strategies per target population



The primary target populations for RSV immunisation include infants, young children, and older and high-risk adults. Active immunisation is the preferred strategy for the paediatric and adult population, whereas for infants, passive immunisation—either by monoclonal antibodies or maternal immunisation during pregnancy—is the preferred approach.RSV, respiratory syncytial virus.*Including younger adults (age 18–60 years) with underlying comorbidity at high risk for severe RSV infection. **Despite young children being recognised as major contributors to RSV transmission, the precise role of specific age groups in transmission remains uncertain. Also, the potential effect of vaccines targeting young children on RSV transmission and their indirect protective effect on infants and high-risk and older adults is yet unknown.

Implementation considerations and challenges in LMICs

Specific implementation considerations arise in LMICs additional to the general considerations (and outlined in the vaccine strategy and implementation per target population section and in the **Supplement**, due to resource constraints, lack of RSV seasonality near the equator, limited vaccine access, and the potential effect of more prevalent maternal comorbidities (eg, HIV and malaria) unaddressed in clinical trials. Maternal vaccines are accessible due to pricing, although maternal vaccination programmes might not be well established in all LMICs. The relatively high prevalence of preterm births and HIV and malaria during pregnancy could affect maternal vaccination efficacy or the duration of protection. The numerical imbalances in preterm births observed with RSVpreF and RSVPreF3 were restricted to

UMICs, necessitating further evaluation in LMICs and possibly delaying introduction in these settings potentially with initial use restricted to later gestational age, which has a major effect on feasibility due to challenges to assess gestational age in LMICs. Recent modelling showed that vaccination at 24 weeks gestational age could prevent 94% of in-hospital RSV mortality among infants younger than 6 months, while delaying it to 32 weeks gestational age could prevent 82% of RSV deaths.[117] The potential safety issues need to be carefully balanced with the potential reduction in the number of deaths caused by vaccinating mothers early in pregnancy using formal risk–benefit analysis. While the high burden of RSV in LMICs favours cost-effective interventions, pricing remains a crucial factor in implementation strategy for both mAbs and maternal vaccination. Seasonal approaches are cheaper as less product is used, but year-round immunisation with either mAb or maternal vaccination might be more achievable in countries with weaker health-care systems. In the end, cost and cost-effectiveness are likely to drive implementation strategy.

There is a high risk of an RSV vaccine gap between HICs and LMICs of several years (**Figure 4a**) further increasing the already existing gap in disease burden. Stakeholders, such as pharmaceutical companies, the Global Alliance for Vaccines and Immunisation (Gavi), WHO, the Bill & Melinda Gates Foundation (BMGF), PATH, regulatory and academic institutions, countries, health-care providers, families, patients, and other relevant health-care stakeholders can take steps to ensure RSV vaccine equity and overcome implementation challenges in LMICs (**Figure 4b**).

First, many LMICs have variable and prolonged regulatory pathways. Recognising this, the SAGE RSV working group evaluates available evidence to inform SAGE in making recommendations for LMICs, given that pivotal clinical trials might not always provide sufficient evidence for decision making in LMICs. The SAGE recommendations expected in September, 2024 will be crucial for Gavi support for RSV vaccines. In parallel, SAGE is undertaking a real-world (ie, few exclusion criteria) impact study in three to four LMICs to better understand the effectiveness and effect of the maternal vaccine against RSV and all-cause LRTI and hospitalisation and safety, including the risk of preterm births. The proposed study is anticipated to start in late 2024, will include two RSV seasons, and its results will be highly relevant to LMIC policy makers, although the trial might also delay vaccine introduction. However, SAGE should broaden their recommendations and impact trial to include mAbs for LMICs to increase the imperative to reduce mAb pricing and thus reduce global inequity.

Second, vaccine pricing is prohibitively high for LMICs. Efforts to introduce clesrovimab beyond HICs and develop RSM01 specifically for LMICs at vaccine-like pricing offer promise in the future,[20,99] but high production costs prove difficult to keep prices to the level needed for global access, necessitating innovations to enhance production efficiency and lower costs for mAbs.[99] Gavi support is indispensable to ensure global accessibility of RSV prevention. Therefore, RSV vaccination was added to the vaccine investment strategy 5.0

(2021–25). SAGE recommendations will be crucial to develop a refined investment case for Gavi board decisions and programme design. Ways to keep pricing down for LMICs include licensure for generics-production, variable pricing mechanisms, and patent pooling.

Third, RSV immunisation products would ideally be co-administered with other vaccines to reduce the strain on the health-care system. However, no data are available on the safety and immunogenicity of co-administration of RSV mAbs with the BCG vaccine at birth. Nonetheless, based on the mechanism of action and experience with palivizumab, nirsevimab is not expected to interfere with the immune response to other vaccines.[123] To date, insufficient data exist on the RSV maternal vaccine administered with the tetanus and pertussis vaccines.

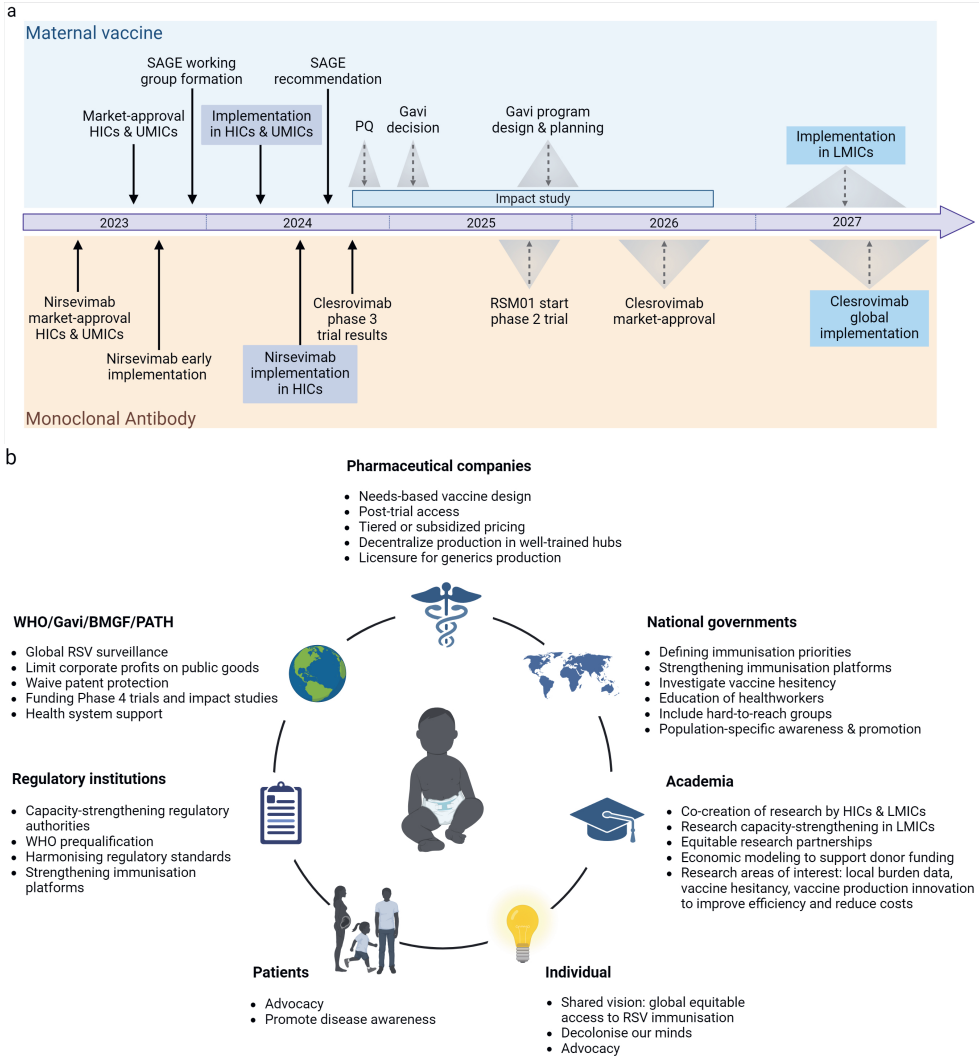
Fourth, LMICs experience competing immunisation priorities: not only are there new vaccines against major pathogens such as malaria, there is also a push to increase uptake of underutilised existing vaccines, such as measles, inactivated polio, and human papillomavirus.

Fifth, inequity in immunisation within LMICs is already a major challenge, particularly affecting hard-to-reach populations, including those residing in remote rural areas, urban low-income communities, conflict-affected regions, and areas facing religion and gender-related barriers. Strengthening of immunisation platforms within countries to include all children and adults might be of higher priority to policy makers.

Sixth, a lack of RSV awareness among both health-care policy makers, health-care providers, and the population in LMICs is a considerable barrier to overcome for successful vaccine implementation. Engagement to build political will is crucial to maximise vaccination uptake as the absence of RSV awareness among policy makers could lead to negative decisions regardless of SAGE recommendations. The RSV Roadshow was created by WHO and PATH as a communications toolkit[124] to advance the RSV prevention conversation to support global, regional, and country decision making around RSV prevention, policy, and implementation preparedness. WHO also generates country-specific burden data from their global surveillance data generation and packaging of existing global RSV data to inform country-level decisions. The perspective of health-care workers is crucial for successful vaccine acceptance,[125] so their education in RSV burden and vaccines could prove beneficial for vaccine uptake.

Additionally, promoting awareness and understanding of RSV among the general population in LMICs, along with improving overall health literacy, should be important for successful uptake of immunisations when available. All in all, barriers including low awareness, lack of country-specific burden data, and cost of products preclude the successful delivery of RSV vaccines in LMICs. The crude birth rates in LICs are 3.5-times higher than in HICs, highlighting the necessity of rapid access to RSV prevention in LMICs.[126]

Figure 4. The road to global access for infant RSV immunisation



a. Projected timeline for global access to RSV prevention for infants with planned (solid arrows) and projected (dashed arrows with uncertainty area) key actions. The WHO SAGE working group was formed in December 2023[118] to draft policy recommendations for RSV prevention in LMICs with a focus on maternal vaccination.[119] Depending on the outcome of SAGE recommendations, it is uncertain if or when following steps will take place. In case of positive SAGE recommendations and WHO prequalification, Gavi might decide to include RSV prevention in their portfolio allowing global access in 2027,[120] 3 years after HICs and UMICs.[121,122] If monoclonal antibodies are not included in the SAGE recommendations, inequitable access to highly effective and safe RSV prevention will remain. Although clesrovimab and RSM01 are dedicated to global affordability, the lag in clinical development delays access in LMICs until at least 2027: it will take at least 2 years to obtain market approval after the end of the clesrovimab phase 3 trial (NCT04767373) in 2024 and another year is estimated to be required to achieve global access. **b.** Key actions per stakeholder needed for equitable global access to RSV prevention. BMGF, Bill & Melinda Gates Foundation; Gavi, Global Alliance for Vaccines and Immunisation; HICs, high-income countries; LMICs, low-income and middle-income countries; PATH, Centre for vaccine innovation and access; PQ, WHO prequalification; RSV, respiratory syncytial virus; SAGE, Strategic Advisory Group of Experts on Immunisation; UMICs, upper-middle-income countries.

Discussion

For the first time since the virus' discovery in 1956 and the tragic results of the first RSV vaccine trial in 1965, we now have effective RSV immunisations for infants and older adults in HICs. However, there is a high risk of years of vaccine gap between HICs and LMICs and urgent action is needed for global access to RSV vaccines. 30 RSV vaccine candidates are still in development, including an LAV in phase 3 for toddlers.

Remaining knowledge gaps in the RSV vaccine landscape include the long-term RSV vaccine effect on early LRTI-induced wheeze and lung health at later ages (**Panel**). Real-world effectiveness data are currently restricted to first experiences with nirsevimab in HICs. However, as additional data emerge in the coming years, across various strategies and settings there is potential for insightful comparisons of real-world implementation, uptake, and effectiveness. As RSV has been associated with both viral and bacterial LRTIs, the protective aspect could be broader than generally anticipated. Moreover, as nasal commensals protect against severe infection via immunomodulatory properties,[127,128] we should investigate how the commensal flora can be leveraged to increase vaccine impact. Although it is most effective to reduce the high RSV burden in infants and older adults using direct immunisation, it is uncertain if the transmission chain could be broken by reducing RSV in toddlers and school-going children (age 2–12 years). The cost-effectiveness of toddler and school-going children vaccination is unknown and would be incremental on previous maternal or infant immunisation. Finally, the potential effect of the introduction of RSV immunisation on the circulation patterns of other respiratory virus infections remains to be elucidated.

Panel. Future RSV vaccine and monoclonal antibody landscape research priorities

- Respiratory syncytial virus (RSV) transmission
- Necessity and optimal timing for booster vaccination
- Co-vaccination strategies
- Safety of maternal vaccination in lower-middle-income countries (LMICs)
- Additional real-world effectiveness data
- RSV genetic surveillance for viral resistance
- LMIC cost-effectiveness data, also driven by out-of-hospital mortality
- Definitive correlate-of-protection
- Effect of vaccination on long-term sequelae, antibiotic use, and microbiome
- Burden of RSV in older adult population
- Tools to increase RSV awareness and vaccine acceptance

To conclude, the RSV vaccine and mAb landscape has come a long way since the first vaccine attempts with formalin-inactivated RSV in the 1960s. We can reduce the economic and clinical RSV burden in infants and older adults in HICs and major collaborative efforts need to be urgently undertaken to reduce the RSV vaccine gap to LMICs.

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Supplement

Supplementary files can be downloaded from:



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Access to highly effective long-acting RSV-monoclonal antibodies for children in low- and middle-income countries – reducing global inequity

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Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infection (LRTI), hospitalisation, and mortality in infants and young children, with the highest burden of severe disease in low-and-middle-income countries (LMICs).[1] The predominant burden of hospitalisation occurs in full-term healthy infants younger than 6 months; however preterm infants are also at risk of severe RSV-LRTI up to the age of 2 years[2] as are children with underlying chronic conditions such as congenital heart disease, lung disease of prematurity, or immunodeficiency. More than 95% of RSV-associated deaths occur in LMICs, with almost two-thirds occurring outside a health facility.[1] A strategy for preventing severe RSV-LRTI in infants in LMICs would have a major impact on child health given the high burden of disease, associated mortality, and limited access to healthcare.

Two new strategies to prevent RSV-LRTI in infants and young children are now available: a maternal prefusion RSV vaccine, RSVpreF, and a long-acting RSV monoclonal antibody (mAb), nirsevimab, given intramuscularly as a single dose to a child prior or during the RSV season.[3-6] Clinical trials found nirsevimab to have a high efficacy (76-80%) against severe RSV-LRTI and medically attended RSV-LRTI through 150 days in preterm and full-term infants, and in young children with underlying conditions.[3-5] Additionally, there was a 35% reduction in all-cause LRTI and 23% reduction in antibiotic prescribing.[3] Use of a long-acting mAb may be useful in infants whose mothers did not receive an RSV vaccine in pregnancy, in preterm babies, or in young children susceptible to severe disease. Population-based effectiveness studies of nirsevimab given to all infants born in the RSV season, with a catch-up campaign for infants born before the RSV season or to children at high-risk aged up to 2 years, confirm that this intervention is highly effective. In Galicia, Spain, where coverage of more than 90% was reached for nirsevimab, substantial reductions in hospitalisation for RSV-LRTI (-82%), hypoxic RSV-LRTI (-87%), all-cause LRTI (-69%), and all-cause hospitalisation (-66%) occurred.[7] Nirsevimab has been approved in at least 35 high-income countries; however, no LMIC has introduced this intervention except China, a upper middle income country (**Figure 1**). This situation exposes glaring disparities in early access and emphasizes the importance of access strategies for RSV prevention for all infants including mAbs in LMICs.

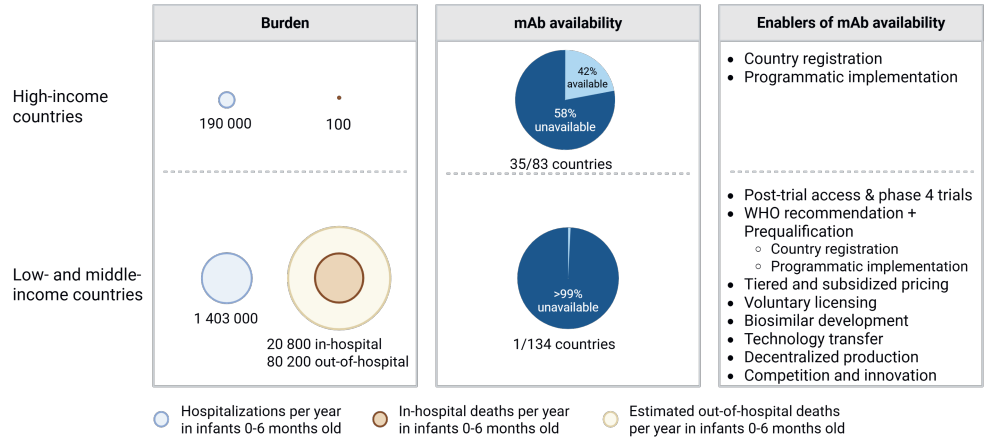
Affordable access to RSV preventive mAbs may be challenging in LMICs.[8] However, public-health-oriented access-to-medicine mechanisms, such as voluntary licensing and technology transfer to multiple manufacturers, have been successful in ensuring at-scale access to affordable therapy in LMICs (eg, for WHO-recommended first-line HIV antiretrovirals that price dropped from several thousand US$ to less than $45 per year).[8] Long-acting RSV-mAbs may provide a unique opportunity for adoption in LMICs and an ideal proof-of-concept of a high-volume model such as the one that enabled broad access to HIV treatment. The single mAb dose (50 mg or 100 mg) for infants suggests a low cost of production per dose (generally referred to as the cost of goods sold [COGS]). The cost of producing a single 50 mg dose of mAb could range from US$5 to $10 and higher volume production could further lower COGS through economies of scale.[9] Even though this estimate excludes other costs (eg, development, regulatory, supply, and general expenses), it highlights the potential

to achieve low COGS that could contribute to an affordable price for mAbs in LMICs. The current capacity constraints faced by suppliers also could be resolved through voluntary licensing and technology transfer, which promotes distributed production, strengthening of the supply chain, and fostering of competition and innovation to drive price reductions and facilitate access in LMICs.

However, achieving successful implementation and ensuring that mAb availability matches the burden of severe RSV disease requires strong collaboration between stakeholders, including originator pharmaceutical companies and public health organizations (**Figure 1**). The COVID-19 pandemic has shown that rapid availability and access to new immunisations are achievable in LMICs. Administration of single-dose RSV preventive mAb to infants could leverage existing national immunisation programmes, many of which already administer a birth BCG vaccine or other vaccines in early infancy. A recommendation by WHO for the use of mAbs for RSV prevention in children is key to promote local and regional approvals and procurement in LMICs. A WHO recommendation would also facilitate the development of a regulatory pathway through WHO prequalification, which might be necessary for biosimilar manufacturers. Supporting regulatory coordination may speed up approval procedures, especially if accompanied by technology transfer maximizing similarity with the originator product. Public sector finance and procurement mechanisms, such as Gavi, the Vaccine Alliance, could play a crucial role in facilitating access and encouraging the adoption of biosimilar products in LMICs that benefit from Gavi funding.

Severe RSV-LRTI is now a preventable disease in infants and young children.[10] Access to affordable preventive interventions for all children, especially those in LMICs, is urgently needed and should be a priority to strengthen child health and global equity.

Figure 1. Availability and enablers of access to long acting RSV preventive mAbs in high and low-middle income countries



Global RSV burden estimates in infants 0-6 months are mismatched with the current mAb availability (i.e. country registration; updated until publication date) in HICs and LMICs. Out-of-hospital deaths for infants 0-6 months were estimated based on the in:out-hospital death ratio of 1:3.86 calculated for children aged 0-60 months.[1] Figure created with Biorender.com. mAb, monoclonal antibody; PQ, prequalification; WHO, World Health Organization.

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Healthcare professionals' recommendations to improve maternal immunisation supply and demand in Soweto, South Africa

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Internal report

Research in context

Evidence before this study

- 5.3 million children under five years died in 2019, with lower respiratory tract infections (LRTIs) and sepsis among the leading causes. Respiratory syncytial virus (RSV) and group B streptococcus (GBS) are major contributors to these deaths, particularly in the first six months of life and in low- and middle-income countries (LMICs).
- Maternal immunisation (MI) offers a means to protect both mother and infant, particularly when infant vaccination is not immediately feasible. MI has already proven effective in reducing neonatal tetanus deaths by 96%, and new MI for RSV and GBS are showing high efficacy. In South-Africa, MI with Tdap (Tetanus-Diphtheria-acellular Pertussis) and influenza vaccines are recommended but barriers prevent 100% uptake.
- Strong implementation of MI is needed to reach the desired improvement in maternal, perinatal, and infant outcomes. Possible strategies to increase MI uptake in LMICs include healthcare provider (HCP) recommendation, digital communication, financial incentives, HCP training, and public–private partnerships. This study aims to co-create context-specific strategies with HCPs to support MI supply and demand.

What this study adds to the existing evidence

- In two co-creation workshops with 13 local HCPs and other community stakeholders each, we established the barriers and facilitators to MI as experienced by the participants.
- Barriers prioritized by workshop participants included lack of HCP education on MI; lack of community awareness; stock shortages; low (human) resources resulting in time constraints for counselling, frustrated staff, and prioritization of South-African citizens over migrants.
- Solutions to the above barriers as proposed by workshop participants include: (1) patient education in waiting rooms, community outreach in familiar settings or via radio/social media, immunization awareness days, or text messaging; (2) HCP education via in-service training or application (app); (3) prevention of stock shortages through improvement of storage facilities, stock system guidelines and prioritization of (high-risk) population groups; (4) better collaboration between HCPs and traditional healers; (5) improved case record documentation; and (6) improved conditions for staff, e.g. stimulating salaries to improve staff shortages, and more effective use of manpower.

Recommendations

- HCPs desire education on current and new vaccines to make informed recommendations. Education should be directly to the staff administering the vaccines rather than via management: in-service training or via micro-education using a daily question in an educational app.
- The community expressed a desire to raise awareness on vaccine preventable diseases. Community outreach at familiar settings, such as churches, require collaboration with community leaders. Immunization awareness days, health promotion in waiting rooms (by posters, pamphlets, televisions, or HCPs), radio/social media coverage by HCPs, or text messages can nudge patients towards MI.
- HCPs and clinics need to be incentivized to provide MI to all patients, including migrants, by mandatory reporting of MI and migrant care and instating these numbers as indicators of good healthcare, to prevent prioritization of South African citizens over migrants.
- Improved documentation in the maternal case record would nudge HCPs to provide the right vaccine at the right time and would inform other HCPs after referrals. The instatement of an electronic patient dossier would be the ideal way to improve patient care.

Introduction

Despite significant declines in mortality in children under 5 years of age over the past 2 decades, there were an estimated 5.3 million deaths in this age group in 2019. The leading causes of death included preterm birth complications, lower respiratory infections (LRTI), intrapartum-related events, and diarrhoea.[1] Respiratory syncytial virus (RSV) is the most common pathogen identified in infants and young children with acute LRTI. With an estimated 3.6 million annual hospitalizations and 101 400 annual deaths, RSV contributes substantially to morbidity and mortality burden globally in children aged 0–60 months, especially during the first 6 months of life and in low- and middle-income countries (LMICs).[2] Group B streptococcus (GBS) is a leading cause of sepsis and meningitis in neonates and young infants and is also an important cause of stillbirth. Globally, 91 900 deaths were estimated to have occurred in children with invasive GBS disease, with the highest numbers in sub-Saharan Africa and Asia.[3]

Although many deaths in the first year of life are attributable to vaccine-preventable diseases, protecting a newborn with active immunization is not always possible - due in part to the maturation of newborn immune system or the requirement of multiple doses, which may be missed or delayed.[4] Maternal immunisation (MI) offers an alternative to combatting neonatal and infant mortality: vaccinating pregnant women induces immunity and protects the mother as well as the foetus and infant against infectious diseases through transplacental transfer of antibodies. MI is a time-tested intervention - maternal tetanus vaccination has contributed to a 96% reduction in the number of tetanus-related neonatal deaths worldwide. In South African guidelines, the TDaP (Tetanus-Diphtheria-acellular Pertussis) vaccine is recommended during pregnancy at 26-34 weeks gestational age or if this window is missed at any later moment including during the immediate post-partum period.[5] During annual influenza campaigns, pregnant women including up to 6 weeks post-partum are among the prioritized groups.[5] New MI are in clinical development for the prevention of RSV disease in infants and group B streptococcus in neonates. A recent Phase 3 study evaluating a RSV prefusion F protein–based vaccine given to pregnant women at 24-36 weeks' gestation showed 82% efficacy against severe RSV LRTI through 180 days after birth.[6] This vaccine has now been approved by the United States (US) Food and Drug Administration (FDA) for use in pregnant women 32-36 weeks of gestational age and is registered in South Africa. A Phase 3b trial is evaluating safety and efficacy of the vaccine in several African countries, including South Africa. A glycoconjugate vaccine is being developed as a maternal vaccine to prevent invasive GBS in young infants and has shown to be safe and immunogenic in a Phase 2 study.[7] As development of these products progresses, vaccine programs will shift away from research and development and towards implementation.

Successful implementation of MI will be key to reach the desired improvement in maternal, perinatal and infant outcomes for RSV and GBS, and reduce neonatal and infant mortality. The COVID-19 pandemic has led to increased vaccine hesitancy in many LMICs and it is important

to understand perceptions of maternal vaccination among pregnant women, communities, health-care practitioners and policymakers, and implement interventions to increase vaccine confidence and demand. Health care professionals' (HCPs) likelihood to recommend the COVID-19 or influenza vaccine was lowest when asked if they would recommend the vaccine to pregnant women compared to adults.[8] Some of the barriers include lack of clear policy guidelines, lack of knowledge among women, and service experience.[9-11] Common facilitators of MI uptake include, for example, a recommendation from an HCP.[9,12] A recent literature review showed that significant increases in vaccine uptake were obtained in LMICs through digital communication and education, financial incentives, HCP recommendations and educational programs, training of HCPs, performance-based financing systems, public-private partnerships, and quality-improvement initiatives (refer to rapid desk review).

This study aims to develop context-specific, tailored strategies to support MI uptake and sustained demand for RSV and GBS vaccination in Soweto, South Africa, through co-creation with HCPs and other key stakeholders.

Methods

Study Design

This study was part of the Maternal Immunisation Readiness Network in Africa & Asia (MIRNA) research project being conducted from 30 September 2024 to 31 December 2026. MIRNA is a collaborative research network working across nine countries in sub-Saharan Africa and South Asia. Within this network, investigators are engaging with key stakeholders to support the country's readiness for future MI. This work includes providing a situational assessment of the health system and delivery platform, a framework for vaccine demand generation and assessing the available and potential evidence on the burden of disease, while considering the cost of vaccine delivery in their context, to inform cost-effectiveness modelling.

For this thesis we used an exploratory qualitative research design by conducting two co-creation workshops on 25 July 25 and 1 August 2025 with 13 participants each as part of the vaccine demand work package of the MIRNA study. Our primary aim was to develop context-specific strategies to support MI uptake in Soweto, South Africa.

Setting

This study was conducted in Soweto (South-Western Townships), a congregation of 29 townships within the Johannesburg Metropolis in South Africa. Soweto is one of the poorest areas of Johannesburg with significant unemployed, underemployed, or employed in the informal economy with youth unemployment standing at 46.3% among the 15-34 year age group. It is inhabited by a low-income, urbanized Black-African community of Zulu, Xhosa, Pedi, Tsonga, Venda, Tswana and Sotho ethnicities, mainly of Christian religious background (mostly Protestant and Charismatic).

Participant recruitment

Participants were purposefully invited to join one of the co-creation workshops. Briefly, health facilities throughout Soweto included in the MIRNA study were visited to obtain approval from facility managers to invite their staff involved with MI to participate. Later, we followed up via email, telephone, or additional visits. We invited HCPs with diverse functions (e.g. nursing, midwifery, management, community health work (CHW), pharmacy, expanded programme on immunisation (EPI), traditional birth attendance) to obtain a broad perspective of faced challenges. Additionally, we invited members of the community advisory board (mothers, traditional healers (THs), and religious leaders) to ensure the relevance of co-created interventions. The inclusion criterion stated that participants are working at least for two years in their current role. The exclusion criteria included presence of serious psychiatric symptoms; visibly distraught or intoxicated at time of consent; unable to read, write and speak in any of the official languages; or unable to provide informed consent. Transportation and refreshments were provided and non-governmental employees received ZAR350.00 to compensate for their time.

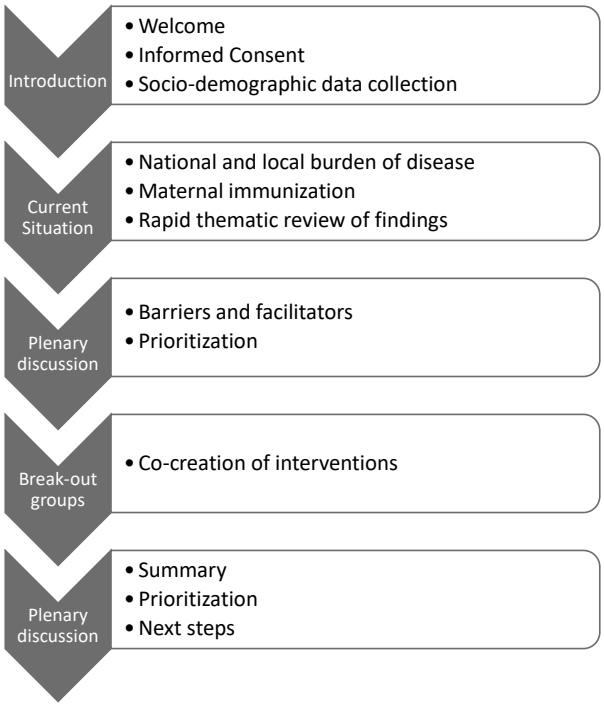
Procedures

Two semi-structured co-creation workshops were hosted at the University of Witwatersrand's Vaccines and Infectious Diseases Analytics (Wits VIDA) Research Unit, Chris Hani Baragwanath Academic Hospital, Soweto. The workshops were informed by a rapid thematic analysis of five previously conducted focus groups discussions with women of childbearing age, including pregnant women, and three key informant interviews with HCPs and community leaders. The rapid thematic analysis focussed on vaccination experiences from women, the HCP perspective on vaccination and their role in vaccine uptake. Preliminary conclusions and excerpts from the transcripts were shared at the start of the co-creation workshops to inform participants of both patient and HCP perspectives.

Measures

After informed consent was obtained, socio-demographic characteristics were captured using REDCap.[13,14] Six Wits VIDA and African Social Sciences Unit of Research and Evaluation (ASSURE) social scientists and one medical doctor co-facilitated all workshops according to the European Commission co-creation principles[15] and a semi-structured workshop guide (**Figure 1**). First, a user-centred design approach was employed to identify, prioritize, and contextualize key barriers in a plenary session with all participants (using the 6As matrix, **Table 1)**[16]. Second, priority barriers were divided over three smaller groups to triangulate known solutions and existing interventions to establish context-specific and relevant strategies for optimized MI in Soweto (**Table 1**). Strategies were scored according to priority scoring card (Appendix A). Finally, the strategies were presented in a plenary session to summarize findings, find overlap between strategies, and decide on next steps.

Figure 1. Schematic overview of semi-structured guide for co-creation workshops.



Results

Participants

Overall, 26 people participated in the two co-creation workshops. All participants identified as female gender and black race (**Table 2**). Participants varied in profession, age (median 43 years, range 26-59), and home language. HCPs were divided over governmental hospitals (9/24, 38%) and community clinics (15/24, 63%); none worked in private healthcare. Non-HCP participants included traditional healers and mothers, who were members of the community advisory board (CAB).

Identified barriers and facilitators in maternal healthcare and immunisation

During the co-creation workshops, perceived barriers and facilitators to MI were identified in a group discussion (**Figure 2**) using the 6As taxonomy to structure discussions (**Table 1**). Accountability barriers were organised according to the levels of the socioecological model by the workshop participants.[17] See Appendix B for a detailed overview of results per workshop. Following the group discussion, participants were invited to anonymously and individually vote on the barriers they considered most critical to target for optimisation of MI in Soweto (**Table 3**).

Table 1. 6As Taxonomy for determinants of vaccine uptake

Domain	Definition	Common barriers	Known solutions
Accountability	The obligation or willingness to accept responsibility for vaccinations by government, organization or person	Lack of political will and accountability Failure to prioritize program performance Poor coordination among partners	Education of HCP Case management Performance-based financing systems Quality improvement principles
Access	Ability of individuals to be reached by, or to reach, recommended vaccines.	Limited or difficult-to-access vaccination sites Inconvenient location or hours of vaccination site	Public-private partnerships
Affordability	Ability of individuals to pay for vaccination in terms of financial or opportunity costs.	Financial costs of vaccine or service delivery Opportunity costs	Financial incentives
Awareness	Knowledge of individuals, communities & societies of the need for, and availability of, recommended vaccines.	Lack of knowledge about diseases or vaccines Insufficient or inappropriate information Lack of knowledge of eligibility for vaccines Misinformation & information gaps	E-Communication materials HCP recommendation Community engagement
Acceptance	The intention to get vaccinated where there is access to, and awareness of, an affordable vaccine.	Low trust Sociopsychological barriers* Risk Communication Disinformation	HCP recommendation
Activation	The degree to which individuals are nudged towards vaccination uptake.	Competing priorities Time constraints	Financial incentives HCP recommendation

6As Taxonomy adapted from Thomson et al, The 5As: a practical taxonomy for determinants of vaccine uptake16 by addition of known solutions based on a literature review (unpublished work Mahoney et al, 2024). Briefly, this taxonomy developed from narrative review of 43 studies organises the multiple root causes of vaccine coverage gaps in the following dimensions: Accountability, Access, Affordability, Awareness, Acceptance and Activation.HCP, health care professional.

Accountability barriers

- Patient level – women do not come in for early/regular ANC visits
- System level – lack of adequate follow-up system (perinatal identification problem)
- System level – inconsistency in seeking care across several clinics (in or out of province)
- System level – variations in maternity case record across public and private care
- System level – Underutilized electronic tracking systems
- Government level – Lack of liaison between the Department of Health (DoH) and HCPs on needs and implementation of new immunization or other health guidelines.
- Governmental level – lack of resources and stimulating salaries for HCPs causing staff issues. HCPs perceive this as healthcare not being a priority for government.

Table 2. Participant socio-demographic characteristics

	N (%)
Gender	
Female	25 (96)
Missing	1 (4)
Age in years (median, range)	43 (26-59)
Race	
Black	26 (100)
Home language	
Zulu	9 (35)
Tswana	5 (19)
Sotho	3 (12)
Venda	2 (8)
Xhosa	2 (8)
Pedi	2 (8)
Tsonga	2 (8)
Ndebele	1 (4)
Preferred language	
English	25 (96)
Afrikaans	0 (0)
Other	1 (4)
Level of education	
Primary education	1 (4)
Secondary education	1 (4)
Post matric diploma	9 (35)
Bachelor's degree	8 (31)
Post-graduate degree	4 (15)
Missing/prefer not to answer	3 (12)
Employment status	
Employed full-time	20 (77)
Employed part-time	4 (15)
Unemployed	2 (8)
Current job description*	
Nurse: Midwife	13 (50)
Nurse: Other	10 (39)
Doctor	0 (0)
Community health care worker	1 (4)
Facility management	6 (23)
EPI	1 (4)
Pharmacy	1 (4)
Community Advisory Board	2 (8)
Traditional healer	1 (4)
Traditional birth attendants	0 (0)
Unemployed	1 (4)
Type of healthcare facility	
Government/public hospital	9 (35)
Community clinic	15 (58)
Private facility	0 (0)
Not applicable	2 (8)

* multiple may apply. EPI, extended programme on immunisation.

Figure 2. Group discussion during co-creation workshops on barriers and facilitators to maternal immunisation in Soweto, South Africa



Access barriers

- Language barriers with foreign nationals that attend the clinic
- High patient volumes with limited staff causing cut lines, unseen patients, and stock-shortages.
- Follow up is affected by people giving incorrect contact details during consultation.
- Some communities are unsafe and out of reach from CHW or even law enforcement.
- Operation hours, especially not accommodating people who are employed
- Stock shortages (sometimes centralized shortages). Poor stocking systems, incorrectly predicting the necessary stock or late orders, also cause stock-shortages. If so, HCPs are reluctant to refer to other (private) options for immunization as it is not free there (see affordability).
- Infrastructure, e.g. currently the maternity case record is out of stock causing a challenge to book patients and keep record of their immunization.
- Load shedding and lack of back-up generators breaks the cold chain of vaccine stocks and spoils the vaccines.
- Inability to refer between TH and HCPs. If women seek care at THs, there is no official hand-over nor do women openly disclose seeking healthcare elsewhere.
- Distance to clinics too far if there are no means of transportation available. Comorbidities might further prevent coming to clinics.

Table 3. Identified barriers and facilitators of maternal immunisation demand and supply

Barriers	**Priority % (n/N)**
Poor infrastructure and shortages of resources (e.g. maternity case records)	62 (8/13)
HCPs lack education to recommend vaccines	42 (11/26)
Community awareness	42 (11/26)
Stock shortages and poor stocking systems	27 (7/26)
Time constraints with patients due to staff shortages	23 (6/26)
Lack of effective follow-up system / perinatal identification problem	23 (3/13)
Personnel vacancies	23 (3/13)
Patient transport to clinics too far	15 (2/13)
Govt providing resources and stimulating salaries for HCPs	15 (4/26)
Pt responsibility to come to ANC early in pregnancy	15 (2/13)
Misinformation	15 (2/13)
Lack of liaison between DoH and HCPs on needs and new health implementation	12 (3/26)
Operational hours	12 (3/26)
Culture and language barriers	12 (3/26)
Late ANC booking past immunisation window	8 (1/13)
Lack of knowledge on traditional medicine	8 (1/13)
Low trust in government	8 (1/13)
HCP-pt relationships and staff attitudes	8 (2/26)
Resistance among HCPs to implement new guidelines or recommend interventions due to lack of liaison from DoH	8 (1/13)
Facilitators	**Priority % (n/N)**
Single dose formulation (3x tetanus vs 1x Tdap) and protect against multiple pathogens with 1 vaccine	23 (3/13)
Public-private consistency of care	23 (3/13)
Collaboration between HCPs and THs or religious leaders	8 (1/13)
THs are cheaper than western healthcare	8 (1/13)
CHW spread information in community	8 (1/13)
Electronic patient dossier	8 (1/13)

Priority was determined based on voting system where all participants received three votes to place next to the identified barriers and facilitators. Priority was calculated as the percentage of participants voting for that barrier/facilitator. The table summarizes the results of both workshops. Barriers and facilitators were only included in this table if they received at least 1 vote; please see Appendix C for all identified barriers and facilitators. ANC, antenatal care; CHW, community healthcare worker; DoH, department of health; HCP, healthcare professional; Tdap, tetanus-diphtheria-pertussis; TH, traditional healer.

Affordability barriers

- Assumption that vaccines are expensive and people cannot afford them, and therefore clinicians never suggest other places to get immunized in case of stock shortages
- Preference for traditional healers due to lower cost
- The false pretence that clinics are financially well when they are actually not (i.e. when the premier is visiting the Clinic)
- Patient lack of finances to commute to clinics

Awareness barriers

- HCP lack of education regarding the benefits and safety of vaccine needed to counsel patients and recommend vaccines. Management might be trained on new guidelines/ vaccines, but this information does not reach the nurses.
- Community lack of awareness on disease and vaccines
- Foreigners unaware of available health services and therefore waiting until delivery to access healthcare
- Misinformation from social media or peers
- Cultural and language barriers
- Lack of knowledge on traditional medicine and how practices affect the human body
- Lack of knowledge on comorbidities affecting vaccine efficacy and safety

Acceptance barriers

- Lack of HCP recommendations
- Cultural beliefs against vaccines
- Language barriers
- Previous healthy pregnancies without vaccines make patients reluctant to take vaccines now as they perceive a low need
- Low trust in vaccines, HCPs, and government
- Belief in traditional medicine over Western medicine and a lack of disclosure to HCPs
- HCP-patient relationships are negatively influenced by frustrated attitudes from HCPs and previous experiences from patients.
- Rules and regulation (e.g. new guidelines or Non-South African citizens' right to access free primary healthcare at government facilities in South Africa, as guaranteed by the Constitution and the National Health Act) imposed without healthcare worker engagement

Activation barriers

- No proactive systems (only reactive responses), waiting for people to be sick and seek treatment rather than educating people about protective measures
- Burden of patients strains the available staff and facilities. E.g. even if vaccines are available staff might have to cut the line for the day and send patients home unseen.
- Lack of liaison between DoH and HCPs causes reluctance of HCPs to make recommendations
- Staff shortages and time constraints hinder adequate counsel for immunization

- Personnel vacancies remain open despite efforts to hire new (untrained) staff
- No emotional wellness support available for the HCPs to deal with emotional stress that comes from their job.

Facilitators

- Maternal case records
- Free public health care
- Community health workers spread awareness on variety of topics and stimulate ANC attendance
- Providing immunisation at other facilities, e.g. pharmacies or private clinics
- Public-private consistency of care: patients' expectations and likelihood to accept MI are largely based on what they hear from others.
- Collaboration between HCPs and traditional healers or religious leaders
- Transition from 3x tetanus to 1x Tdap (single dose formulation and protects against multiple pathogens)

Strategies to optimise maternal immunisation in Soweto

Subgroups of participants proposed strategies to address the barriers that received the most votes (see **Appendix C** for a complete list of recommended strategies). The most promising strategies are discussed below for each barrier.

Community awareness

- TVs in waiting rooms: Install televisions in clinic waiting areas to show short, informative videos—subtitled in local languages for accessibility—on the importance and safety of MI and other health topics.
- Health promoters in waiting rooms: Deploy trained health promoters to engage with pregnant women in waiting rooms, providing tailored information and answering questions about vaccines.
- Community outreach: Use community health workers for regular, scheduled outreach in familiar settings, such as churches or community gatherings, with tracking systems to monitor subsequent clinic visits and impact.
- Radio and social media platforms: Leverage trusted voices—such as clinic matrons—via local radio and social media to disseminate reliable information about MI and available services.
- Immunisation awareness days: Organise special clinic days, similar to existing WorldAIDS or breastfeeding weeks, focused on MI, with health talks, educational materials, and opportunities for direct interaction with healthcare providers.
- SMS pregnant women during flu season: Send targeted SMS messages to pregnant women during relevant seasons (e.g. flu season) to remind them about vaccination and its benefits, encouraging timely uptake.

HCP awareness and education

- In-service training: Conduct weekly in-service training sessions, ideally integrated into existing routines such as morning prayer, and facilitated by a mix of internal staff and external experts (e.g. Wits VIDA, Department of Health, or academic trainees) to provide regular updates and build capacity on MI.
- Knowledge app: Introduce a mobile app that delivers a daily multiple-choice question related to MI, followed by a brief explanation—designed to reinforce learning through micro-education and continuous engagement.

Stock shortages

- Generators to overcome load shedding for vaccine fridges: Equip clinics with backup generators to ensure continuous refrigeration during load shedding, preventing spoilage and maintaining vaccine availability.
- Stock system guidelines and dedicated staff: Implement clear quality control protocols for stock management and assign dedicated staff to monitor inventory and respond proactively to potential shortages.
- Consistent suppliers: Maintain consistent vaccine suppliers where possible, and provide targeted training for healthcare workers whenever a supplier changes to ensure correct dosing and handling.
- Prioritizing South African citizens: HCPs expressed that limited time, personnel, and resources (including vaccines) should first go to South African women before providing healthcare to (illegal) immigrants.

Consistency in care across private and public healthcare as well as THs

- Collaboration between THs and HCPs: Foster collaboration between THs and HCPs by establishing referral systems, formal handovers, and monthly joint meetings to build mutual understanding and improve continuity of care, including the joint attendance of THs at clinic visits with their patients.
- Recognition of THs: Promote the formal recognition of THs by integrating them into clinic settings, where feasible, to enhance trust, communication, and culturally sensitive care.
- Monthly meetings: Hold regular multidisciplinary meetings—including public and private HCPs and THs—to provide joint training, align practices, and discuss complex cases to ensure consistent and coordinated maternal care.
- Government resources, infrastructure, and financing

Documentation improvement

- Maternity case record: Revise the maternity case record to include dedicated space for recording MI (e.g. specifying Tdap instead of TeTox), with areas for stickers, time, date, and staff signatures to ensure clear tracking.
- Electronic patient dossier: Expand access to electronic health records in Gauteng to streamline administrative tasks, enable easier follow-up, and accurately track immunisation status over time.

- Separating immunisation from the counselling rooms: Alleviate pressure on clinic counselling rooms by setting up separate immunisation stations or drives, supported by trained THs and retired nurses to expand capacity and reduce patient waiting times.
- Checklist on MI experiences: Develop and implement a standardised checklist to capture MI experiences and pregnancy outcomes, supporting better data collection and programme evaluation.
- Quicker hiring of staff after resignation or retirement: Streamline hiring processes to ensure that vacant posts due to staff departures are filled promptly, avoiding long-term staff shortages that disrupt service delivery.
- Overtime financing: Allocate funding for overtime pay to ensure continued service coverage during periods of staff shortages, maintaining consistent access to MI services.

Recommendations

Based on the barriers and solutions provided by the HCPs and other stakeholders of Soweto, we make the following overall recommendations:

- HCPs desire education on current and new vaccines, the need for them, their mechanism of action, the benefits, and side effects. Education should be directly to the staff administering the vaccines rather than via management: in-service training or via micro-education using a daily question in an app. Webinars through the Knowledge Hub for HCPs and integration of training on MI in HCP curriculums can alternatively improve MI knowledge among current and newly trained HCPs.
- The community desires raised awareness on diseases and how to prevent them. Community outreach at familiar settings require collaboration with community leaders. Immunization awareness days, radio/social media coverage by HCPs, or text messages can nudge patients towards MI and ANC in general.
- HCPs and clinics need to be incentivized to provide MI to all patients, including migrants, by mandatory reporting and instating MI and migrant care as an indicator of good healthcare, to prevent prioritization of South African citizens over migrants.
- Improved documentation in the MCR would nudge HCPs to provide the right vaccine at the right time and would inform other HCPs after referrals. The instatement of an EPD would be the ideal way to improve patient care.

Lessons learned of the research process

- HCPs should be included in the strategy design before any new vaccines are implemented.
- Participants could identify many barriers to MI or general challenges in providing care in the public health sector. It required a lot of probing to move on from naming specific, detailed barriers to coming up with targeted solutions. It was difficult to obtain information on the next steps: to figure out how to test the success of strategies as well as prioritizing strategies. It might require several workshops with the same participants to train them into the methodology of the workshops and work with a stepwise approach to achieve the workshop objectives.

- There was a benefit to having both social scientists and medical professionals on the research team as they complemented each other's knowledge in organisation and facilitation of discussions.
- Participant recruitment was facilitated via health facility managers. Paper copies of invitation including the objectives of the co-creation workshops were left behind in clinics and digital copies were sent to clinics via email afterwards to be spread among employees. However, some participants seemed unaware of the purpose of the study or the duration of the workshop, which caused some resistance to be present. Despite some resistance, those that agreed to partake in the co-creation workshops, signed informed consent and all were vocal during the discussions.
- The Human Research Ethics Committee (HREC) recommended to only provide reimbursements for non-governmental employees, as governmental employees are paid to be present during workhours. However, we experienced resistance from governmental HCPs as they would be making unpaid overtime by being present (e.g. participants were scheduled to work until noon but present at the workshop until 2pm, leading to 2 unpaid hours). For equality, we recommend HRECs to also reimburse government employees for the time committed to research on top of their scheduled working hours.

Reflexivity statement

As a white healthcare provider and researcher from a high-income country, I was aware of the potential power imbalances and cultural dynamics present during the facilitation of the workshops with predominantly black healthcare providers in Soweto, South Africa—a low to middle-income setting. My social and professional positioning may have influenced how participants engaged with me and with each other, particularly in relation to sensitive topics. To mitigate these dynamics, deliberate efforts were made to create an open, respectful, and inclusive environment where all voices were welcomed and valued.

Workshops were co-facilitated with local colleagues, who supported my understanding of the culture and language, where needed. Participants were explicitly encouraged to share their lived experiences, perspectives, and concerns—including those considered socially sensitive or taboo. Notably, the space enabled open discussions around xenophobia, cultural practices, and systemic inequities, which enriched the depth and contextual relevance of the findings.

Conclusion

These findings, recommendations, and lessons learned will inform future co-creation workshops at a national level with more varied stakeholders such as government policy makers, Department of Health Representatives from EPI and ANC, academics, obstetricians, midwives, professional societies. Lessons learned may form the basis for identifying, formulating and developing targeted solutions to improve supply and demand of existing and future MIs in the South African setting. Lessons will also apply more broadly to future MI studies.

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Appendix

Appendix A: Priority scoring card criteria

Appendix B: Identified barriers and facilitators in maternal immunisation and healthcare in Soweto according to the 6As taxonomy

Appendix C: Recommended solutions and priority scoring

Appendix A: Priority scoring criteria

Criteria	Considerations	Score
Impact potential	Lives saved Population reach Sustainability of benefits Addresses critical needs	3: high impact (significant lives saved, wide reach, sustainable) 2: moderate impact (moderate reach, some sustainability) 1: low impact (limited reach or unsustainable)
Feasibility	Resource requirements Technical complexity Timeline Existing capacity Scalability	3: highly feasible (resources available, technically simple) 2: moderately feasible (some challenges but manageable) 1: Challenging (significant resource/technical barriers)
Implementation readiness	Available expertise Health system integration Required partnerships Regulatory requirements Current infrastructure	3: easy to implement (existing systems, expertise available) 2: moderate effort (some new resources/systems needed) 1: difficult (requires significant new infrastructure)
Cost-effectiveness (value)	Cost per impact Resource efficiency Funding sustainability Value for money Return on investment	3: high value (low cost, high return) 2: moderate value (balances cost/return) 1: low value (high cost, lower return)

Appendix B: Identified barriers and facilitators in maternal immunisation and healthcare in Soweto, South Africa, according to the 6As taxonomy

Workshop 1

6As	Barriers	Facilitators
Accountability	Patient – responsibility to come to ANC early in pregnancy and to follow-up System – lack of effective follow-up system System – inconsistency in where women seek care disrupt immunisation schedule Lack of liaison between DoH and HCPs on needs and new implementations	Maternity case record helps keeping track of immunisation
Access	Stock shortages (often centralized so no other place to send women that are due for immunisation) Operational hours Poor infrastructure and shortages of resources (e.g. the maternity case record) Staff shortages Lack of referral system between TH and clinics	
Affordability	In case of stock shortage, nurses experience reluctance to refer women to private clinics where they would have to pay for vaccines False pretence that clinics are financially well e.g. when the premier is visiting	THs are cheaper than western healthcare
Awareness	HCPs lack the information needed to counsel their patients on the safety and importance of vaccines and make recommendations Culture and language barriers Lack of awareness in community/patients	CHW spread information in community
Acceptance	Cultural differences Language barriers Low trust in HCPs, especially among foreign patients	
Activation	Amount of patients mismatch the staff capacity Time constraints with patients due to lack of staff Resistance among HCPs to implement new guidelines/ interventions due to lack of liaison from DoH Lack of emotional wellness support to deal with high burden of the job	

Results from the first workshop with healthcare providers and community representatives obtained via group discussion structured around the 6As. ANC, antenatal care; CHW, community health workers; DoH, Department of Health; HCP, healthcare provider; TH, traditional healer.

Workshop 2

6As	Barriers	Facilitators
Accountability	Follow-up problem (Perinatal identification problem) Patient – responsibility to come to ANC early in pregnancy and to follow-up Lack of liaison between DoH and HCPs on needs and new implementations Government providing resources and stimulating salaries for HCPs	
Access	Transport to clinics Comorbidities preventing visiting clinics Stock shortages in facilities Poor stocking systems Operational times Load shedding causing spoiled vaccines Overpopulation (incl health migration) leading to stock-outs	Public-private consistency of care Collaboration between HCPs and TH or religious leaders
Affordability	Pt lack of financing for transport to clinics	Free public health care
Awareness	HCPs lack the information needed to counsel their patients on the safety and importance of vaccines and make recommendations Lack of community awareness on disease, vaccines, and need for vaccines based on previous healthy pregnancies when vaccines were not available Misinformation Lack of knowledge on traditional medicine Lack of knowledge on comorbidities affecting vaccine efficacy/safety	
Acceptance	Cultural differences and language barriers Previous healthy pregnancies without vaccines Low trust (in vaccines, HCPs, or government) Lack of HCP recommendations HCP-Pt relationships and staff attitude Pt disclosure of consulting TH	TDaP: Single dose formulation and protects against multiple pathogens in one vaccine
Activation	Time constraints with patients due to lack of staff hinders adequate counseling Personnel vacancies	

Results from the second workshop with healthcare providers and community representatives obtained via group discussion structured around the 6As. ANC, antenatal care; CHW, community health workers; DoH, Department of Health; HCP, healthcare provider; Pt, patient; TDaP, tetanus, diphteria, acellular pertussis; TH, traditional healer.

Appendix C: Recommended solutions and priority scoring

Workshop 1: subgroup 1. **Barrier/facilitator:** Inadequate infrastructure and insufficient resources **Date:** 25 July 2025

Solution/idea	Outcome How to measure success	Timeline	Key driver What is needed	Key partners Who needs to be involved
Improved stock systems	Tracking system how many vaccines administered. Reduction in stock shortages	Monthly checks	Quality control guidelines Dedicated staff Tracking system	Enrolled nurse Pharmacy assistant
Division of duties among staff. Duties might include health care education, immunisation, measurements, etc. Also, taking breaks in shifts so there is always someone on the floor.	Efficiency in work leads to more patients seen in a day		Ownership among staff. Rotation and daily delegation Management monitors	Current staff, unit management
Improved collaboration between TH and HCPs. This includes accompaniment of TH when referring to HCP, official hand-over (paper or electronic), monthly maternity meetings to understand each others practices and discuss cases.	Referrals between TH and HCPs, meeting attendance, TH available in ANC clinics	Immediate	Monthly meetings, referral system, record keeping by TH, reduction on stigma to see TH	THs, COGTA, HCPs, government
Accreditation of TH	Accredited THs, recognised practices and remedies	Multiyear	Training and exposure in ANC clinics	THs, COGTA, HCPs, government
Documentation – Maternal case record (MCR) improvement	Improved space for immunisation: Tdap rather than TeTox, new vaccines, stickers, time and date, signature	Year	New MCR edition	Government, HCPs
Documentation – Electronic patient dossier	Available to all staff, trained staff, no more paper MCR	Multiyear	Computers/tablets in clinics and hospitals	
Healthcare promotion and immunisation outside of the counselling rooms	Administered vaccines, patients asking about vaccines and diseases	Months	Trained health promotors	Government, HCPs, academics (to train health promotors), CHWs, THs (to show collaboration between THs and HCPs to patients)
Vaccine drives in community (road show)	Administered vaccines	Monthly drives	Mobile clinics, coolers, sufficient stocks, translators	Government, HCPs, call back retired nurses, CHWs, collaborate with TH and religious leaders

Priority scoring card:

Barrier: Inadequate infrastructure and insufficient resources **Date:** 25 July 2025

Solution/idea	Impact potential	Feasibility	Implementation readiness	Cost effectiveness
Improved **stock systems**	2	3	3	2
Division of duties among staff. Duties might include health care education, immunisation, measurements, etc. Also, taking breaks in shifts so there is always someone on the floor.	3	3	3	3
Improved **collaboration between TH and HCPs**. This includes accompaniment of TH when referring to HCP, official hand-over (paper or electronic), monthly maternity meetings to understand each others practices and discuss cases.	3	N.A	N.A	N.A
Accreditation of TH	3	N.A	N.A	N.A
Documentation – **Maternal case record** (MCR) improvement	N.A	N.A	N.A	N.A
Documentation – **Electronic patient dossier**	N.A	N.A	N.A	N.A
Healthcare promotion and **immunisation** outside of the counselling rooms	N.A	N.A	N.A	N.A
Vaccine drives in community (road show)	N.A	N.A	N.A	N.A

HCP, healclth care professional; MCR, maternal case record; N.A., not available; TH, traditional healer.

Workshop 1: subgroup 2. **Barrier/facilitator:** Community education **Date:** 25 July 2025

Solution/idea	Outcome How to measure success	Timeline	Key driver What is needed	Key partners Who needs to be involved
TVs in waiting rooms teaching various topics incl maternal immunisation and importance of follow-up	Increased knowledge among patients		TVs, videos/slides	DoH, HCPs and researchers with credible knowledge, clinic management
Healthcare Promoters in Clinic waiting rooms educating people about the importance of maternal immunization	People to have more knowledge and information about maternal immunisation and the importance to increase uptake		More healthcare promoters More Funds allocated to them because there is already a set budget for this	The department of health and the government
Community healthcare workers to go into communities educating people about maternal immunisation	To limit misinformation and misconceptions about maternal immunisations in communities. Promote ANC visits and requesting immunisation.		More community healthcare workers More funds	The department of health and the government
Radio Platforms where matrons from local Clinics can share information about maternal immunisation and the services they offer at the Clinic	This is to spread information from reliable sources and services Clinics offer to surrounding communities		The government to allocate the **budget** for this platform to spread information on important information.	The **government** and **local Clinics**
Social media platform for healthcare professionals to share reliable information to the people	To spread trustworthy information from trusted professionals		Medical professionals to come on board and collaborate	Medical professionals (i.e. Doctors and Nurses) The department of health
Host **wellness open days** to educate on different topics like maternal immunisation	To spread awareness about maternal immunisation and prioritize it so that it is a norm in communities		Educational pamphlets	The department of health to provide with educational pamphlets

DoH, department of health; HCP, healcth care professional; MCR, maternal case record; N.A., not available; TH, traditional healer.

Priority scoring card:

Barrier: Community education **Date:** 25 July 2025

Solution/idea	Impact potential	Feasibility	Implementation readiness	Cost effective
Healthcare Promoters in Clinic waiting rooms educating people about the importance of maternal immunisation	3	Very feasible. Health promoters to educate patients in waiting areas about immunizations	There is already existing healthcare promoters. More staff for this is needed.	Budget already in place allocated for this. More funds needed to allocate more staff for this
Community healthcare workers to go into communities educating people about maternal immunisation	3	Very feasible. Community healthcare workers to spread information about maternal immunisation in communities	Community healthcare workers already in the community, we just need more of them	Budget already in place allocated for this. Need more funds for more staff
Radio Platforms where matrons from local Clinics can share information about maternal immunisation and its importance	3	Feasible given that radio is a popular platforms in our communities.	There are local radio stations which we can work with for the purpose of spreading information on immunizations	Cost effective because platforms like SABC belong to the government and can be used for this
Social media platform for healthcare professionals to share reliable information to the people	3	Feasible. Healthcare workers to come together in collaboration	Social media is a very popular platform in communities. This would not be a challenge to implement as long as we can get healthcare professionals on board and work together.	Cost effective
Host wellness open days to educate on different topics like maternal immunisation	3	Open days to be hosted in all local Clinics maybe 2x a month to educate people on different topics such as maternal immunisation	Already there are WorldAids day and Cancer day where awareness is spread on those days, there is structure in place. Maternal immunisation just needs to be added onto the list.	The government and the Department of Health to allocate funds for the educational pamphlets and posters for these open days

Workshop 1: subgroup 3. **Barrier:** Loss to follow-up **Date:** 25 July 2025

Solution/idea	**Outcome** **How to measure success**	**Timeline**	**Key driver** **What is needed**	**Key partners** **Who needs to be involved**
Mandatory proof of address	Less loss to follow-up. However, it might also limit access to care especially for non-South Africans or illegal residents	Immediate	Change of procedures during registration	Clinicians, clerks
WBOTs have **community registers**, where they can update if people move around.	Less loss to follow-up	Months/years	Approval to edit community registers	Government, municipality, WBOTs, translators (to assist with reading cards and communicate with non-South Africans), community leaders
Outreach and campaigns: Vaccinate community members within the community to reach more people. Mid-month and on one day (e.g. Wednesday) for consistency.	Monthly stats to trace impact. Outreach resisters can be compared to clinic register to determine how many patients from the outreaches actually came to the clinic		Resources (pamphlets, tents, vaccines) and staff. Involvement of community members.	Wellness coordinator, Health promoters and WBOTs to educate the community Communication officers – promote the events on community social media pages. Community members – to share information of WhatsApp groups Nurses – to vaccinate during outreaches Pharmacy – to ensure there is enough stock for these campaigns

Current satiation: Tracing patients by using addresses. Clinicians work with WBOTs to trace patient for follow-up visits, but patients register wrong address or move around. WBOTs give **feedback** to clinicians regarding the patients that were (not) reached. WBOT, ward-based outrach team.

Priority scoring card:

Solution/idea	**Impact potential**	**Feasibility**	**Implementation readiness**	**Cost effective**
Mandatory proof of address WBOTs have **community registers**, where they can update if people move around.	2 - this will have moderate impact because of wrong addresses and difficulty to reach and conduct follow-up in non-South African dominant areas that are dangerous	2 – due to patients who move around	2 – will need more cars, additional staff member.	3 – the systems are already available, they just need a better way of utilizing them.
Outreach and campaigns: Vaccinate community members within the community to reach more people. Mid-month and on one day (e.g. Wednesday) for consistency.	2 – Some areas are hard to access	2 – More resources required Educating patients at the clinic level	2 – Training on communicating with communities, translator might also be required	2- more staff member means more money required, and the impact might not be as much as expected.

Workshop 2: subgroup 1. **Barrier/facilitator:** Community education **Date:** 01 Aug 2025

Solution/idea	**Outcome** **How to measure success**	**Timeline**	**Key driver** **What is needed**	**Key partners** **Who needs to be involved**
Outreach: multiple health representatives (PNs, CHWs, health promoters, THs), several platforms and using community events to promote maternal immunisation	Vaccines accepted/consented, women asking about vaccines including non-pregnant women, post-natal feedback at the EPI.	Weekly for consistency and building rapport	Pamphlets (lay-person language, in different languages), gazebos (take into account climate), tables, cooler box, transport, train the educators	THs, WBOTs, community leaders, HCPs, government
Facility based education: in waiting rooms by nurses or trained health promoters, pamphlets	See above		Train the educators	Academics/students to train the nurses/health promoters Nurses and health promoters
Immunization awareness days	Community coming for immunisation. Increased uptake	Quarterly event	Budget (goodie bags and t-shirts)	Nurses, WBOTS, community leaders
Billboards in public spaces (e.g. Spaza shops)	Community coming for immunisation. Increased uptake	June and December during peaks of pregnancies	Billboards and posters	DoH, suppliers
SMS for influenza vaccines	Community coming for immunisation. Increased uptake	Winter	Budget	DoH

CHW, community healthcare workers; EPI, extpanded programme on immunisation;HCP, health care professional; N.A., not available; PN, professional nurses; THs, traditional healers; WBOT, ward-based outreach team.

Priority scoring card:

Solution/idea	**Impact potential**	**Feasibility**	**Implementation readiness**	**Cost effectiveness**
Outreach	3 – reach higher numbers, more sustainable when resources can be used long-term. High impact on prevention	3	2 – WBOTs/staff and transport needed	3 – less costs, reduce costs even for patients
Facility based education	3 - Reach both pregnant women and others (men)	3 – all resources are availabe	2 – need pamphlets, information, printers	2 – some resources are available
Immunization awareness days	N.A.	N.A.	N.A.	N.A.
Billboards in public spaces	N.A.	N.A.	N.A.	N.A.
SMS for influenza vaccines	N.A.	N.A.	N.A.	N.A.

N.A., not available.

Workshop 2: subgroup 1. **Barrier/facilitator:** Government resources and financing **Date:** 01 Aug 2025

Solution/idea	**Outcome** **How to measure success**	**Timeline**	**Key driver** **What is needed**	**Key partners** **Who needs to be involved**
Motivate population to work in healthcare by providing higher salaries	Less vacancies	N.A.	Budget	Government
Checklist on MI experiences including information on pregnancy outcome				
Immediate hiring after resignation or retirement as budget is already available	Shorter delays in re-hiring vacancies		Governments to hire faster	Government
Overtime financed	Stimulated staff in times of employment issues		Budget from DoH	DoH

DoH, department of health; N.A.- not available.

Priority scoring card: not available

Workshop 2: subgroup 2. **Barrier/facilitator:** Stock shortages **Date:** 01 Aug 2025

Solution/idea	Outcome How to measure success	Timeline	Key driver What is needed	Key partners Who needs to be involved
Proper equipment, such as fridges designed for vaccines and back-ups when there is load shedding	Less spilled vaccines. More vaccines administrated	Initiate now for next financial year	Matron nurse writes to area manager who sends request to the director	Nurse, matron, director
Guidelines for timely orders and proper stock monitoring, such as first in first out (FIFO) rules, emergency stock-up availability.	No more stock shortages. Service satisfaction	Monthly orders	Timely checks, FIFO	Nurse, chief clerk (sends orders to Hillbrow, as pharmacy stocks run out more quickly), in-house pharmacy
Consistent supplier (currently packaging is different from paediatric vaccine, which is confusing if it is the same vaccine)	Communicate supplier changes to prevent over-/ underdosing	N.A.	Short training of staff when changes occur	Supplier to train pharmacy, pharmacy to train staff, government to pay supplies.
Prioritize South African citizens during low stocks	Less stock-outs	N.A.	Tighten borders to reduce health migration, make immigrants pay for services	Government to change laws

FIFO, first-in, first-out; N.A.- not available.

Priority scoring card:

Solution/idea	Impact potential	Feasibility	Implementation readiness	Cost effective
Proper equipment, such as fridges designed for vaccines and back ups when there is load shedding	3	3	2	3
Guidelines for timely orders and proper stock monitoring, such as first in first out (FIFO) rules, emergency stock-up availability.	3	3	3	3
Consistent supplier (currently packaging is different from paediatric vaccine, which is confusing if it is the same vaccine)	N.A.	N.A.	N.A.	N.A.
Prioritize South African citizens	N.A.	N.A.	N.A.	N.A.

N.A.- not available.

Barrier: Consistency of healthcare across private/public/TH **Date:** 01 Aug 2025

Solution/idea	Outcome How to measure success	Timeline	Key driver What is needed	Key partners Who needs to be involved
National health services and equipment consistency (e.g. some services are not available in other provinces such as sonar while some Gauteng clinics have monthly sonar services available)	Procedures used throughout healthcare	N.A.	N.A.	N.A.
Collaboration between private and public	Procedures used throughout healthcare	N.A.	Private to visit public to see how things are done. Standardize procedures and protocols	Government (DoH), nursing counsil
TH consistency	More collaboration, lessons on dosage, clinics have in-house THs	ASAP	Health education to TH and mothers. TH to give placebo. Documentation. Meetings	Registered THs, nurses, government, mediators

ASAP, as soon as possible; DoH, department of health; N.A.- not available; TH, traditional healer.

Priority scoring card:

Solution/idea	Impact potential	Feasibility	Implementation readiness	Cost effective
National health services and equipment consistency (e.g. some services are not available in other provinces such as sonar while some Gauteng clinics have monthly sonar services available)	3	3	2	3
Collaboration between private and public	N.A.	N.A.	N.A.	N.A.
TH consistency	N.A.	N.A.	N.A.	N.A.

N.A.- not available; TH, traditional healer.

Workshop 2: subgroup 3. **Barrier:** HCP education **Date:** 01 Aug 2025

Solution/idea	**Outcome** **How to measure success**	**Timeline**	**Key driver** **What is needed**	**Key partners** **Who needs to be involved**
In-service training. Once weekly there should be a training to keep HCPs up to date with vaccinations. Currently training management who don't pass on the knowledge to staff	Knowledge gains, attendance	Months	The Department of health	External people (e.g. Wits VIDA) should come to train. DoH.
Immunization awareness days.	Response from community by coming for immunisation	N.A.	Budget for goodies/t-shirts	DoH, nurses
Radio-programs (by maternal immunisation co-ordinator)	HCP knowledge on new vaccines and changes. HCP confidence for recommendation	Monthly	Collaboration to give different talks. HCP radio stations	Nurses, maternal coordinators
Knowledge app with information on various topics, incl MI	N.A.	N.A.	App development.	ICT

DoH, department of health; HCP, healthcare professional; N.A.- not available.

Priority scoring card: not available





Part III

The value of simplicity: innovation for inclusive RSV research





RSV neutralizing antibodies in dried blood

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Abstract

Background

The key correlate of protection of respiratory syncytial virus (RSV) vaccines and monoclonal antibodies (mAb) is virus neutralization, measured via sera obtained through venipuncture. Dried blood obtained with a finger prick can simplify acquisition, processing, storage, and transport in trials and thereby reduce costs. In this study, we validate an assay to measure RSV neutralization in dried capillary blood.

Methods

Functional antibodies were compared between matched serum and dried blood samples from a phase 1 trial with RSM01, an investigational anti-RSV prefusion F mAb. Hep-2 cells were infected with a serial dilution of sample-virus mixture using RSV-A2-mKate to determine half-maximal inhibitory concentration. Stability of dried blood was evaluated over time and during temperature stress.

Results

Functional antibodies in dried blood were highly correlated with serum (R^2 = 0.98, p < .0001). The precision of the assay for dried blood was similar to serum. The function of mAb remained stable for 9 months at room temperature and frozen dried blood samples.

Conclusions

We demonstrated the feasibility of measuring RSV neutralization using dried blood as a patient-centered solution that may replace serology testing in trials against RSV or other viruses, such as influenza and SARS-CoV-2.

Clinical Trials Registration. NCT05118386 (ClinicalTrials.gov)

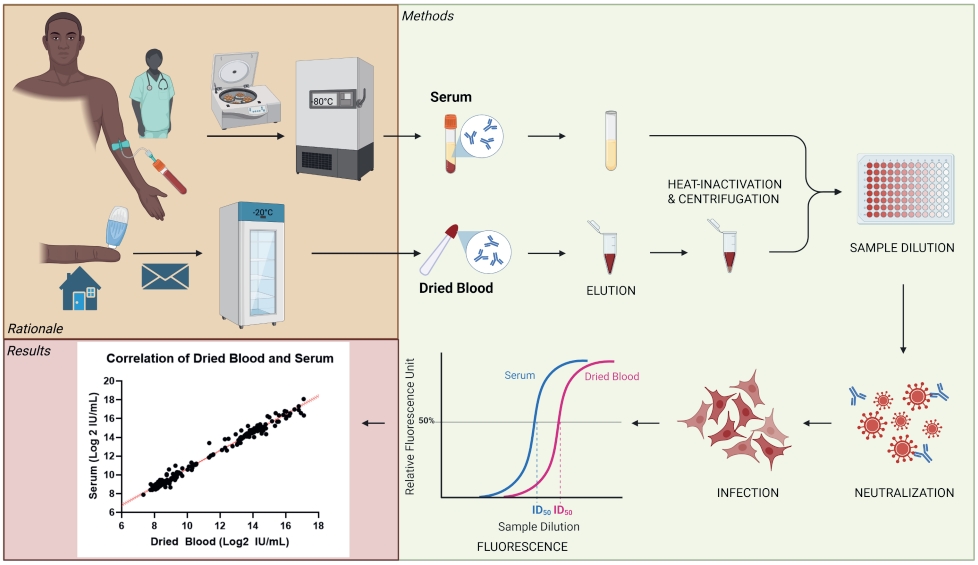
Introduction

The respiratory syncytial virus (RSV) vaccine and monoclonal antibody (mAb) landscape has rapidly expanded with 16 candidates currently in late-phase clinical development.[1] A next-generation, long-acting mAb was approved in 2022 and expected to be commercially available in 2023,[2,3] soon followed by vaccines for pregnant women and older adults. All late-phase vaccines are in development for high-income countries due to the high costs, so a delay in implementation is expected in areas with the highest infant RSV burden: low- and lower-middle income countries (LMICs).[4] RSM01 (an anti-RSV prefusion [PreF] protein site ø mAb) is intended for infants in LMICs and recently completed a phase 1a trial in healthy adults.[5]

Trials largely monitor neutralizing antibody concentrations in serum as a putative correlate of protection. The functional antibodies are measured by a broad variety of neutralization assays harmonized with an international standard.[6] Blood draws are burdensome for trial participants due to the large amount of blood taken through painful venipunctures that can fail or cause a hematoma. The cost of personnel and equipment, the immediate processing, and the necessity of a -80°C cold chain is resource-intensive and complicates implementation of trials in LMICs. Moreover, venipuncture is difficult to perform in children. Alternatively, dried blood samples can simplify trials, especially in remote areas (**Figure 1**): 1 drop of blood is obtained with a finger or heel prick by self-sampling or minimally trained personnel. Samples can be shipped by regular mail without temperature control and stored at -20°C before analysis, while serum samples require clotting and centrifugation before storage or analysis.

Dried blood using filter paper or Mitra volumetric absorptive microsamples (VAMS) is validated to measure mAb concentrations measured by ELISA,[7] but the neutralizing capacity of antibodies after drying of blood is less well established. Five studies compared neutralizing antibodies in dried blood spots (DBSs) on filter paper or hemaPEN with plasma or serum for SARS-CoV-2, rabies, and human papilloma virus.[8-12] Samples sizes were small (range, 7-48 matched samples, total, 128 pairs) with varying correlations (R^2 = 0.51-0.99). This study aimed to determine if anti-RSV antibodies retain their function in dried blood using VAMS devices in a large sample size using clinical samples from the first-in-human trial with RSM01 (Clinical Trials.gov NCT05118386). Second, we validated the use of dried blood vs serum using a simple tool – a reporter virus-based neutralization assay – to measure neutralizing antibodies against RSV.

Figure 1. Study rationale and experimental set-up.



Trials conventionally monitor neutralizing antibody levels in serum, which requires a professional to draw blood, immediate processing in a laboratory, and storage and transport at very low temperatures until analysis. Alternatively, dried blood samples can be obtained from a simple finger prick either by non-medical personnel or study participants themselves. Transport through regular post and storage at room temperature or in a regular freezer reduces the necessary resources, allowing trials in remote areas. Antibody function was evaluated in dried blood vs serum on the neutralization assay. This figure was created with Biorender. ID_{50}, half-maximum inhibitory dilution; mAb, monoclonal antibody; PreF, prefusion protein; RSV, respiratory syncytial virus.

Methods

Sample handling and preparation

Individual samples for validation of the assay were obtained from healthy adults from the University Medical Center Utrecht healthy donor service. Serum and whole blood were unaltered or spiked with RSM01, a mAb against RSV PreF site ø (Bill & Melinda Gates Medical Research Institute). After 30 minutes on a rocker to mix, Mitra 20 µl VAMS (Neoteryx Clamshell, 20004; Trajan Scientific) were touched to the whole blood sample surface to wick up the fixed volume[13] and left to dry for 3 hours at room temperature (RT). Samples were used immediately or stored at -80°C or higher temperatures (RT or -20°C) for stability assessments.

Clinical samples at matched timepoints (day 1 pre-dose, day 91, day 151) were collected from 56 participants in clinical study GATES-MRI-RSM01-101 (NCT05118386) as follows: by venipuncture blood in serum separator tubes, which were processed to serum, or by finger-prick via lancet, followed by touching the Mitra 20 µl VAMS (Neoteryx Cartridge, 20103; Trajan Scientific) to the blood drop to wick up the fixed volume. Of note, the adult participants in this study were expected to have preexisting RSV neutralizing antibodies

from natural infection. Samples were stored at -80°C. Prior to analysis on the neutralization assay, dried blood samples were thawed for 30 minutes before opening the air-tight bag to prevent condensation.

Dried blood elution for antibody recovery

Dried blood was rehydrated with a phosphate buffered saline (PBS)-only elution method: 1 microsampler tip was transferred to a 2-mL Eppendorf tube with 200 µL 1xPBS and incubated overnight at RT on a shaker at 300 rpm. The PBS-only elution method was compared to a validated elution method, which incubates microsampler tips in 400 µL of 1xPBS + 1% bovine serum albumine (BSA) + 0.5% Tween 20 at 4°C overnight as previously published.[14] Antibody recovery was measured on an RSV PreF enzyme linked immunosorbent assay (Supplementary Methods). The elimination of Tween from the elution buffer is important to enable use in cell-based assays such as this RSV neutralization assay. The elimination of BSA from the elution buffer is important to enable use in resource-limited regions where acquisition of animal-based reagents is limited.

Percentage absolute recovery is the percentage of measured concentration divided by the spiked concentration (100 µg/mL). Percentage recovery is calculated as follows: [(measured concentrations of the spiked sample – unaltered sample)/measured concentration of the spiked standard diluent] × 100%. Elution efficiency was defined as the percentage recovery with preferred PBS-only elution method divided by the percentage recovery with the validated elution method. Boundaries for acceptance were set at 80% to 120%.

Neutralization assay

Neutralizing capacity of samples was tested using a neutralization assay described previously.[15,16] Briefly, Hep-2 cells (CCL-23; ATCC) were seeded at a concentration of 0.5×10^6 cells/well in 384-well black optical bottom plates (142761; Thermo Scientific) and incubated for >4 hours. Dried blood eluate and serum were heat-inactivated at 56°C for 30 minutes. Dried blood eluate was then centrifuged at 15 000g for 10 minutes to remove cell debris. Samples were added in duplicate to dilution plates with a starting dilution of 1:5 for serum (12 µL of sample in 48 µL of medium) and 1:2 for dried blood eluate (30 µL of sample in 30 µL of medium; total 1:20 dilution after elution). Samples were serially diluted 1:3 in Dulbecco's Modified Eagle's Medium containing 10% fetal calf serum (High Glucose, D6429 [Sigma]; or Glutamax, 31966-021 [Gibco]). Recombinant mKate-RSV-A2 (2.23×10^6 $TCID_{50}$ [50% tissue culture infectious dose]/mL)[17,18] was added 1:1 to the sample dilution series and incubated for 1 hour at 37°C before addition of 50 µL of sample-virus mixture to the adherent Hep-2 cells. After 26 hours of incubation at 37°C, we recorded relative fluorescence units (RFU) with excitation at 584 nm and emission at 620 nm (FLUOstar OMEGA; BMG Labtech). T he neutralization curve and 50% inhibitory concentration or dilution (IC_{50} or ID_{50}, respectively) of samples were analyzed using a log (inhibitor) vs response 4-parameter nonlinear regression curve in Prism version 8.3 (GraphPad Software Inc.). International units (IU) were calculated

according to manufacturer's instruction (**Supplementary Methods**) and conversion to IU obviates the need to adjust for absolute volumes.

Sample stability

Stability of mAb in dried blood was tested with dried blood and serum samples spiked with 10 or 100 µg/mL of RSM01. Dried blood samples were stored per 2 VAMS in a generic zip-lock bag, including generic desiccant sachet of 10 g of silica gel and humidity cards (12665157; Fisher Scientific).[19]

Long-term stability was evaluated because the opportunity to delay sample processing could ease logistical challenges of trials in remote areas. Dried blood was stored at -20°C and RT, while serum aliquots were stored at -80°C. Storage temperature was logged to ensure correct conditions. Samples were measured on the neutralization assay at baseline (1 day after sample preparation) and at 7 follow-up time points over 1 year. One replicate per stability sample (4 dried blood and 2 serum) was measured per time point except at baseline (n=2). Stability was defined as <30% difference in ID_{50} per time point compared with baseline ID_{50}.

Short-term stability with a stress test was evaluated to simulate potential varying storage conditions during sample transport. Spiked dried blood samples were stored at -20°C and underwent temperature stress at various degrees (4°C, RT, 37°C, or 45°C) for a total of 48 hours before returning to -20°C storage. Stability was defined as <30% difference in ID_{50} between stressed samples and samples that were not stressed but remained at -20°C.

Statistical Analysis

Critical reagents were determined by paired *t* test of linear ID_{50} values of the same sample measured with 2 reagents (SPSS Statistics, version 26.0.0.1; IBM). Precision was calculated as the percentage coefficient of variance (%CV) of RFU of sample duplicates (intra-assay precision) or %CV of the linear ID_{50} between runs (inter-assay precision) or between operators (inter-operator precision; **Supplementary Methods**). Dried blood and serum from the RSM01 Phase 1 clinical trial were compared through simple linear regression (SPSS Statistics, version 26.0.0.1; IBM).

Ethical considerations

Informed consent was obtained from healthy donors and study participants. The healthy donor service was approved by the Ethical Committee of Biobanks (study 18-774) and medical ethical committee (07-125/O). The RSM01 phase 1 clinical trial (NCT05118386) was conducted in accordance with the United Department of Health and Human Services guidelines and the ethical standards of the Helsinki Declaration (2013).

Results

Validation of Elution Method

We first validated our PBS-only elution method against an existing validated elution method[14] by testing PreF binding by samples recovered from dried blood with or without spiked RSM01. Acceptance limits were met by 90% of the samples for elution efficiency and absolute recovery (**Figure 2A** and **2B**). Comparison of elution across 2 days in the low-tech elution method show a mean reproducibility of 5.4% ± 4.3% CV compared to 12.7% ± 7.0% CV in the validated method (**Figure 2C**). Based on our findings, our PBS-only method was considered noninferior to the validated method for recovery of RSV PreF-binding antibody and was used for future sample processing.

Validation of Dried Blood on RSV Neutralization Assay

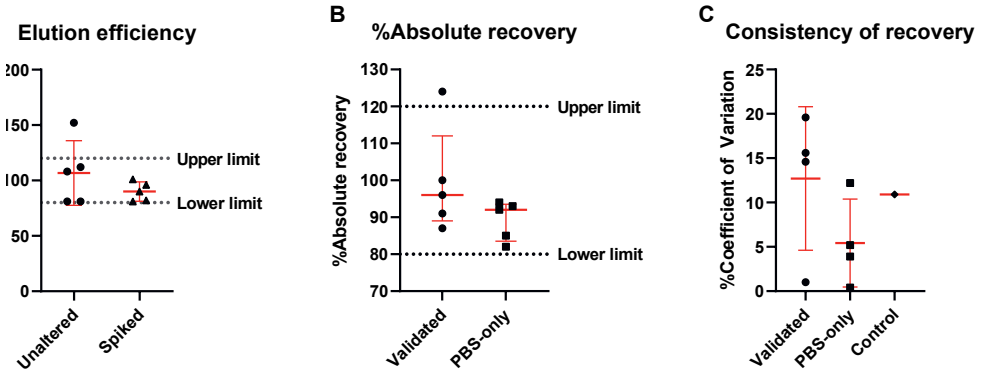
Validation of RSV Neutralization Assay

We determined the critical reagents for the RSV neutralization assay. Two virus batches cultured from the same master stock appeared to affect the ID_{50}, but it did not achieve significance ($p = 0.057$; **Figure 3A**). Two brands of medium (Sigma and Gibco) for cell culture affected the ID_{50} of samples significantly ($p = 0.009$; **Figure 3B**), while it had no effect when used for the dilutions ($p = 0.2161$; **Figure 3C**). Based on this data, culture medium and virus batch are considered critical reagents that require characterization, while the dilution medium is not.

A range of concentrations of RSM01 in PBS was evaluated to determine the limits of detection on the neutralization assay. We found a wide dynamic range of the assay, enabling measurement of RSM01 in concentrations ranging from 1 µg/mL up to >10 mg/mL (**Figure 4**).

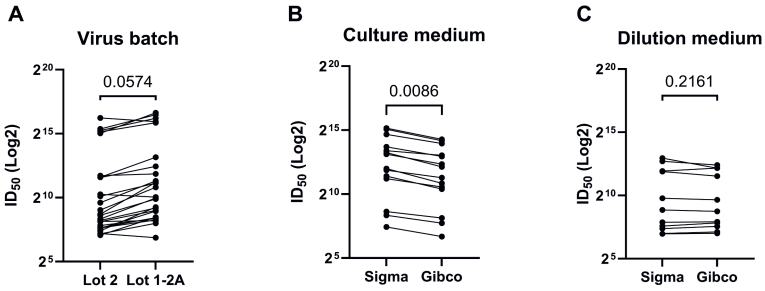
Precision of the RSV neutralization assay with dried blood (≤28.1%CV) was noninferior to serum samples (≤34.0%CV) for spiked (1-100 µg/mL) or unaltered samples (**Table S1**). Inter-operator variation was lower than inter-assay variation for all sample types except spiked serum.

Figure 2. Comparison of two elution methods on dried blood samples unaltered or spiked with 100 µg/mL RSM01 in healthy donor peripheral blood measured by RSV preF ELISA.



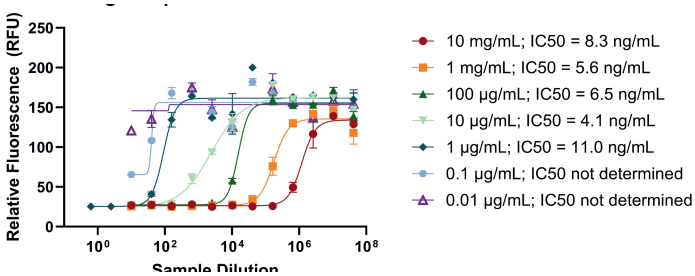
Comparison of validated vs PBS-only elution method (5 samples from 3 healthy donors). Data are presented as mean ± SD. A. Elution efficiency was defined as the percentage recovery with preferred PBS-only elution method divided by the percentage recovery with the validated elution method. B. Percentage absolute recovery per elution method. Percentage absolute recovery is the percentage of measured concentration divided by spiked concentration. Percentage recovery is calculated as follows: [(measured concentrations of the spiked sample – unaltered sample)/measured concentration of the spiked standard diluent] × 100%. C. Consistency of recovery expressed as % CV between 2 days. RSM01 (100 µg/mL) in PBS served as control. % CV is the SD divided by the mean × 100%. CV, coefficient of variance; ELISA, enzyme linked immunosorbent assay; PreF, Prefusion glycoprotein; RSV, respiratory syncytial virus; SD, standard deviation.

Figure 3. Characterization of critical reagents for RSV neutralization assay.



Comparison of assay performance: **A.** with two virus batches; **B.** with two brands of culture medium, a critical reagent; **C.** with old vs new dilution medium, a non-critical reagent. Significance determined by paired *t* test. ID_{50}, half-maximal inhibitory dilution.

Figure 4. Characterization of limits of detection of RSV neutralization assay.



Dynamic range of quantitation of RSM01 antibodies in PBS on the neutralization assay. A range of concwentrations of RSM01 in PBS (0,01 µg/mL – 10 mg/mL) with equal dilution series (1:5 starting dilution, 4-fold serial dilutions) was measured to obtain IC50 values. Error bars resemble technical duplicates. The lowest 2 concentrations did not qualify the assay quality control criteria, and IC50 values could not be determined. IC50, half-maximal inhibitory concentration.

Stability of Dried Blood

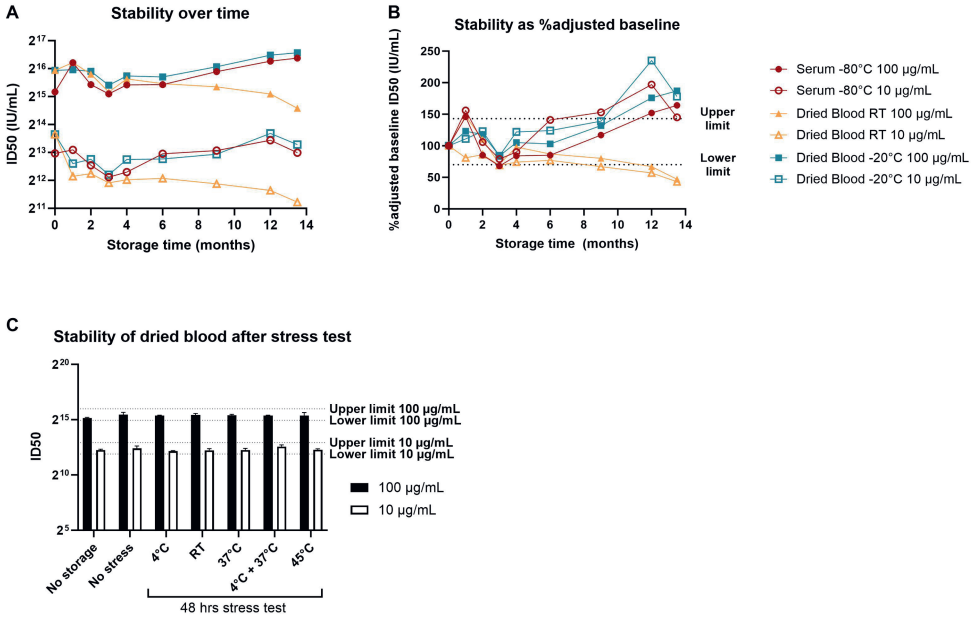
We evaluated long term storage of dried blood samples compared to serum at room temperature (RT) and -20°C. Dried blood with 100 µg/mL (high) or 10 µg/mL (low) concentrations of RSM01 retain stable functionality when compared with serum (Figure 5A). An outlier of serum (100 µg/mL) at 1-month storage is likely an artifact due to faulty measurement (as this is unlikely to be an increase in concentration and because subsequent points show stability). What appears as loss of neutralizing capacity after 1 month storage could also be caused by faulty baseline measurement. Therefore, we calculated an adjusted baseline using all available data of heathy donor samples spiked with 100 µg/mL RSM01 (n = 18 of 3 healthy donors) or 10 µg/mL (n = 11 of 2 healthy donors), as natural immunity is negligible when spiked with these concentrations of RSM01. Antibodies at higher concentrations retain their function for 9 months in frozen and RT dried blood as well as in frozen serum, but at lower concentrations mAb the loss of function is already observed after 6 months storage (**Figure 5B**). Long term storage (>6 months) of dried blood at RT showed discoloration that increased over time from maroon to brown-gray. Humidity was less than 10% over time in dried blood samples at either storage condition over time.

Short term storage at -20°C followed by 48 hours of temperature stress did not induce sample instability at either spiked concentration (**Figure 5D**). Dried blood samples were kept at humidity <10% throughout the temperature stress and the samples at higher temperatures > 37°C showed brown-gray discoloration similar to that observed at > 6-month storage at RT.

Comparison of functional antibodies in dried blood and serum on RSV Neutralization Assay

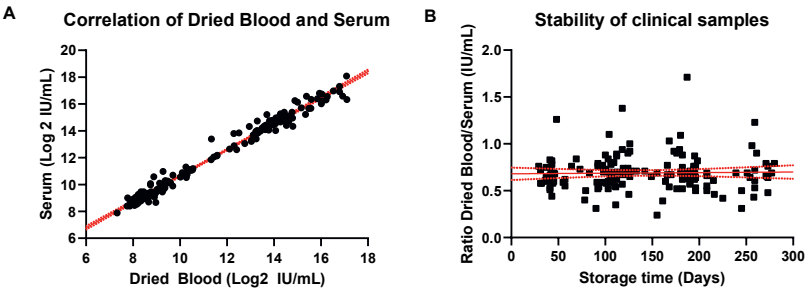
We compared the neutralizing activity in dried blood eluate against serum at 3 different time points for 56 participants of a phase 1 clinical trial evaluating RMS01 in healthy adults. Neutralizing activity in dried blood samples correlated strongly with serum neutralization (R^2 = 0.98, p < .0001, n = 165 matched samples; **Figure 6A**). The duration of storage between sample collection and measurement did not have an effect on the ratio between the

Figure 5. Stability of antibody function in dried blood samples compared to serum samples.



Stability of functional antibodies in dried blood stored at room temperature and -20°C vs serum stored at -80°C (baseline, n = 2; other timepoints, n = 1). Preset upper and lower limit of acceptance are 143% and 70%, respectively (dotted lines). **A**. Neutralizing capacity after long-term storage. **B.** Neutralizing capacity of dried blood expressed as the percentage of adjusted baseline neutralizing capacity. Adjusted baseline was calculated as the geometric mean titer of all available data of healthy donor blood spiked with 100 µg/mL (n = 18) or 10 µg/mL (n = 11), excluding the long term stability data. **C**. Dried blood sample stability after short-term storage at -20°C with 48-hour stress test at various temperatures before returning to storage at -20°C. Upper and lower limit of acceptance are based on stable storage at -20°C (no stress) condition. ID50, half-maximum inhibitory dilution; IU, international units; RT, room temperature.

Figure 6. Antibody function in dried blood samples compared to serum samples.



A. Simple linear regression of Log_2 ID50 values of RSM01 phase 1 clinical trial serum and dried blood samples at three time points (R^2 = 0.981, n = 165 matched samples) with 95% CI (red dotted lines). **B.** Simple linear regression of the ratio between dried blood and serum ID50 (R^2 < 0.001, slope ≈ 0) with 95% CI (red dotted lines). CI, confidence interval; ID50, half-maximum inhibitory dilution; IU, international units.

matched dried blood and serum samples (slope ≈ 0; mean ratio 0.6; 95% CI, 0.66-0.72), with a storage time of 29 to 280 days (**Figure 6B**).

Discussion

In this study, we show for the first time that antibodies stored in dried blood retain their neutralizing capacity against RSV, and we validated an RSV neutralization assay for reconstituted dried blood. The strong correlation between the two sample types collected in a clinical trial (R^2 = 0.98) indicates that dried blood could be used to measure RSV neutralizing antibodies. Dried blood samples are highly stable as compared with serum, for at least 9 months storage at -20°C and 6 months at RT. Short term temperature stress did not induce instability.

This study is the first to use dried blood samples to measure anti-RSV antibody function. To date, antibody concentration, not function, has repeatedly been studied in dried blood. The concentrations of therapeutic mAbs are reliably recovered from VAMS.[7] Anti-SARS-Cov-2 antibody titers in VAMS highly correlate with serum, suggesting that VAMS are a valid method to evaluate antibody titers.[14,20] The varying correlation and precision in previous head-to-head comparisons of neutralizing antibodies[8-12] using DBS on filter paper instead of VAMS could be explained by the smaller sample sizes or by the hematocrit effect. This effect is a well-known limitation of DBS where the hematocrit-dependent blood flow through the paper complicates reproducible recovery of the serum fraction containing the antibodies of interest when a fixed area of the card is punched.[21] VAMS circumvent these limitations by ensuring homogenous sample uptake, and the entire sample is eluted allowing more consistent recovery.[21] Moreover, the volume taken up from a test tube or finger prick is adequately consistent: 107% ± 3.4% of the reported blood volume when taken from a test tube and 102% ± 6.1% when taken from a finger prick.[7] Other devices that control total volume are anticipated to have acceptable precision similar to that shown here with VAMS.

Antibody function is widely assumed to be stable over time in serum, but it is less known if the assumption holds for dried blood. According to a systematic review, DBS are most stable with the least variation at -20°C and -70°C regardless of the intended measurements.[22] No clear decrease in mAb concentration is observed in VAMS up to 1-month storage at RT and 4°C[7] and HIV antibodies are stable when stored for 6 weeks at 4°C, -20°C, or -70°C in DBS.[23] We showed that functional antibody in dried blood stored at RT and -20°C is stable compared with the adjusted baseline up to at least 6 months storage. However, the neutralizing capacity drops between baseline and month 1 raw data (**Figure 5A**). We believe that limitations of our study design underestimate stability when compared with a single baseline measurement at T=0, which was unexpectedly high. Therefore, we calculated a theoretical baseline using various healthy donors to correct this limitation. After short-term storage in the stress test no loss in neutralizing capacity was observed, supporting the potential faulty baseline measurement in the long-term stability test (**Figure 5C**). Moreover,

the neutralizing capacity of dried blood samples plateaus between months 1 and 9 (**Figure 5A**); therefore, the samples are most likely stable. The reason for the upward trend observed for frozen serum and frozen dried blood is unknown. One possibility is that aggregates formed during long-term frozen storage may better neutralize RSV, which was observed for another monoclonal antibody.[24,25] Although adding Tween-20 to the extraction buffer could potentially disrupt aggregates, this hypothesis was not directly tested, as detergent is inconsistent with the cell-based assay. We observe that 48 hours of temperature variation – for example, during shipments of samples – is acceptable in terms of sample stability, although, like neutralizing capacity, IgG concentration in DBS on filter paper was shown to decrease drastically after 7 days storage at 45°C.[26] Our data suggest that -20°C is more suitable for storage >6 months, but shorter storage is also acceptable at RT. These findings need to be confirmed in repeat experiments.

This study has several strengths and limitations. The neutralization assay is sensitive to several reagents due to the live cells and virus, which showcases the importance of same batch measurements and the use of assay controls. The enrichment of the media source might affect viral infection and/or replication and thus influence the read-out. The in-house controls allowed for conversion of the results to international units to compare with other RSV neutralization data worldwide and correct for batch variations during long studies. As the hematocrit fraction is removed during serum processing, the mAb concentration in serum is higher than it is in the capillary blood and thus in the dried blood samples. The normal hematocrit is 40 to 54% for men and 36 to 48% for women,[27] so the serum is expected to be 1.56 to 2.17 times more concentrated than dried blood. The ratio of dried blood/serum is therefore naturally expected to vary between 0.46 and 0.64, which we consider to be similar to the ratio of 0.69 (95% CI, 0.66-0.72) between dried blood and serum. Another limitation is that we have validated the assay on dried blood only from healthy adults. Considering the intended use in infants – with maturing immune systems and possibly with comorbidities – we need to continue validation on blood from healthy infants and those with comorbidities, such as prematurity, coinfection, or immune deficiencies. The scope of this study did not include isotypes of antibodies other than immunoglobulin G, so it remains unknown if dried blood is a valid sampling method for studying immunoglobulin M, A, or E. Last, as assay precision did not meet the 30%CV target for serum samples, it suggests that the ±30% target may have been overly conservative for a bioassay with two living components: cells and virus.

We demonstrated RSV neutralization on dried blood as a patient-centered solution that may replace serology testing in trials in LMICs and potentially be used as a tool to monitor protection against RSV globally. Our study has implications for the future of vaccine and mAb development against RSV and other respiratory viruses, such as influenza and SARS-CoV-2, as blood draws can be challenging in remote areas, especially in young children. One obstacle for worldwide vaccine and mAb access is globally representative trials that need to take place in countries with the highest disease burden because efficacy can differ between

regions.[28] The potential to use dried blood obtained with a simple finger prick can reduce logistical burden of trained phlebotomists and cold chain requirements and thereby reduce cost and complexity. We plan to validate the assay in a LMIC setting as proof of concept that sample analysis is independent of an advanced immunology lab in a high-income country.

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Supplement

Supplementary files can be downloaded from:

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Replacing serum with dried blood microsampling for pharmacokinetics, viral neutralisation and immunogenicity bioanalysis supporting future paediatric development of RSM01, a candidate respiratory syncytial virus neutralising monoclonal antibody

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BMC Infectious Diseases, 2024

Abstract

Background

Virus neutralising antibodies in serum are considered key correlates of protection for vaccines and monoclonal antibodies against respiratory syncytial virus (RSV). RSM01 is a novel, highly-potent, half-life-extended and fully-human monoclonal antibody candidate targeting the RSV prefusion F protein. Currently in Phase 1 development, RSM01 is primarily being developed to potentially provide an effective and affordable RSV prevention strategy in low- and middle-income countries. To evaluate the ability of dried blood collection to generate data sets and conclusions comparable to serum collection, we compared pharmacokinetics (PK) of RSM01, immunogenicity, and virus neutralisation for dried capillary blood samples with serum samples.

Methods

RSM01 PK, anti-drug antibodies (ADA), and RSV-neutralising antibodies from the Phase 1 trial were analyzed and compared between matched serum and dried blood samples. Deming regression analysis was performed using baseline-corrected values to evaluate correlation between measurements in liquid serum versus dried blood.

Results

The analysis showed good correlation ($R^2 > 0.95$) between individual RSM01 concentrations measured in both serum and capillary blood. Analysis of RSM01 PK parameters in capillary blood yielded equivalent conclusions as from serum. A strong correlation ($R^2 > 0.95$) was observed between RSV neutralising activity measured in both serum and capillary blood. In addition, RSV neutralising activity was correlated with RSM01 concentrations in both serum and capillary blood data sets. For ADA, individual sample results had 96% agreement (290/302) and overall participant ADA status had 93% agreement (52/56).

Conclusions

Both RSM01 concentrations and RSV neutralising activity showed a strong correlation between the serum and blood measurements. ADA measurements also had an agreement of > 90% for individual samples and overall participant status. Our results demonstrate that dried blood is a suitable specimen type for collection and evaluation in the RSM01 clinical development program and shows promise as a useful approach to reduce patient burden in clinical trials, particularly for infants in low- and middle-income countries.

Background

Respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory tract infection (LRTI) in infants and young children worldwide, particularly in low- and middle-income countries (LMICs).[1] Current approaches to prevent RSV disease include vaccination of pregnant individuals and passive immunization with monoclonal antibodies (mAbs).[2] RSM01 is a novel, highly potent, fully human, RSV-neutralising mAb candidate with an extended half-life and is being developed primarily for LMICs.[3]

Given that the clinical development of RSM01 focuses on the infant population experiencing their first RSV season,[4] we considered aspects of patient-centricity within the RSM01 clinical development program. Two key aspects to patient-centricity within the bioanalytical sciences include (1) optimal sample collection to make sure that every sample being collected has a clear use and (2) minimizing sampling requirements (both number of samples and volume of samples) for trial participants.[5] Reducing sample volumes is particularly important for infant populations due to lower weight and blood volume, as oversampling can lead to iatrogenic anemia.[6]

While one approach is to reduce sample collection volume while maintaining traditional venipuncture techniques, this still requires personnel trained in venipuncture, on-site or nearby equipment to process the venous blood to serum, and cold chain storage and shipment of the serum samples. The venipuncture procedure itself is particularly challenging in patients with difficult venous access, for which risk factors in children include younger age, female sex, darker skin color, prematurity, and sickle cell disease.[7,8] As these factors align with our target population of infants in LMIC, capillary collection was evaluated as a preferred approach to both reduce sample volume and the impact of the venipuncture procedure in vulnerable populations.

Dried blood spot (DBS) sample collection is the most well-known and researched form of microsampling, with multiple applications across different analytes including nucleic acids, peptides and proteins.[5,9,10] DBS is one of the least invasive blood sample collection methods, with samples easily transported and stored.[11,12] One of the most common and widely accepted clinical uses of DBS sampling is in neonatal screening programs. DBS has also been used to assess infectious disease serology from both natural immunity and vaccines.[10,13,14] While these serology assays have been conducted as binding assays rather than functional assays, other functional enzyme assays have previously been assessed successfully by DBS.[15]

Development of volumetric based dried blood collection has expanded the use of dried blood to now include quantitative drug analysis applications, including therapeutic antibodies.[16-19] A key advantage of volumetric sampling versus DBS is precise collection of a fixed volume of blood rather than a fixed diameter of dried blood, which reduces variability due to haematocrit viscosity effect impacting spread of DBS.[20,21] Several of the volumetric sampling devices also employ a polymeric substrate, which has been helpful to facilitate

complete recovery of hydrophilic antibodies without requiring the use of additional steps to disrupt the paper substrate of DBS.[17,18,22] The specific technology evaluated in this work was volumetric absorptive microsampling (VAMS), which also has the advantage of using capillary action to wick up the blood from the finger stick or heel stick directly to minimize collection volume.[17,18,23]

In conjunction with the advances in microsampling has come increased familiarity and acceptance of these approaches by health authorities. Validation of methods on dried matrices is included in ICH M10.[24,25] If data from both serum and blood specimens are used within a program, a bridging experiment is required, however it does not require nearly as many samples as the fully matched trial design conducted herein.

Since the RSM01 development program targets an infant population with the eventual goal of global enrollment at a variety of clinical sites with different resource constraints, we decided to use small volumes of capillary blood as the primary specimen type for the entire RSM01 clinical development program. As such, we took the opportunity in the first in human (FIH) trial Gates MRI-RSM01-101 to collect both capillary blood and venous serum for the bioanalytical program. The analytes tested included RSM01, anti-drug antibodies (ADA) against RSM01, and RSV-neutralising activity whether facilitated by RSM01 or the result of natural immunity. We selected the specific cell-based RSV neutralisation assay for the low sample volume required, the short incubation time, the relatively high throughput, and use of common instrumentation.[26]

Within the Gates MRI-RSM01-101 trial, we did fully matched sample collection in this population of healthy adults to expand analyses beyond correlation of individual sample results to also enable comparison between conclusions derived from the pharmacokinetics (PK) analysis. While this duplicated sample collection design far exceeds the requirements of a bridging trial and is not patient-centric for the participants in Gates MRI-RSM01-101, the patient-centric focus was targeted towards the infant populations planned for enrollment in the subsequent development program of RSM01.

This manuscript describes the results of the exploratory objective in Gates MRI-RSM01-101 to evaluate the feasibility of using capillary blood in future RSM01 clinical development that will be conducted entirely in infants.

Methods

First-in-human trial

The first-in-human, double-blind, phase 1 trial of RSM01 enrolled healthy adults of 18–49 years of age.[4] In brief, participants were randomised 6:1 to receive a single dose of RSM01 or placebo in dose ascending cohorts of 300 mg intravenously (IV), 300 mg intramuscularly (IM), 1000 mg IV, 3000 mg IV, or in a dose expansion cohort of 600 mg IM. The primary objective was to evaluate the safety of a single dose of RSM01. Secondary objectives

included characterization of the PK and anti-drug antibodies. Exploratory objectives included characterization of RSV-neutralising antibodies as well as comparison of PK and pharmacodynamics between serum and capillary blood samples.

Serum and blood samples were collected at fully matched time points through 151 days (**Supplement Table 1**). Serum samples were collected by venipuncture in serum separator tubes. Dried blood samples from finger sticks were collected by touching the Mitra VAMS (Trajan Scientific, Australia) from above the blood spot to wick up the fixed volume. Both serum and VAMS samples were stored at -70 °C and shipped on dry ice.

RSM01 concentration

Separate validated immunoassays were used for the quantification of RSM01 in serum and in dried blood extract. In summary from full method (Supplemental Methods), dried blood was extracted from the 20 µL VAMS device into 200 µL buffer. Calibrators, quality control (QC) samples, and clinical trial samples (serum or extracted blood in separate assays) were diluted to the minimum required dilution (MRD) and captured on coated MSD plates. After incubation and washing, detection antibody conjugated to Ruthenium (MSD Sulfo Tag Gold, Mesoscale Discovery, Gaithersburg, MD, USA) was added to the plate and incubated. Plates were then washed followed by the addition of MSD Read Buffer. Relative light units (RLU) were captured on an MSD Sector 600 Imager. The concentration of RSM01 was calculated from a 5-parameter nonlinear regression curve with 1/Y2 weighting of calibrators.

PK non-compartmental analyses

The PK analysis included all participants who received RSM01 and had baseline and at least one post-baseline serum or blood PK result. PK parameters were determined using noncompartmental analysis (NCA) of concentration-time data performed with Phoenix® WinNonlin® v.8.4. PK parameters analysed included maximum drug concentration (C_{max}), time to C_{max} (T_{max}), and area under the curve up to the last measurable concentration (AUC_{last}) in serum and blood. Some PK parameters (AUC_{inf}, CL, CL/F, Vz, Vz/F and $t_{1/2}$) were not calculated due to unreliable terminal phase (AUC extrapolation > 20%).

RSV-neutralising antibodies

Separate qualified viral neutralising assays were used to determine neutralising capacity as described previously.[27] In brief, 20 µL of dried blood was eluted overnight at room temperature into 200 µL PBS. Both serum and dried blood eluate were heat-inactivated at 56 °C for 30 min before serial dilution i n medium. Recombinant mKate-RSV-A2[28] was added 1:1 to the sample dilution series and incubated for 1 h at 37 °C before addition of 50 µL of sample-virus mixture to a monolayer of Hep-2 cells. After 26 h incubation, fluorescence was recorded and 50% inhibitory dilution (ID_{50}) was calculated from a 4-parameter nonlinear regression curve.

Anti-drug antibodies

Separate validated bridging immunoassays were used for the detection of anti-drug antibodies (ADA) against RSM01 in serum and in dried blood extract. In summary from full method (Supplemental Methods), dried blood was extracted from the 20 µL VAMS device into 200 µL buffer. Samples were diluted to the MRD and incubated with biotin-labelled RSM01 and ruthenium-conjugated RSM01. Complexes of labeled RSM01 and ADA were captured on streptavidin MSD plates. Samples above the threshold in the screening assay were subsequently analysed in the confirmatory assay to evaluate a drop of signal in the presence of excess unlabeled RSM01. Samples that were positive in the confirmatory assay were titrated by serial dilution to determine the reciprocal value of the highest dilution factor considered positive.

Overall ADA status was derived across the individual results for each participant. For the agreement analysis, participants were considered positive if they had any ADA positive samples, whether pre- or post-baseline.

Correlation analyses

Correlation analysis was performed using Deming regression,[29] which minimizes the perpendicular distances between the data points and the regression line, thus incorporating known variability in measuring both variables. The variance ratio was set to one since both variables had similar value ranges, making the Deming regression equivalent to an orthogonal regression. Additional discussion on selection of Deming regression is in the Supplemental Methods.

Regressions were performed primarily on the PK population, except for comparing the RSV neutralising activity between serum and blood, which was performed on the per protocol population. The per protocol population included all participants who did not have a significant protocol deviation. The PK population included all participants who received RSM01 and had a baseline and at least one post-baseline PK assessment.

Due to the wide range of values within the regression and estimated errors being proportional to values rather than fixed across the range, the Deming regressions were performed on natural log transformed data. For RSM01 concentrations, the natural log was performed on ng/mL units. The regression lines were then converted to exponential lines for plotting with raw data.

Results

We measured three types of antibodies in serum and dried blood samples from RSM01-101 clinical trial: RSM01 mAb candidate, ADA against RSM01, and RSV-neutralising antibodies. As antibodies including RSM01 are generally not expected to partition to the cellular component, concentrations measured in whole blood were expected to be numerically lower than concentrations in serum.[22] This is because for a fixed collection of blood with a fixed amount of antibody present, processing the blood to serum would retain nearly all

of the antibody in the serum fraction while the total sample volume would be decreased by approximately the cellular fraction in the clot. With serum samples having the same numerator and a smaller denominator versus blood samples, the reported concentration should always be higher.

During assay development for all analytes, we were able to attain 100% recovery using standard buffers (detailed methods in Supplementary information and as described earlier by Terstappen et al.[27]). This complete recovery helped maintain assay sensitivity even while reducing the total sample size.

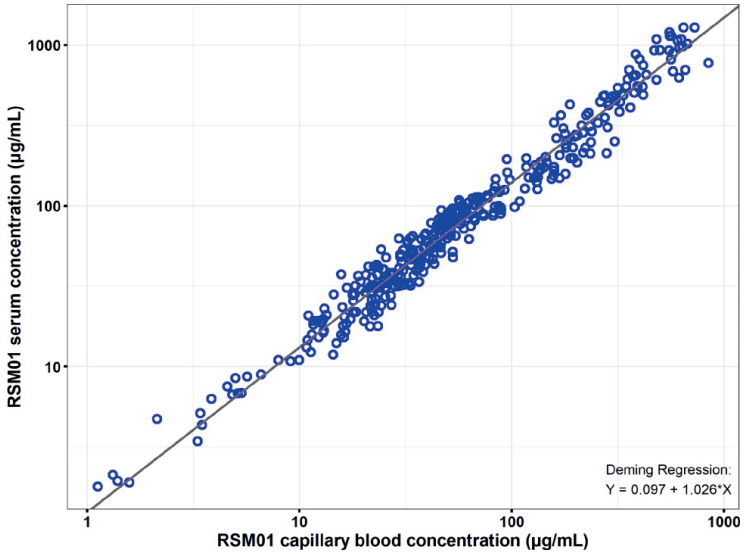
RSM01 PK in serum and blood

As previously seen for other monoclonal antibodies,[17,30] there was good correlation (R^2 = 0.985) between individual concentrations of RSM01 measured in both serum and capillary blood (**Figure 1**). In addition, the residuals were equally distributed above and below the regression line across the concentration range.

To further understand the impact on the specimen type on PK analysis, the non-compartmental analysis was conducted separately on both serum and blood data sets. Peak concentrations in capillary blood (T_{max}) were reached between approximately 6 days and 8 days post dose for the intramuscular cohorts (**Supplement Table 2**). RSM01 was eliminated gradually, in a monophasic manner following IM administration and in a biphasic manner following IV administration (**Supplement Figure 1**, **Supplement Table 3**). The observed between-participant variability was in line with the anticipated variability for a mAb following IM administration (**Supplement Figure 2**). RSM01 concentration and AUC increased dose-proportionally following IV and IM administration with similar profiles across individual participants (**Figure 2**). These PK results and conclusions are similar to those observed for serum samples.[4]

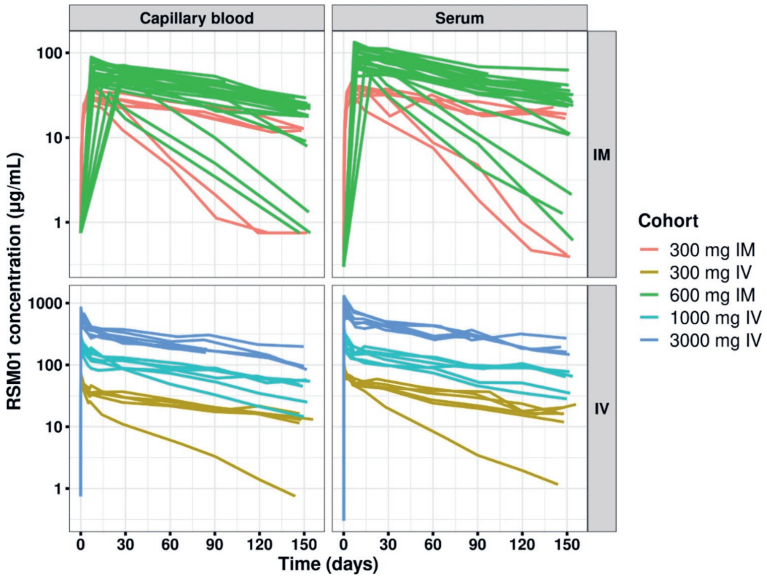
The ratio of blood to serum PK parameters were performed for AUC_{0-D91}, $AUC_{0\text{-}D151}$, and C_{max} (**Table 1**). The geometric mean ratio was similar across all three parameters and cohorts.

Figure 1. Deming Regression of RSM01 Concentration in Serum versus Blood.



Log-log scale scatter plot of RSM01 concentration (µg/mL) for serum versus capillary blood. The Deming regression was performed with pooled data across all matching timepoints and from all cohorts, with variance ratio = 1 on log-transformed concentrations to yield the regression line of log (serum RSM01 concentration) = 0.097 + 1.026*($\log_{10}$[capillary blood RSM01 concentration]). This regression line is shown in black.

Figure 2. RSM01 concentration in serum and capillary blood over time by cohort, stratified by administration route.



The left column shows individual profiles derived from capillary blood concentrations and the right column shows individual profiles derived from serum concentrations. The top row shows individual profiles from IM administration and the bottom row shows individual profiles from IV administration.

Table 1. Ratios between blood PK parameters to serum PK parameters

Serum vs blood PK Parameters	Cohort 1 300 mg IV n = 6	Cohort 2 300 mg IM n = 6	Cohort 5 600 mg IM n = 24	Cohort 3 1000 mg IV n = 6	Cohort 4 3000 mg IV n = 6
AUC_{0-D91}	0.674 (14.9)	0.825 (8.9)	0.677 (13.2)	0.815 (10.3)	0.643 (10.6)
AUC_{0-D151}	0.703 (15)	0.797 (8.6)[a]	0.693 (13.4)[b]	0.783 (8.9)	0.665 (7.9)[c]
AUClast	0.700 (15.3)	0.776 (9.7)	0.690 (14.3)	0.783 (8.9)	0.653 (11.7)
Cmax	0.633 (16.7)	0.791 (17.8)	0.622 (15.3)	0.843 (19.8)	0.622 (22.1)

Summary statistics were performed on ratios from individual participants' PK parameters [a]n = 5; [b]n = 23; [c]n = 4. Data are presented as geometric mean (CV%). CV, coefficient of variation.

RSV-neutralising activity in serum and blood

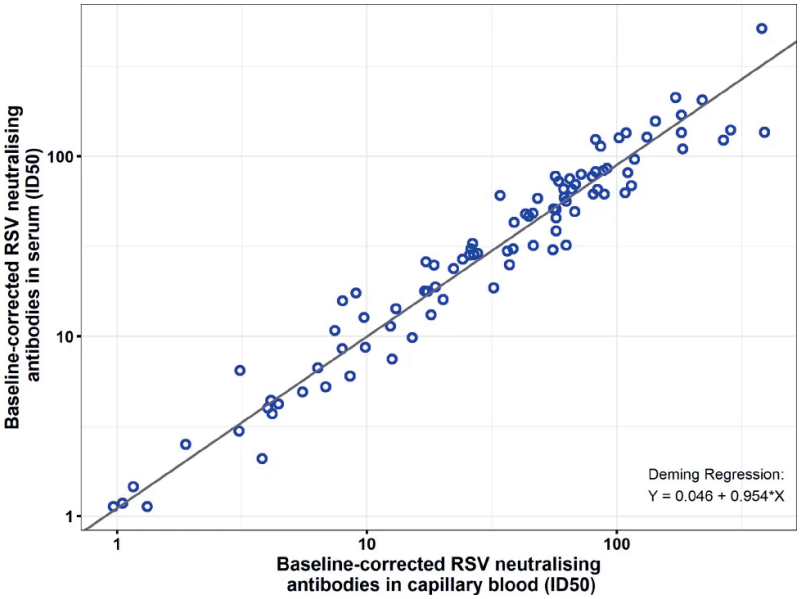
RSV neutralising activity was detected in all samples including prior to first dose for both serum and blood, with 10- to 100-fold increase over baseline at day 151 (**Supplement Figure 3**). There was good correlation (R^2 = 0.964) between the baseline-corrected RSV neutralising activity measured in serum and capillary blood (**Figure 3**). In addition, the residuals were equally distributed above and below the regression line across the concentration range. (Note that the smaller n for baseline corrected ID50 reflects that all Day 1 values are normalised to 1 and therefore excluded from the correlation analysis.)

To evaluate the relationship between RSV neutralising activity and RSM01 concentration, the Deming regression was performed for values derived from serum (**Figure 4A**) and blood (**Figure 4B**). As the RSV neutralising activity detects both RSM01 and any RSV neutralising antibodies from natural infection, there was no expectation that the values would correlate well with the RSM01 concentrations. While RSV neutralising antibodies generated by the participants' adaptive immune systems may vary during the trial, a uniform baseline correction was performed within a participant by dividing post-administration ID_{50} values by their pre-dose baseline ID_{50}. The Deming regression of baseline corrected RSV viral neutralising activity versus the RSM01 concentration showed a correlation between the two assessments over the range of values in both specimen types. While the regression was generally strong (R^2 = 0.834 and R^2 = 0.800 for serum and blood, respectively), the RSV neutralising activity exhibited proportionally more positive residuals at lower concentrations of RSM01 and more negative residuals at higher concentrations of RSM01.

ADA in serum and blood

Because immunogenicity test results are not reported as continuous numeric data, the comparison was conducted by positive versus negative categorical results across both individual samples (**Table 2**) and overall immunogenicity status of subjects (**Table 3**). Individual sample results had 96% agreement (290/302) and overall participant status had 93% agreement (52/56).

Figure 3. Deming Regression of RSV Neutralising Activity in Serum versus Blood.



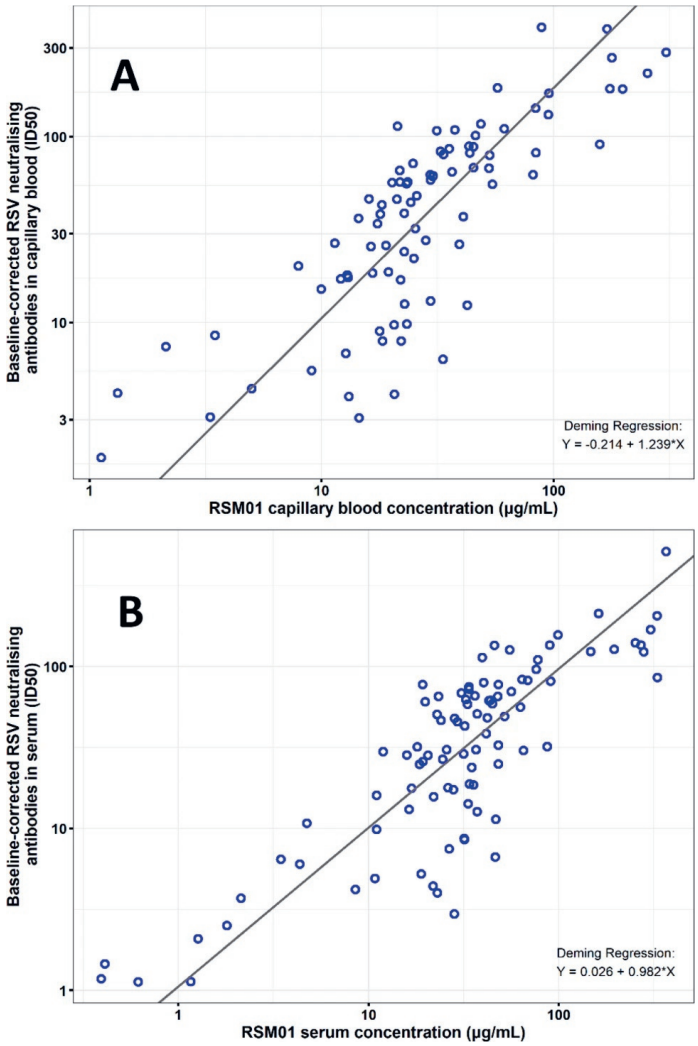
Log-log scale scatter plot of baseline-corrected RSV neutralising activity (ID_{50}, reciprocal dilution corresponding to 50% inhibition of viral activity) in serum versus blood The Deming regression was performed with pooled data across all matching timepoints and from all cohorts, with variance ratio = 1 on log-transformed ID_{50} to yield the regression line of $\log_{10}$(serum RSV neutralising ID_{50}) = 0.046 + 0.954*($\log_{10}$[blood RSV neutralising ID_{50}]). This regression line is shown in black.

For serum measurements, the baseline ADA-positive rate was 2/48 (4.2%) with RSM01.[4] The two baseline ADA positive participants were in the RSM01 600 mg IM cohort and one remained ADA-positive after RSM01 administration. One baseline ADA-negative participant in the RSM01 1000 mg IV cohort was categorized as postbaseline ADA-positive, for a treatment-emergent ADA-positive rate of 1/48 (2.1%) with RSM01.

For blood measurements, the baseline ADA-positive rate was 1/48 (2.12%) with RSM01. The baseline ADA positive participant was in the RSM01 1000 mg IV cohort and remained ADA-positive after RSM01 administration. Two baseline ADA-negative participants, one each in the RSM01 1000 mg and 3000 mg IV cohorts, were categorized as postbaseline ADA-positive, for a treatment-emergent ADA-positive rate of 2/48 (4.2%) with RSM01.

To better understand the potential source of discrepancies in ADA status, the ADA results from all individual samples for the 5 patients positive in at least one specimen type were examined. For the participant in the 1000 mg IV cohort characterized baseline ADA negative and post-baseline ADA positive in both serum and blood, seroconversion was detected at the same trial day with the same persistence of response with no discrepancies in individual results. For the 4 participants with discrepant overall status from discrepant individual sample results, the results were all close to the assay limit of detection which was established using an expected false positive rate (see **Supplemental Methods**).

Figure 4. Deming Regression of RSV Neutralising Activity versus RSM01 concentration.



Log-log scale scatter plot of baseline-corrected RSV neutralising activity (ID_{50}) versus RSM01 (µg/mL) for blood **(A)** and serum **(B)**. The Deming regression was performed with pooled data across all matching timepoints and from all cohorts, with variance ratio = 1 on log-transformed RSV neutralising activity and RSM01 concentration to yield the regression lines of $\log_{10}$(blood RSV neutralising ID_{50}) = -0.214 + 1.239*($\log_{10}$[blood RSM01 concentration]) and $\log_{10}$(serum RSV neutralising ID50) = 0.026 + 0.982*($\log_{10}$[serum RSM01 concentration]). These regression lines are shown in black.

The potential impact of immunogenicity on PK parameters was evaluated across both serum and blood data sets. In both specimens, concentration-time curves for participants who were ADA positive (either baseline or treatment-emergent) were within the limits of the other ADA negative participants in their respective cohorts (**Figure 4**).[4]

Table 2. Agreement of serum and blood ADA results from individual samples

ADA in serum	ADA in blood	
	Positive[a]	Negative
Positive[a]	3 (1.0%)	4 (1.3%)
Negative	8 (2.6%)	287 (95%)

[a]Positive status includes positive samples with titre not reportable

Table 3 Agreement of overall participant status derived from serum and blood ADA sample results

Participant Status from Serum ADA results	Participant Status from Blood ADA results	
	Positive[a]	Negative
Positive[a]	1 (1.8%)	2 (3.6%)
Negative	2 (3.6%)	51 (91.1%)

[a]Positive status includes positive samples with titre not reportable

Discussion

Gates MRI RSM01-101 is the first-in-human, phase 1 trial of RSM01 mAb evaluating the safety, PK and immunogenicity of RSM01 in healthy adults.[4] In the present analysis, we evaluated the feasibility of using capillary blood instead of venous serum for bioassay measurements in future RSM01 clinical trials that will be conducted entirely in infants. We compared RSM01 PK, ADA, and RSV-neutralising antibodies between matched serum and dried blood samples collected in the RSM01- 101 trial. Correlation between measurements in liquid serum versus dried blood was evaluated using Deming regression analysis.

Both RSM01 concentrations and RSV neutralising activity exhibited strong correlation between the serum and blood measurements. Lower concentrations and RSV neutralising activity were observed in blood compared with serum as expected, with antibodies primarily partitioning to the liquid but not to the cellular component of blood.

Notably, the RSV neutralising activity was retained in the cell-based neutralisation assay throughout the VAMS collection, processing, storage, and extraction steps. To our knowledge, this is the first published cell-based activity assay showing equivalent results for serum and dried blood. While we have only tested the blood extracts in one viral neutralising activity assay, we anticipate that retention of function would also be observed in other RSV neutralising activity assays employed for other mAbs and vaccines.[31-35]

The PK analysis yielded similar conclusions in both blood and serum specimens. Variability within cohorts was similar. Dose-proportionality was observed in both data sets. While females have lower average haematocrit than males,[36] no gender-based differences were observed in the correlation analysis or in pharmacokinetic analyses from either serum or blood.

Immunogenicity results from blood and serum specimens were also in high levels of agreement. It is difficult to determine explicitly whether the individual samples or categorization of the 4 participants with discrepant results are false positive or false negative. Certainly, the detection of ADA near the threshold is consistent with either hypothesis. Since both methods required use of non-parametric cut point factors, which are challenging to establish and highly dependent on the included samples, it is possible that this contributed to the Type I and Type II error rates in one or both assays.

Of note in this first-in-human trial in adults, we expected that the participants would have RSV neutralising activity at baseline from antibodies generated against prior natural infection. Therefore, baseline-correction was applied for comparison with measured RSM01. While this correction may still be required for future trials in infants with pre-existing neutralisation activity from maternal natural immunity, the baseline signal may not be constant over time as it depends on infant gestational age at birth, maternal natural immunity, and breastfeeding status.[35] For the infant population, the correlation with RSM01 will be evaluated with and without baseline-correction.

The correlation between RSM01 and RSV neutralising activity did exhibit bias as a function of RSM01 concentration. This bias was observed in both the serum and blood matrices, so does not disqualify either specimen collection. As this trial was conducted in healthy adults and overlapped with the COVID-disrupted RSV season, this bias may be due to the incomplete correction for natural immunity being more evident at lower RSM01 concentrations.[37] In the infant populations, the bias in neutralising activity versus RSM01 concentration may or may not remain. Additional hypotheses independent of trial population include bias of incurred samples in one or both assays which was not previously detected with spiked samples in validation, or an inherent non-proportional property of RSM01 in the viral neutralising activity.

The ratio of blood to serum may be lower and with greater variability in the infant population due to higher average haematocrit at birth that declines over the first 2 months of age,[38,39] although these trends are less dramatic in African infants.[40,41] It is currently unknown whether haematocrit variability will meaningfully contribute to pharmacokinetic variability in infants. Use of a well-controlled immunoassay optimized and validated for blood measurements, a precise volumetric based collection device, and shipments at established stability conditions helps minimize preanalytical and analytical variability to better assess measures of biological variation. The potential contribution of haematocrit can then be evaluated by comparing Deming regressions and total variability from NCA derived from concentrations with and without haematocrit adjustment.

Conclusions

For the RSM01 candidate, use of whole capillary blood sampled on VAMS is a suitable specimen type for the full clinical development program. Variability in blood was no greater

for RSM01 concentrations, PK parameters, or RSV neutralizing activity than observed from serum. We encourage other sponsors to consider this approach to reduce the patient burden in clinical development programs. While the impact of reducing collection volume is greatest in the target infant population, other benefits of patient-centric sampling are broadly applicable, including streamlined processing at the clinical site and the potential to collect samples at locations with limited or no access to trained phlebotomists for venipuncture.

Implementing dried blood microsampling more broadly across clinical trials and post-marketing research on RSV prevention could help advance research into the most vulnerable patients who need these treatments, both premature infants and older adults with chronic medical conditions. Since these individuals also are at higher risk for difficult venous access,[7,8] these individuals may inadvertently be excluded from research into efficacy and safety clinical trials due to the inability to complete extensive venipuncture sample collection.

In addition, collection of microsamples during the registrational clinical trial enables direct translation to future sample self-collection by individuals in real world post-marketing trials, surveillance efforts, or even monitoring individual therapeutic or vaccine response to determine whether they need an additional monoclonal dose or vaccine boost. With both concentration and RSV neutralizing activity assays performing well in dried blood matrix, whichever measure is the best predictor of protection in the clinical trial can be measured from the self-collected dried blood sample.

While this manuscript focuses on implementation in the clinical trials, it is also possible to expand the blood microsampling into nonclinical research.[42-45] Implementation of this approach in nonclinical studies supports the 3R principles (Replacement, Reduction, and Refinement)[46] by enabling collection of exposure information in the pharmacology studies such as the cotton rat infection model.

As with every innovative approach, switching to patient-centric sampling approaches currently places more requirements on the sponsor including proactive engagement with trial investigators and institutional review boards, establishing a supply chain with the central laboratories for the nontraditional devices, education and training of clinical site personnel, and additional assay validation at analytical laboratories. Shifting the burden from the patient and site towards the sponsor early in development is anticipated to reap rewards in later stages of clinical development, including enabling hybrid and decentralized trial designs where biospecimen collection is required or adds scientific value.

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Supplement

Supplementary files can be downloaded from:

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Proof-of-principle of a technology transfer of a dried blood virus neutralization assay to a Gavi-eligible country

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Abstract

Background

Global health clinical research is commonly led by high-income countries (HICs) as low- and middle-income countries (LMICs) face barriers to participate, including lack of financial and human capacity and lack of research environment. Respiratory syncytial virus (RSV) vaccine development is also led by HICs, while LMICs carry the burden of life-threatening disease. Representative trials and research capacity strengthening in LMICs are needed to ensure global vaccine access and equity. This study aims to transfer an RSV neutralization assay, which uses live cells and virus with inherent high variation, to a country eligible to receive support from the Gavi, the Vaccine Alliance.

Methods

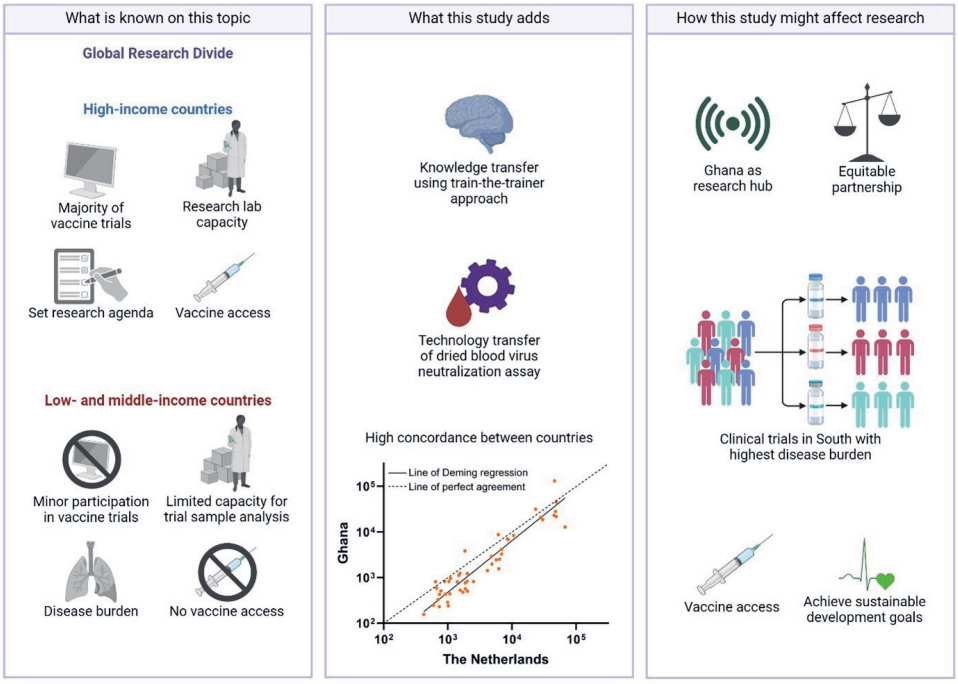
Using a train-the-trainer approach, a Ghanaian researcher was trained in the Netherlands on the dried blood-based RSV neutralization assay. Subsequently, a Dutch researcher visited Ghana to support the process of adapting the technique to the Ghanaian setting. In a previously validated RSV neutralization assay on dried blood, Hep-2 cells were infected with a serial dilution of sample-virus mixture to determine the half-maximal inhibitory concentration. Fifty-one dried blood and serum samples were tested in parallel in both countries to assess concordance.

Results

Training and technology transfer was deemed successful, which was defined as neutralization measurements by the Ghana team and high concordance (Lin's concordance correlation coefficient (CCC) > 0.8). Neutralizing capacity measured in identical samples in Ghana and the Netherlands correlated highly (Lin's CCC = 0.87; Spearman rho = 0.89) but was systematically lower in Ghana than the Netherlands.

Conclusion

We show successful transfer of an RSV neutralization assay, thereby strengthening the laboratory research capacity in a Gavi-eligible country. Reliable measurement of RSV neutralizing antibodies in a Gavi-eligible country and the use of dried blood can contribute to inclusion of LMICs in RSV vaccine development and access.



What is already known on this topic

- The global health research divide is evident in respiratory syncytial virus (RSV) vaccine development, where vaccines are developed and available in high-income countries (HICs), despite low- and middle-income countries (LMICs) bearing the majority of the disease burden.
- Representative trials and research capacity strengthening in LMICs are needed to ensure global vaccine access and equity.

What this study adds

- In this newly established partnership, we used a train-the-trainer approach to transfer a low-tech dried blood live-virus neutralization assay from the Netherlands to Ghana, a country eligible to receive support from the Gavi, the Vaccine Alliance.
- A sample panel measured in parallel in both countries showed high concordance and correlation despite the difficulties of running a bioassay with inherently high variability while using distinct materials.

How this study might affect research, practice, or policy

- The successful implementation of this low-tech virus neutralization assay may facilitate local in-country participation in future trials, sample analysis and management of clinical trial samples.
- The expertise and set-up would serve as a capacity strengthening platform for researchers in Ghana and other LMICs. The country's participation in trials can stimulate local manufacturing companies to produce current or future vaccines or monoclonal antibodies (mAbs), which ultimately can reduce barriers to equity in vaccine access and the ability to respond to pandemics.

Introduction

The global divide in resources, including research funding and scientific capacity, results in a research agenda set by high income countries (HICs).[1] Low- and middle-income countries (LMICs) experience substantial barriers to participate in clinical research, including lack of funding, lack of skilled personnel, and lack of research environment (**graphic abstract**).[2] Equitable partnerships can facilitate research capacity strengthening by sharing knowledge, technology, expertise, and funding to effectively address pertinent research questions and enhance self-sufficiency.[1,3]

The global research divide is visible in the respiratory syncytial virus (RSV) vaccine development: vaccines and immunization are predominantly developed in HICs, where RSV is now a vaccine-preventable disease,[4-7] although more than 95% of RSV-associated hospitalizations and deaths occur in LMICs.[8] Registration trials included participants in LMICs but sample and data analysis were performed in HICs. Consequently, no RSV vaccine or monoclonal antibody (mAb) is within reach in LMICs despite the high burden.[9,10] Representative clinical trials in LMICs are key to ensure global access and affordability of an effective RSV vaccine.[9,11] Trials largely monitor neutralizing antibody (nAb) levels as a correlate of protection, however a simple tool to measure protection after RSV vaccination is lacking in LMICs due to prohibitive costs and lack of research capacity.[12] Bioassays can measure nAbs with live cells and virus but the live components inherently increase the complexity and the variation in results. Cell-based assays are recommended to have a target coefficient of variation (CV) of < 50% with an average of 25%,[13] whereas industry guidelines are stricter with a CV < 20%.[14] Capacity strengthening for vaccine trial research in Gavi-eligible countries, i.e. LMICs that qualify for support from Gavi, the Vaccine Alliance to improve access to vaccines and immunization services, could accelerate access to RSV vaccines while promoting research equity.

Serum obtained through venipuncture is generally considered the gold-standard sample to measure surrogates of vaccine-induced protective immunity.[15] Dried blood spots (DBSs) obtained through finger-prick offer attractive alternatives to serum antibody testing and have already been found to be appropriate tools for the antibody surveillance and/or diagnosis of other viral pathogens such as hepatitis C virus (HCV) and human immunodeficiency virus (HIV).[16,17] DBS samples provide numerous advantages over serum, as they are cheaper, less invasive, and easy to (self-)collect in clinical and nonclinical settings. DBS do not require specialist equipment for processing and storage, have long-term stability, are readily transportable and hence may facilitate clinical trials in LMICs.[18,19] Our recently validated neutralization assay using dried blood offers a patient-centric and financial solution to logistical barriers to immunization research in LMICs.[20]

Here, we describe a technology and knowledge transfer of a dried blood-based RSV neutralization assay as part of a new partnership to strengthen the research capacity in a Gavi-eligible country. The low-tech low-cost assay can be used to measure clinical trial

endpoints in a low resource setting and can set a platform for future development of broader respiratory virus surveillance, vaccine impact, and correlate of protection studies in LMICs.

Methods

Site selection and training

The Virology Department of the Noguchi Memorial Institute for Medical Research (NMIMR) situated in Accra, Ghana was selected as a site after a screening process involving ten sites within the international GOLD-III ICU network.[21] Thorough inquiry ensured availability of the required facilities to conduct the assay: cell and viral culture systems, biosafety level 2 cabinets (including lamina air flow hoods), fluorescence plate reader with required filters, and available research staff experienced in cell culture and virus handling.

Using the train-the-trainer approach,[22] a researcher from NMIMR visited the University Medical Center Utrecht (UMCU), The Netherlands, for one month to be trained on the dried blood-based RSV neutralization assay.[20] Subsequently a Dutch expert researcher visited Ghana for one month to support the process of adapting the technique to the Ghanaian setting (**Table 1**). Successful training was defined as measurement of neutralizing capacity in dried blood using the neutralization assay by the Ghana team.

Sample panel preparation and handling

Individual samples for validation of the assay were obtained from healthy volunteers to be measured in parallel in both laboratories. Whole blood was either left blank or spiked with anti-RSV mAbs palivizumab and RSM01 (Bill & Melinda Gates Medical Research Institute) at concentrations ranging 5-200 µg/mL. After 30 minutes on a rocker to mix, Mitra 20 µl volumetric absorptive microsampling tips (VAMS; Trajan Scientific, Neoteryx Clamshell, 20004) were touched to the whole blood sample surface to absorb the fixed volume and left to dry for 3 hours at room temperature.[20] The dried blood samples were packaged in zip-lock bags containing desiccant to allow further drying. Blood of eight healthy donors provided five unique samples per donor for a total of 40 dried blood samples. Additionally, four archived dried blood samples from a previous study,[20] four in-house serum pools, and three commercially available serum pools (BEI Resources NR-4021, NR-4022, and NR4023) were added to the sample panel for a total of 51 samples. Samples were shipped to NMIMR on dry ice within five days and stored at -80°C until analysis. Prior to analysis on the neutralization assay, dried blood samples were thawed for 30 minutes before opening the air-tight bag to prevent condensation. Dried blood was rehydrated by incubating VAMS tips in 200 µL of PBS in Eppendorf tubes overnight at room temperature on a shaker at 300 rpm.

Table 1. Distinct materials between the two laboratories.

Distinct materials potentially increasing variation	Distinct materials unlikely increasing variation	Actions to reduce variation
Virus stocks cultured from the same master stock	Fluorescence plate-reader	Triplicates rather than duplicates
HEp-2 cell stocks	Single-channel pipettes	WHO international standard
Culture medium	Non-critical reagents, e.g. PBS	Verification of data analysis*
Multichannel with securely fitting tips	General laboratory equipment, e.g. rocker, centrifuges, heat block	

*Raw data from Ghana was analysed in parallel by both Ghana and the Netherlands teams and compared to cross-validate the analysis methods. PBS, phosphate-buffered saline; WHO, World Health Organization.

Neutralization assay

Neutralizing capacity of the dried blood samples was tested using a neutralization assay described previously.[20,23,24] Briefly, Hep-2 cells (ATCC CCL-23) were seeded at a concentration of 0.5 x 10^6 cells/well in 384-well black optical bottom plates (Thermo Scientific, 142761) and incubated for > 4 hours. Dried blood eluate and serum controls, including World Health Organizations (WHO) 1st International Standard for Antiserum to RSV (NIBSC code:16/284),[25] were heat-inactivated at 56°C for 30 minutes. Dried blood eluate was then centrifuged at 15.000g for 10 minutes to remove cell debris. Serum and dried blood eluate (1:10 during elution) were serially diluted in 3-fold from starting dilution 1:5 or 1:2 (total dilution 1:20), respectively, in Dulbecco's modified Eagle's Medium containing 10% FCS. Recombinant mKate-RSV-A2[26,27] was added 1:1 to the sample dilution series and incubated for 1 hour at 37°C before addition of 50 µL of the sample-virus mixture to the adherent Hep-2 cells. After 48 hours incubation at 37°C, relative fluorescence units were recorded using excitation at 584 nm and emission at 620 nm (FLUOstar OMEGA, BMG Labtech and Tecan SparkControl™ V3.2). The neutralization curve and 50% inhibitory dilution (ID50) of samples were analysed using a log(inhibitor) vs response 4-parameter nonlinear regression curve in GraphPad Prism version 8.3 or higher (GraphPad Software Inc., San Diego, CA, USA). International units (IU) were calculated according to manufacturer's instruction.[25] The bioassay using live cells and virus is inherently subject to high variability compared to non-biological assays, e.g. ELISA, with <50% variance considered as the target precision in cell-based assays.[13] Healthy adults were expected to have pre-existing anti-RSV antibodies detectable in the assay at baseline (ID50 of approximately 10^3) with an increase post-spiking with palivizumab or RSM01.[28]

Sample size and statistical analysis

Results reported as IU/mL from both laboratories were compared using the Lin's concordance correlation coefficient (CCC) to test how well the pairs of observations conform relative to perfect concordance using DescTools package in R (version 4.4.1).[29] In case the data do not follow (near-to) normal distribution, Deming regression is performed using SimplyAgree package in R additional to Lin's CCC. Deming regression takes into account the

measurement errors in both the dependent and independent variables simultaneously.[30] Additionally, Bland-Altman plots using the Log10 transformed data are used to describe the agreement between the two measurements.[31,32] A small bias is expected between the two laboratories due the live biological components of the assay resulting in a difference unequal to zero. Simulations performed in R using precision data previously obtained at the UMCU showed that a sample size of 50 samples would provide acceptable Lin's CCC values (≥0.9) and precision (confidence interval width ≤ 0.1).[29] Successful technology transfer was defined as Lin's CCC > 0.8. Assay precision was calculated as the percentage %CV of relative fluorescence units of sample duplicates (intra-assay precision) or %CV of the linear ID_{50} between runs (inter-assay precision). Plots were created in GraphPad Prism version 10.1.2.

Ethical approval

The use of blood samples from healthy adults from the UMCU healthy donor service was approved by the ethics committee of the UMCU, reference number 07-125/O. The healthy volunteers provided informed consent before taking part of the healthy donor service.

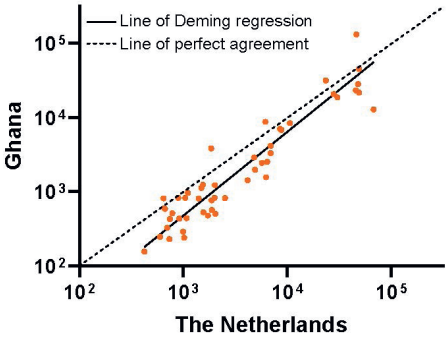
Patient involvement

Patients were not involved in the design or conduct of the study. Interim study results were presented to the ReSViNET patient advisory board.

Results

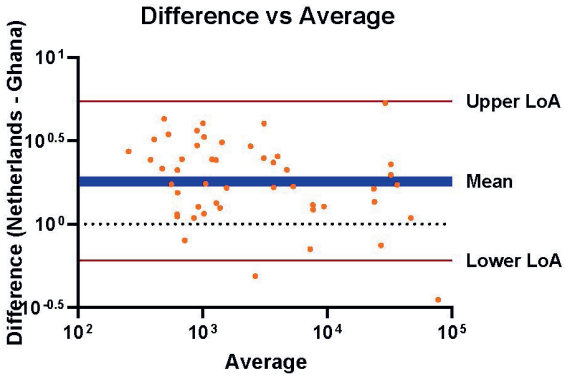
Successful training was achieved within the planned time frame of one month of remote training of the trainer and one month on-site training of other personnel, according to the pre-specified definition of measuring neutralizing capacity in dried blood using the neutralization assay by the Ghana team. The RSV neutralization assay performed in Ghana and the Netherlands detected neutralizing activity in all blood samples of the sample panel including blank dried blood samples. Two samples gave unreliable ID_{50} values according to quality control criteria[20] upon repeated measurements in Ghana and were therefore excluded from analysis. Lin's CCC was 0.87 (95% confidence interval (CI) 0.80-0.92), which is above the defined value of 0.80 for a successful transfer (**Figure 1**). As the data were minimally skewed, we also performed Deming regression resulting in the following equation: Ghana [IU/mL] = 1.13 x Netherlands – 2.37 (95% CI of slope 0.9795 to 1.280, 95% CI of Y-intercept -4.057 to -0.6775). Values in Ghana highly correlated with those in the Netherlands over the range of ID50 values (Spearman rho = 0.89, 95% CI 0.81-0.94, p<0.0001) but were systematically lower. The measurement differences in RSV neutralizing capacity in dried blood have a significant bias, better illustrated in the Bland-Altman plots (**Figure 2**). The Log_{10} mean difference (Netherlands – Ghana) was 0.26 (**Figure 2**), corresponding to a geometric mean difference of 1.81 with more bias seen in samples with lower neutralizing capacity. Inter-assay and intra-assay precision were lower in Ghana than in The Netherlands (**Table 2**).

Figure 1. Scatter plot of RSV neutralizing capacity in blood samples measurements in Ghana over measurements in the Netherlands.



Scatter plot for Log_{10}-Log_{10} transformed RSV neutralizing antibodies (ID_{50} in IU/mL) in dried blood measured in Ghana over measurements in the Netherlands (n=49 paired measurements). Deming regression (solid line) is compared to the line of perfect agreement (dashed line) to calculate the Lin's concordance correlation coefficient of 0.87. ID_{50}, dilution factor with 50% viral neutralization activity; IU/mL, international units per milliliter.

Figure 2. Bland-Altman plot of the agreement for RSV neutralizing antibody measurements between the Netherlands and Ghana.



Scatter plot of difference between Log_{10} transformed neutralizing antibodies (ID_{50} in IU/mL) in dried blood samples measured the Netherlands and Ghana vs. the average of the two measurements. The mean difference (bias) with 95% CI in blue. Limits of agreement are calculated as mean bias ± 1.96*standard deviation. CI, confidence interval; ID_{50}, dilution factor with 50% viral neutralization activity; LoA, limit of agreement.

Table 2. Assay precision in the two laboratories.

	Ghana	The Netherlands
Inter-assay precision	35.6 (30.5 - 37.5)	23.6 (22.5 - 26.4)
Intra-assay precision	12.9 (5.1 – 26.2)	3.5 (1.6 - 6.6)

Precision expressed as the %coefficient of variation (%CV, median (IQR)) where lower %CV equals higher precision. IQR, interquartile range.

Discussion

We show successful strengthening of the laboratory research capacity in Ghana by implementing a virus neutralization assay with dried blood. The neutralizing antibodies in matched dried blood samples obtained at the laboratories in Ghana and the Netherlands had high concordance (Lin's CCC = 0.87) and were highly correlated (Spearman rho = 0.89) in the context of a biological assay with high expected variance due to the use of live virus and live cells. The reliable measurement of neutralizing antibodies acts as proof-of-principle that knowledge and technology can be successfully transferred and thereby promote the participation of LMICs in RSV clinical trials.

The five recently available RSV vaccines and mAbs are only in use in HICs, despite the high burden in LMICs.[9] Urgent measures are needed to minimize the existing vaccine gap, including conducting representative trials and reducing pricing.[9,10] Notably, all phase 3 registration trials of the recently market approved RSV vaccines were recruiting participants in upper middle-income countries (UMICs)[5-7,33,34] yet these participants still lack post-trial access to these vaccines and no low or lower-middle income countries participate in the trials, except for The Gambia and the Philippines. To our knowledge, all trial samples are shipped to HICs for neutralizing antibody measurements, further exposing the disparities and limited roles left for LMICs in addressing global health challenges posed by RSV. During the COVID-19 pandemic, LMICs played a crucial role in the genetic surveillance identifying mutants of concern.[35-37] However, vaccines developed in HICs tend to induce lower immune responses in LMICs,[38] underscoring the importance to conduct vaccine trials in LMICs. The research community shares the responsibility to include LMICs in global health research efforts, for example by establishing partnerships.[1] The current assay set-up in Ghana, a Gavi-eligible country, and use of dried blood could facilitate clinical trials in LMICs, thereby ensuring access to effective vaccines and mAbs, as well as addressing other research priorities within the local research agenda. Dried blood sampling, more accepted than serum due to its common use in HIV testing,[17] is also more practical and safer for self-sampling or acquisition by field technicians and does not require cold-chain transportation, making it more suitable for LMIC settings. However, the lack of local production of VAMS devices and the price could pose a challenge in the procurement of VAMS for large-scale trials.

The current study had several strengths and limitations. The on-site training of the leading Ghanaian investigator in the Netherlands allowed an active practical hands-on experience and collaborative knowledge sharing. These fostered real time feedback, troubleshooting, seamless transfer of expertise between the two labs within a short time and contributed to the overall success of the tech transfer. By training the trainer, consistency in methodology and peer-to-peer learning was obtained in all Ghana trained personnel. Moreover, the use of the WHO international standard facilitated direct comparison of the assay results. Although identical methodology was used, certain critical reagents such as growth medium, virus, and cell culture, varied between the laboratories. The variations observed in the data

were partly corrected by normalizing the ID_{50} values to IUs with the international standard. In industry, it is commonly considered best practice to acquire identical equipment and materials in the context of a technology transfer, which was not feasible in the current study. The lower resource setting in Ghana led to discrepancy in the availability of materials, such as a multichannel with securely fitting pipette tips (**Table 1**). Inter-assay precision was higher in the Netherlands than in Ghana potentially due to higher quality of materials (**Table 2**). Despite these limitations of using varying materials of varying quality with identical methodology, we were able to obtain a high correlation and concordance. A third limitation was the lack of cross-validation from Ghanaian samples shipped to the Netherlands, which is planned in a future study. The shipment of samples and consequently later measurement of samples in Ghana potentially caused the systematically lower neutralizing capacity in Ghana than in the Netherlands. As there was no thawing during the shipment and antibodies are generally stable, we expect limited impact of the lack of cross-validation. Fourth, we transferred a neutralization assay using RSV-A that cannot measure RSV-B neutralizing antibodies. Assessment of protection against both RSV subtypes would require adaptation of the assay. Fifth, we observed a bias in measurements between the labs with higher measurement bias in samples with lower neutralization capacity. As the number of observations is small for higher averages, the variation in bias could potentially be an artefact of lack of normal distribution in ID_{50} values. Cell-based bioassays typically target a variance below 50%, averaging around 25%,[13] so the observed 81% mean bias is likely partially attributable and within acceptable assay variation. As the neutralization is best used to measure relative nAb differences in clinical trials, the lack of full agreement might be acceptable. Sixth, we acknowledge that technology transfers are more easily done to facilities with established research capacity (capacity strengthening) than facilities without any research capabilities (capacity building). The availability of the assay in the NMIMR research lab does not yet make the technology widely available to the entire country. Lastly, comprehensive assay validation is needed before the NMIMR can analyze samples for regulatory purposes, such as in vaccine trials.

The current research project has significantly impacted RSV clinical trial capacity in Ghana, establishing the laboratory as a research hub supporting other African countries. By successfully transferring the dried blood-based RSV neutralization assay technology to Ghana, local researchers are now equipped and planning to analyze RSV clinical trial samples. Taking away some of the barriers to active participation of LMICs, especially Gavi-eligible countries, in clinical trials improves the access to vaccines where the disease burden is highest. This capacity strengthening not only enhances Ghana's role in regional health research but also opens opportunities for expanding similar capabilities to labs in other regions and countries. Importantly, establishment of the neutralization assay empowers Ghanaian scientists to participate in and lead research relevant to the population, ensuring that local health challenges are addressed with local expertise. Currently, intellectual property rights are predominantly owned by HICs often preventing local manufacturing

of products, which could be resolved by active participation by LMICs. Localizing research and development with this project contributes to decolonizing global health by shifting the balance of research capabilities and decision-making power from HICs to LMICs through equitable partnerships with team engagement at all stages of the study. Consequently, it fosters a more equitable global health landscape where research and innovation are driven by those most affected by the diseases studied in order to achieve the 2030 sustainable development goals.[1]

Panel: Key next steps to LMIC participation in vaccine research and ultimate vaccine access

- Assay qualification and laboratory qualification for licensure (e.g. good clinical laboratory practice, GCLP)
- Obtain intellectual property rights (IPRs), e.g. through voluntary licensing
- Hubs for local manufacturing of vaccines or mAbs
- Clarification of regulatory standards for registration trials in LMICs
- Equitable partnerships between HICs and LMICs, including clinical research organizations (CROs) based in HICs with existing research infrastructure in LMICs

See also Partnerships for African Vaccine Manufacturing (PAVM) Framework for Action.[39]

Conclusion

We provide a proof-of-principle that a bioassay with inherently high variability can be transferred to resource limited settings such as in Ghana with limited time and financial investment. Strengthening research-capacity in LMICs offers a platform for future development of broader vaccine development, vaccine impact, and correlate of protection studies in LMICs. The transfer of this assay is a key step in minimizing the RSV vaccine gap between HICs and LMICs.

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Part IV

Equity-by-design: development of affordable RSV mAbs





Daily Intranasal Palivizumab to Prevent Respiratory Syncytial Virus Infection in Healthy Preterm Infants: a Phase 1/2b Randomized Placebo-Controlled Trial

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Abstract

Background

Mucosal administration of monoclonal antibodies (mAbs) against respiratory pathogens is a promising alternative for systemic administration because lower doses are required for protection. Clinical development of mucosal mAbs is a highly active field yet clinical proof-of-concept is lacking.

Methods

In this investigator-initiated, double-blind, randomized placebo-controlled trial, we evaluated intranasal palivizumab for the prevention of RSV infection in preterm infants (Dutch Trial Register NTR7378 and NTR7403). We randomized infants 1:1 to receive intranasal palivizumab (1 mg/mL) or placebo once daily during the RSV season. Any RSV infection was the primary outcome and RSV hospitalization was the key secondary outcome. The primary outcome was analyzed with a mixed effect logistic regression on the modified intention-to-treat population.

Findings

We recruited 268 infants between Jan 14, 2019 and Jan 28, 2021, after which the trial was stopped for futility following the planned interim analysis. Adverse events were similar in both groups (22/134 (16.4%) palivizumab arm versus 26/134 (19.4%) placebo arm). There were 6 dropouts and 168 infants were excluded from the efficacy analyses due to absent RSV circulation during the SARS-CoV-2 pandemic. Any RSV infection was similar in infants in both groups (18/47 (38.3%) palivizumab arm versus 11/47 (23.4%) placebo arm; aOR 2.2, 95% CI 0.7-6.5).

Interpretation

Daily intranasal palivizumab did not prevent RSV infection in late preterm infants. Our findings have important implications for the clinical development of mucosal mAbs, namely the necessity of timely interim analyses and further research to understand mucosal antibody half-life.

Research in context

Evidence before this study

Systemic IgG antibodies lack efficient access to the mucosa. The need for high doses of systemic monoclonal antibodies (mAbs) to reach a therapeutic level in the respiratory tract may be overcome through local administration. Preclinical studies support efficacy of local administration of mAbs against respiratory pathogens yet the highly active clinical development of mucosal mAbs is lacking proof-of-concept. Respiratory syncytial virus (RSV) is the second cause of death in the infant period. Palivizumab, a humanized mAb against the surface F protein of RSV, has been market-approved for more than 20 years, but access is limited to high-risk infants due to prohibitive costs. Administration via monthly intramuscular (i.m.) injections is burdensome and still allows for significant breakthrough infections occurring at low trough antibody levels. 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.

Added value of this study

This study is the first trial to investigate the efficacy of i.n. administration of antiviral monoclonal antibodies to prevent respiratory infection. We show palivizumab is stable in nose drop formulation and that daily intranasal palivizumab (50 µL per nostril of 1 mg/mL solution) is safe for clinical use. In the planned interim analysis, we do not find efficacy against lab-confirmed RSV infection for this child-friendly and affordable route of administration. Although the sample size was smaller than planned due to lack of RSV circulation, we expect this limitation to have had no impact on the conditional power of the study as the trial was terminated early due to a planned interim analysis.

Implications of all the available evidence

Intranasal antibody drug development is highly active with 11 drug candidates in development for SARS-CoV-2, RSV, and influenza. Our study demonstrates the necessity of a timely interim analysis to evaluate efficacy and avoid wasted time and capital. Further research on pharmacokinetics and medication dosage is needed to understand lack of efficacy of i.n. administration.

Introduction

Globally, respiratory syncytial virus (RSV) is the second cause of death in the infant period,[1] yet there is no vaccine or treatment available for children in low- and lower-middle income countries (LMICs), where disease burden is highest.[2] Of the vaccine candidates and monoclonal antibodies (mAbs) in late-stage clinical trials—including the recently registered mAb nirsevimab[3] and RSV maternal vaccine[4]—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 20 years, but access is limited to high-risk infants due to its prohibitive costs and limited efficacy. Administration via monthly intramuscular (i.m.) injections is burdensome and still allows for significant breakthrough infections occurring at low trough antibody levels.

According to Ku et al.,[5] circulating IgG antibodies lack efficient access to mucosal compartments. Antibody levels in the lung are 200–500 times lower than in blood after intravenous infusion and 30–70 times lower in the nose after i.m. monoclonal antibody injection6 resulting in the need for high doses of potent neutralizing mAbs with only a small antiviral effect in the respiratory tract. The need for high doses of systemic mAbs to reach a therapeutic level may be overcome through local administration. Mucosal administration of mAbs may offer a key solution for major respiratory pathogens: a dose-sparing highly targeted prevention stopping infection at the site of viral entry. Preclinical studies support efficacy of local administration of mAbs against respiratory pathogens.[7] 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.[8] Recently, low doses of i.n. hyper-enriched anti-RSV IgG were reported to inhibit infection in mice.[9] Multiple preclinical studies show that i.n. SARS-CoV-2 neutralizing mAbs provide protection in mice.[5,10]

Intranasal antibody drug development is a highly active field with 11 drug candidates in development for SARS-CoV-2, RSV, and influenza. Development is led by pharmaceutical companies, public private partnerships, and non-profit networks with seven preclinical candidates, two candidates in phase I trials, and one candidate in a phase I/II trial (Supplemental Appendix 1). This manuscript reports the first published data from a proof-of-concept trial in infants and the findings are highly relevant to ongoing clinical development.

Intranasal palivizumab offers a child-friendly and affordable alternative for i.m. palivizumab.[11] In the MAKI trial, we have shown 80% efficacy against RSV hospitalization through i.m. administration of palivizumab in late preterm infants 32–35 weeks gestational age.[12] We hypothesized that local administration of palivizumab to the airways prevents RSV infection in infants because it is delivered directly to the main viral point of entry and decreases the chance of breakthrough infection.

Here, we describe product development, a phase I trial and 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.

Methods

Study design

We conducted a double-blind, randomized, placebo-controlled cross-over phase I safety trial (Narsyn Study A, Dutch Trial Register NTR7378) in the Netherlands from September to November, 2018. Subsequently, we intended to obtain proof–of-concept that i.n. palivizumab prevents RSV infection in infants. Study B (Dutch Trial Register NTR7403, see Supplemental Appendix for full protocol), a double-blind, randomized, placebo-controlled proof-of-concept phase IIb trial was initiated based on the 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).

Participants

Study A included 20 healthy adult volunteers between 18 and 60 years of age (**Supplemental Figure 1**). Exclusion criteria were nasal obstructions, history of any respiratory symptoms within 4 weeks prior to drug administration, nasal surgery, immunocompromised subjects, simultaneous use of other nasal drops or spray or other nasal drugs ever (including cocaine and 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 March 31st). For this reason, recruitment occurred from the end of August to the end of January (**Supplemental Figure 2**). To limit the required sample size, the trial was performed in a high-risk population of infants with at least one older sibling under 18 years of age.[13] Exclusion criteria were known cardiac anomalies, Down syndrome or other serious congenital disorders as well as simultaneous use of other nose drops or spray except normal saline drops.

Randomization and masking

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.

Procedures

Commercial saline nasal drops (0.9% sodium chloride, Fagron) with a concentration of 1 mg/mL palivizumab were used as study medication and saline nasal drops without palivizumab were used as placebo. We showed the drug formulation is stable at 4 °C (intended use) in the investigational medicinal product dossier (Supplemental Appendix 2). For study B, parents were instructed to administer one drop (50 µL) of study medication in each nostril daily from the beginning of the RSV season (October 1st) or directly post-discharge for children born during the RSV season. Parents were instructed to take a nasal swab (stored in Copan universal transport medium (UTM)) in case of respiratory symptoms lasting more than one day. Parents recorded medication adherence, presence of respiratory symptoms, doctor visits, and the use of airway medication in a daily log. Weekly follow-up calls were performed to minimize missed infections and to maximize study medication compliance.
Nasal swabs were transported in UTM by regular mail to the laboratory and were stored at −80 °C until analysis as done in our previous trial.[12] Polymerase-chain-reaction (PCR) assays were performed to determine the presence of RSV RNA as described previously with minor modifications (Supplemental Appendix 3).[14]

Drug dose

We determined the dose based on best knowledge available from clinical studies of trough levels upon therapeutic efficacy, which were used for intramuscular dose determination for current market approval. Serum trough concentrations are minimally 30 μg/mL and ideally greater than 40 μg/mL (as a margin of safety for person-to-person variability) for clinical efficacy.[15] Dose-dependent increases in concentration of anti-RSV antibodies in bronchoalveolar lavage fluid (BALF) have been observed to be 500–1000x less than steady-state plasma concentrations of antibody.[16,17] Consequently, a protective dose of palivizumab on the airways may be presumed to be 500x less than serum concentration or 0·08 μg/mL for therapeutic efficacy. Nasal epithelial lining fluid (ELF) is estimated to be 800 ul per nostril.[18] Thus, in order to achieve a minimal trough concentration of 0·08 μg/mL in 800 μL, 0.064 μg is needed per nostril as a minimal protective dose. In this study we administer nasal drops with a concentration of 1 mg/mL of palivizumab with 50 μL administered daily per nostril, resulting in a daily dose of 50 μg per nostril, easily above the minimal threshold needed for therapeutic efficacy (780x more than minimal trough concentration). Other therapeutic antibodies that have been used locally utilize doses 1/100th of the required systemic dose to allow for reduced costs and side-effects.[19]

Outcomes

For study A, the primary outcome was self-reported local and systemic adverse events (AEs; Supplemental Appendix 4.1–4.3). In the case of objectifiable symptoms, researchers performed a home visit. An independent DSMB and investigators considered if (S)AEs were treatment related. For study B, the primary outcome was any lab-confirmed RSV infection. The key secondary outcome was lab-confirmed RSV hospitalization. Other secondary outcomes are defined in the Statistical Analysis Plan (Supplemental Appendix 4.4–4.7 and Supplemental File Study Protocol and SAP).

Statistical analysis

Nearly all analyses were performed (DC and PvdV) according to the statistical analysis plan (Supplemental File Study Protocol and SAP); we specified if the analysis was performed post-hoc (Supplemental Appendix 6 and 7). The primary outcome was analyzed with a mixed effect logistic regression including treatment, season, prognostic factors at baseline (having more than one sibling, date of birth between August 14—December 1st, neonatal respiratory support), as fixed effects and a random effect for siblings. The primary analysis was performed on the modified intention-to-treat (mITT) population. The predefined target sample size of 408 infants provided 85% power to detect a relative risk reduction for RSV infection of 62·5%.[12] An interim analysis was performed according to protocol (Supplemental File Study Protocol and SAP) to assess futility or efficacy when 50% of the expected events had been observed. 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.

Role of funding source

The funder of the study 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.

Results

In study A, 20 subjects were enrolled in the first-in-human study between October 1–4, 2018 (**Supplemental Table 1**). One subject was excluded before any study medication was administered due to symptoms of a respiratory infection. Airway patency after 10 min was 100% in both the palivizumab (10/10) and placebo (9/9) arm. Local and general symptoms were tabulated per arm per study participant (**Supplemental Table 2A**). There were no SAEs in either trial arm and no AEs were considered to be treatment-related by the study staff or DSMB (**Supplemental Table 2B** and **2C**).

In study B, 268 of the 4403 eligible preterm infants were enrolled in the study between January 14, 2019 and January 28, 2021 (**Figure 1**). Eleven infants were enrolled during season 1 (2018–2019), 89 during season 2 (2019–2020) and 168 during season 3 (2020–2021). Infants enrolled during season 3 were excluded from the efficacy analyses due to absence of RSV circulation in the winter of 2020–2021 in the Netherlands during the COVID-19 pandemic.[20] 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 (6%) infants who discontinued the study early (3 in the intervention group and 3 in the placebo group) for whom the primary endpoint was unknown. 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. 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 futility (Supplemental Appendix 8).

Baseline characteristics were similar in both study arms (**Table 1**). Median age of infants at start of treatment was 2.3 months (interquartile range (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 (**Supplemental Figure 3**). Parents administered nasal drops for a mean duration of 3.9 months (SD: 1.1 months) in the intervention arm and 3.7 months (SD: 1.1 months) in the placebo arm.

Figure 1. Study B enrollment

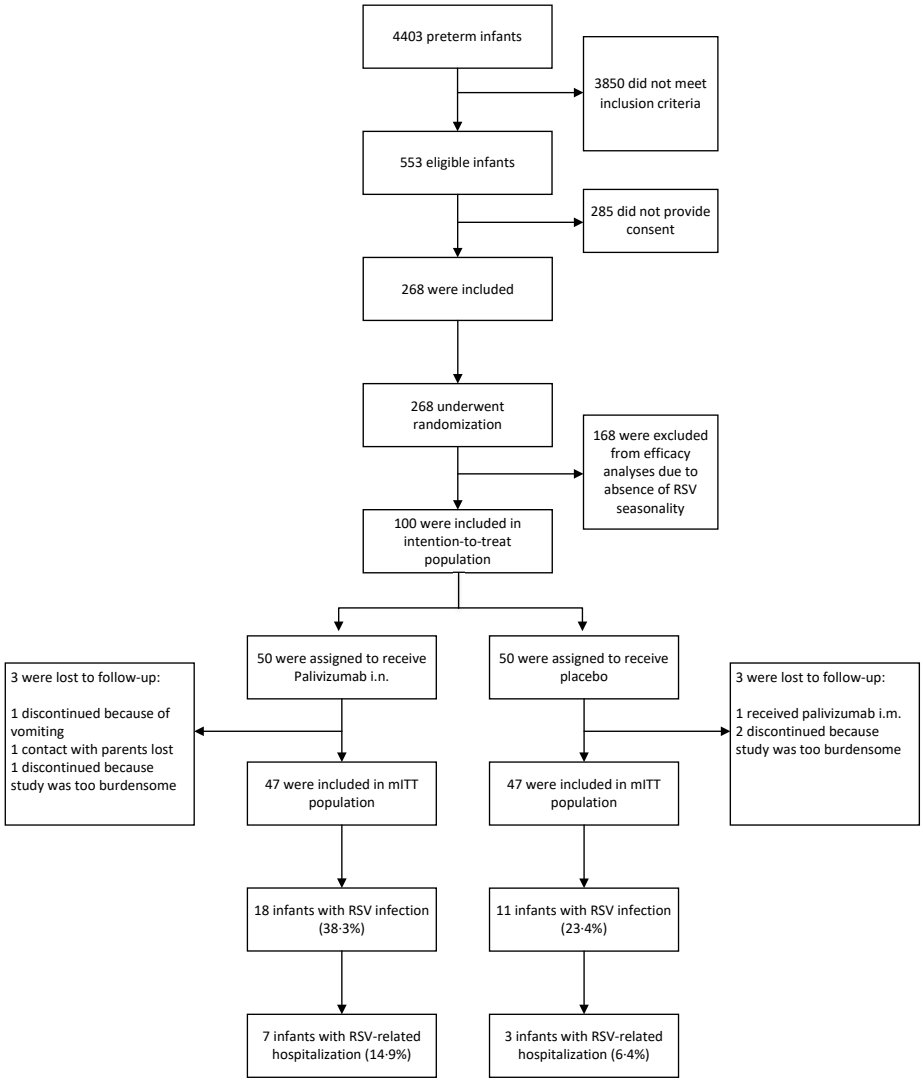


Table 1. Baseline characteristics of Study B participants

	Palivizumab (*N* = 50)	**Placebo (*N* = 50)**	**Total (*N* = 100)**
Female, n (%)	22 (44)	26 (52)	48 (48)
Age in months, median (IQR)1	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)	2343 (1954–2590)
Multiple birth, n (%)	16 (32)	13 (26)	29 (29)
Complication(s) during pregnancy, n (%)	23 (46)	27 (54)	50 (50)
Antenatal corticosteroids, n (%)	20 (40)	22 (44)	42 (42)
Complication(s) during delivery, n (%)	26 (52)	29 (58)	55 (55)
Antibiotics during delivery, n (%)	8 (16)	7 (14)	15 (15)
Vaginal Delivery, n (%)	24 (48)	27 (54)	51 (51)
Apgar score 5 min, median (IQR)	9 (8–10)	9 (8–10)	9 (8–10)
Respiratory support after birth, n (%)	26 (52)	24 (48)	50 (50)
Received antibiotics after birth, n (%)	19 (38)	22 (44)	41 (41)
Maternal age at birth child, median (IQR)	32 (30–36)	32 (30–35)	32 (30–35)
Exclusive breastfeeding, n (%)2	21 (42)	23 (46)	44 (44)
Breastfeeding and formula, n (%)	22 (44)	17 (34)	39 (39)
Exclusive formula, n (%)	7 (14)	10 (20)	17 (17)
Maternal level of education – postgraduate, n (%)	46 (92)	46 (92)	92 (92)
Paternal level of education – postgraduate, n (%)	42 (88)	46 (92)	88/98 (90)
Maternal smoking during pregnancy, n (%)	5 (10)	4 (8)	9 (9)
Smoking inside, n (%)	1 (2)	0 (0)	1 (1)
Total number of persons in household, median (IQR)	4 (4–5)	4 (4–5)	4 (4–5)
More than one older siblings, n (%)	25 (50)	18 (36)	43 (43)
Day care attendance, n (%)3	28 (56)	27 (54)	55 (55)
Siblings attending day-care, median (IQR)	1 (1–2)	1 (1–1)	1 (1–1)
Maternal atopy, n (%)	26 (52)	16 (32)	42 (42)
Paternal atopy, n (%)	20/47 (43)	18 (36)	38/97 (39)

Denominator was shown only if there was missing data. [1]Age in months is reported at start of intervention. [2]Breastfeeding was determined based on the question which feeding was given from birth and duration was not taken into account. [3]Day care attendance includes both current and intended day care attendance.

Adverse events were determined to be unrelated to study medication in all participants in the intervention arm (n = 3/134, 2.2%) [**Supplemental Table 3**]. No SAEs were determined to be related to study medication; the number of SAEs was similar in the palivizumab arm and placebo arm (22/134 versus 26/134, non-significant (NS)).

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**). There were more RSV infections in the intervention arm but this was not significantly different (adjusted odds ratio (aOR): 2.2; 95% CI: 0.7–6.5) and per protocol population (aOR: 1.9; 95% CI: 0.6–6.3) (**Table 2**). Sensitivity analyses assessing the impact of missing outcome (**Table 3**), different CT-value cut-offs and adherence (Supplemental 4-5) also showed no statistical significant difference between both trial arms. The key secondary outcome, RSV hospitalization, was similar in the palivizumab arm and placebo arm (7/47 (14.9%) versus 3/47 (6.4%)). Other secondary outcomes were also similar in both trial arms (**Table 2**, **Supplemental Table 6**).

There was no difference in any wheezing, fraction of wheezing days (**Supplemental Table 7**), or wheezing episodes between the trial arms (incidence rate ratio (IRR): 1.2; 95% CI 0.8–1.7) [**Supplemental Figure 4** and **Supplemental Tables 8–9**]. The proportion of infants with recurrent wheezing and physician-diagnosed wheeze were similar across trial arms (**Supplemental Table 8**). Occurrence of any wheezing was similar for RSV-infected and non-infected infants (post-hoc analysis) [**Supplemental Table 10**].

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

	Palivizumab (N = 47)	Placebo (N = 47)	Adjusted odds ratio[a] (95% CI)	P value	Crude odds ratio (95% CI)	Risk difference % (95% CI)	RRR[B] % (95% CI)
Primary Endpoint							
RSV infection, n (%)	18 (38.3)	11c (23.4)	2.2 (0.7 – 6.5)	0.14	2.0 (0.8 – 5.0)	14.9 (-3.5 – 33.3)	-63.6 (-207.8 – 13.0)
Secondary Endpoints							
Hospitalization for RSV infection, n (%)	7 (14.9)	3 (6.4)			2.6 (0.6 – 10.6)	8.5 (-3.8 – 20.9)	-133.3 (-748.2 – 35.8)
Medically attended RSV infection without hospitalization, n (%)	8 (17.0)	2 (4.3)			4.6 (0.9 – 23.0)	12.7 (0.1 – 25.0)	-300.0 (-1685.2 – 10.4)
RSV infection without medical attention, n (%)	3 (6.4)	7 (14.9)			0.4 (0.1 –1.6)	-8.5 (-20.9 – 3.8)	57.1 (55.8 – 88.2)
All-cause RTI[d]							
Any RTI, n (%)	46 (97.9)	45 (95.7)			2.0 (0.2 – 23.3)	2.1 (-5.0 – 9.2)	-2.2 (-10.0 – 5.0)
RTI hospitalization, n (%)	9 (19.2)	6 (12.8)			1.6 (0.5 – 5.0)	6.4 (-8.4 – 21.1)	-50.0 (-288.1 – 43.0)
Medically attended RTI, n (%)	26 (55.3)	22 (46.8)			1.4 (0.6 – 3.2)	8.5 (-11.6 – 28.7)	-18.2 (-76.1 – 21.7)
Non medically attended RTI, n (%)	43 (91.5)	45 (95.7)			0.5 (0.08 – 2.7)	-4.3 (-14.1 – 5.6)	4.4 (-6.2 – 14.1)

[a] 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. [b] The following formula was used to calculate relative risk reduction: RRR=((CER-EER))/CER. [c] 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. [d] Number of children per type of RTI are reported. Children can have several types of RTI during study period. CER, control event rate; EER, experimental event rate; RTI, respiratory tract infection; RRR, relative risk reduction.

Table 3. Sensitivity Analyses including infants with missing outcome

Analysis	Population	Palivizumab	Placebo	aOR (95% CI) or RR (95%)
Primary analysis replacing all missing outcomes with no RSV, aOR	ITT (*N* = 100)	18/50 (36.0)	11/50 (22)	2.2 (0.8 – 6.3)[a]
Primary analysis replacing all missing outcomes with RSV, aOR	ITT (*N* = 100)	21/50 (42.0)	14/50 (28.0)	2.0 (0.7 – 5.5)[a]
Primary analysis replacing all missing outcomes with RSV in the placebo group and no RSV in the treatment group, aOR	ITT (*N* = 100)	18/50 (36.0)	14/50 (28.0)	1.5 (0.6 – 4.0)[a]
Primary analysis replacing all missing outcomes with no RSV in the placebo group and RSV in the treatment group, aOR	ITT (*N* = 100)	21/50 (42.0)	11/50 (22.0)	2.8 (1.0 – 7.8)[a]
Primary analysis in the per protocol population, aOR	Per Protocol Population (*N* = 86)	16/42 (38.1)	11/44 (25.0)	1.9 (0.6 – 6.3)[a]
Relative risk	mITT (*N* = 94)	18/47 (38.3)	11/47 (23.4)	1.6 (0.9 – 3.1)[b]

[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 siblings was used to calculate OR for CT-value sensitivity analyses. [b] Crude relative risk for treatment arm. aOR, adjusted odss ratio; CI, confidence interval; ITT, intention to treat;, mITT, modified intention to treat;, RR, risk ratio; RSV, respiratory syncytial virus.

DISCUSSION

This study is the first trial to investigate the efficacy of mucosal administration of antiviral mAbs to prevent respiratory infection. Development of the investigational product was investigator-initiated and was conducted without funding from industry or capital investment, contrary to common practice in late-phase product development. Intranasal prophylaxis in late preterm infants was not effective to prevent lab-confirmed RSV infection despite high rates of adherence. The lack of efficacy has important implications for ongoing and future trials in the rapidly expanding field of mucosal mAb clinical development. However, affordability of RSV prevention may be within reach due to a commitment to ensure access for MK-1654[2] and support of the Bill and Melinda Gates Foundation for affordable maternal PreF vaccination.[21]

The rate of total RSV infection (30.8%) was higher than expected at the time of sample size calculation (expected rate 16%) but similar to the rate reported in a recently published European birth cohort study (26.2%).[22] The proportion of infants with any wheezing in the placebo arm (43.5%) was similar to the MAKI trial (47%).[12]

The observed lack of efficacy may be explained by several non-exclusive mechanisms. First, it is uncertain whether the mucosal half-life of i.n. mAbs is sufficient for protection. At the time of the trial there was no accepted sampling technique to define the half-life of palivizumab in the airways for i.m. administration or measure an effective medication dosage by measuring trough antibody concentrations. The half-life of IgG in the nasal ELF is not well established, but due to mucociliary clearance it is expected to be substantially shorter than in serum. However, it remains unclear whether measurement of half-life corresponds to the clinical outcome of interest, namely, protection against RSV infection. In vivo we previously showed[8] that despite full protection against RSV for at least one week, palivizumab administered (0.5 mg/kg) into the lungs of naïve wild-type BALB/c mice is detected at low levels or not at all in the nasal airway or lungs on day 7 [unpublished data]. Recent data also suggests that mAbs administered via nasal spray retain neutralizing capacity against SARS-CoV-2 for 24 hours in a small clinical trial.[23] Second, it is known that the eyes and mouth are potential, though less effective, routes of inoculation for RSV[24,25] and i.n. administration unlikely protects via these routes. Third, study medication adherence may have been overestimated due to desirability bias. We minimized this bias by measuring objective adherence (weighing bottles before and after use) as well as subjective adherence (parent-reported). Fourth, inadequate dosing might have contributed to lack of efficacy. However, the trial dosage of i.n. palivizumab is expected to be higher than airway medication concentrations achieved through current market-approved i.m. administration. The daily dose of 50 µg per nostril in this study is easily above the minimal threshold needed for protective efficacy (0.064 µg per nostril) as explained in the methods. In summary, lack of efficacy may most likely be explained by a short half-life of study medication in the nasal cavity although recent trial results show viral neutralization until 24 h after study drug administration.

The strength of this study is the investigator-initiated and industry-independent testing of the "bouncer" hypothesis: i.n. mAb administration stops infection at the site of viral entry and provides a solution to prevent infection of respiratory pathogens with significant cost reduction. It is estimated that 100 µL of 1 mg/mL daily would add up to 15 mg/season, while current i.m. administration of 15 mg/kg for an average weight of 5 kg adds up to 375 mg/season. Therefore, the cost reduction is minimally 20 fold (275 versus 7000 USD per child per season).

Limitations of the study include no circulation of RSV during the third season due to the COVID-19 pandemic. Although the sample size was smaller than planned due to lack of RSV circulation, we expect this limitation to have had no impact on the conditional power of the study as the trial was terminated early due to a planned interim analysis. Baseline incidence of RSV infection was sufficiently high to support our negative trial results. Second, administration of study medication depends on parental adherence. Parent-reported subjective adherence may have been subject to social desirability bias, underreporting missed doses and overestimating adherence. To minimize the impact of this limitation we collected objective adherence data based on weight of dosage. Objective adherence was high, indicating that insufficient adherence is not the main driver of lack of efficacy. Third, nasal swabs were collected by parents as previously done in the MAKI trial.[12] To mitigate the risk of missed outcomes, parents were contacted on a weekly basis by dedicated study staff and general practitioners were contacted to collect information on medical visits and respiratory episodes during the first year of life. Despite intensive follow-up, there were 175 (36.9%) respiratory episodes without a swab. However, we expect limited impact on the primary outcome because these episodes had a shorter median duration of symptoms than episodes with a swab (4 vs 13 days) and were therefore unlikely to be RSV-positive as the median duration of symptoms was 16 days for RSV-positive episodes. Lastly, the nasal drops would ideally coat the large nasal cavity surface for maximum efficacy. Although a spray could increase nasal cavity deposition in adults,[26] nasal drops penetrate the nasal valve to reach the turbinates more effectively than sprays.[27]

Our results show a trend towards higher RSV infection and hospitalization in the intervention arm. We considered four mechanisms that may explain the observed trend. We considered mechanistic damage to the nasal epithelium is unlikely as palivizumab solely targets virus and the two other components present in palivizumab (histidine and glycine) are amino acids.[28] Antibody-dependent enhancement in which suboptimal RSV medication concentrations may enhance viral replication[29] is unlikely as the dosage used was higher than the minimum threshold for protective efficacy established for i.m. administration. Increased RSV exposure may explain the observed trend as infants who received palivizumab had more siblings than infants who received placebo (50% vs 36% respectively). Finally, an underlying predisposition for RSV infection in the intervention arm is unlikely by study design (randomization).

Several gaps in knowledge make it difficult to confirm the mechanism for lack of efficacy of i.n. palivizumab against RSV infection. First, the mucosal pharmacokinetics of antibodies is unknown. In future trials, it will be important to measure antibody half-life in the nose as improved sampling devices have recently been validated to collect neat mucosal lining fluid.[30] Second, it is important to understand whether the neonatal Fc receptor (FcRn), which is critical to extended half-life of antibodies, is present in the infant's nasal mucosa. Research in vitro and in animal studies suggests this may be the case.[31-34] Intranasal extended half-life mAbs have recently been shown to block COVID-19 infection after experimental infection in mice.[5] Third, an alternative to therapeutic IgG is IgA, which has been associated with protective immunity against RSV.[35] However, we previously showed in mice that re-engineering palivizumab into monomeric and secretory IgA is a less effective i.n. prophylaxis.[36]

In conclusion, daily i.n. palivizumab prophylaxis did not show protection against RSV infection in late preterm infants. Our findings imply that a timely interim analysis is essential to evaluate efficacy to avoid wasted time and capital. Further research is needed on nasal antibody half-life in the highly active field of i.n. drug development. Trials with other therapeutic antibodies are needed to understand whether our findings are specific to palivizumab or generalizable to any mucosal antibody administration.

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Supplement

Supplementary files can be downloaded from:



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Intranasal monoclonal antibodies do not prevent respiratory infection in a randomized, controlled experimental infection trial

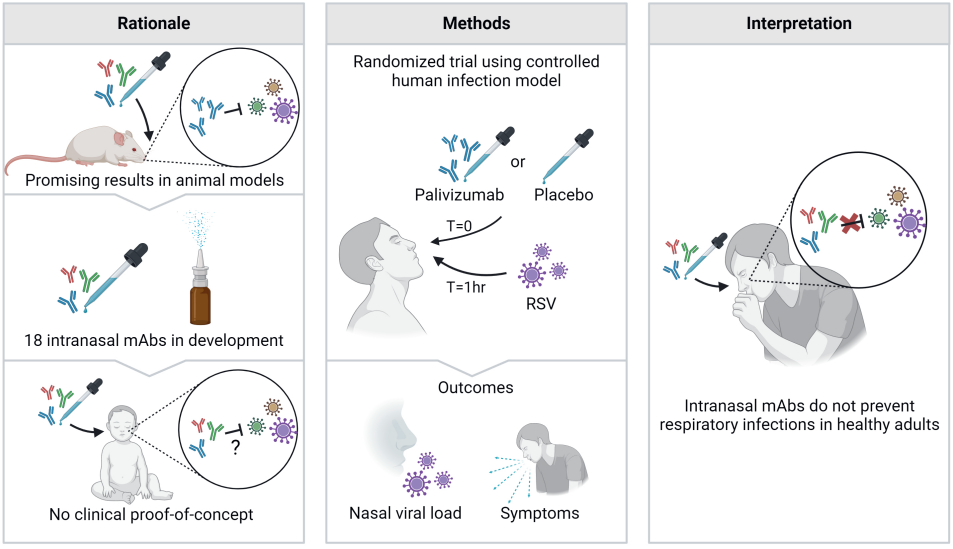
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Abstract

Intranasal monoclonal antibodies (mAbs) offer a promising cost-reducing and non-invasive alternative to systemic administration. Intranasal palivizumab prevented respiratory syncytial virus (RSV) infection in mice but not in healthy infants. This proof-of-concept study evaluates efficacy of intranasal palivizumab to prevent RSV infection. In this randomized, double-blind, placebo-controlled, experimental infection model, 28 healthy adults (18-55 years old) were randomized 1:1 to receive 1 mg/mL palivizumab in saline nasal drops or placebo one hour prior to RSV-A Memphis 37b challenge (EU clinical trial register 2020-004137-21, August 14, 2021). The primary outcome was viral load over time determined by RT-qPCR. Intranasal palivizumab had no effect on viral load despite a 72% reduction in symptomatic infections and marginally reduced upper respiratory tract symptoms compared to placebo. Intranasal palivizumab does not protect against RSV infection under optimally controlled conditions. Intranasal mAbs are highly unlikely to be a feasible and cost-effective method of protection against respiratory infection.

Intranasal monoclonal antibodies to prevent respiratory infections



Introduction

Lower respiratory tract infections are the main cause of death in children under 5 years old globally[1] and a major cause of morbidity and mortality in the elderly.[2] Active immunization with vaccines remains the preferred method of infection prevention due to the long lasting effect, immune memory, and relatively affordable production. However, not all populations benefit from vaccines: young infants have immature immune systems that require passive immunization, such as monoclonal antibodies (mAbs) against respiratory syncytial virus (RSV).[3] Aging people with immunosenescence or immunocompromised individuals who do not respond as well to vaccines might also benefit from mAbs.

Although the aim is protection against infections of the respiratory tract, mAbs are administered intramuscularly. Systemic IgG antibodies lack efficient access to the respiratory mucosa: IgG levels are 30-70x lower in the nose than in serum after intramuscular mAb injection[4] and at least 200-500x lower in the lung after intravenous infusion.[5,6] Mucosal mAb levels are significantly lower in infants with breakthrough RSV infections than in non-RSV infected infants.[7] The need for high intramuscular doses to reach a therapeutic level in the respiratory tract drives high costs and prevents global access.[8] Local administration of mAbs, i.e. intranasal, would require lower doses, making it an affordable non-invasive alternative to intramuscular mAbs.

The clinical development of intranasal mAbs is a highly active field yet clinical proof-of-concept is lacking. Currently, 18 mAbs are in development to prevent respiratory infections with SARS-CoV-2, influenza virus, RSV, and Middle East Respiratory Syndrome (MERS) virus (**Supplementary Table 1**). Two candidates have completed efficacy trials with negative or unreliable results, one candidate has completed a phase 1/2a trial, three candidates are in phase 1 trials and three candidates are in undefined clinical trials. Preclinical development in animal models supports the prophylactic efficacy of local administration of mAbs against respiratory infection after viral challenge.

Intranasal palivizumab, a registered IgG1 mAb against the RSV F-protein, protected mice against experimental RSV infection in a dose-dependent manner[9] but healthy late-preterm infants were not protected by daily intranasal palivizumab in a placebo-controlled trial.[10] There are multiple possible reasons why this phase 2b trial did not show efficacy: short half-life, low virus circulation, lack of virus exposure, or potential low drug adherence which are inherent to the trial design.

A controlled human infection model (CHIM) addresses these experimental design problems and allows a proof-of-concept of intranasal mAbs to prevent respiratory infections in a highly controlled setting. A CHIM provides an unparalleled study design to assess efficacy in a controlled setting with fixed dosing, adequate viral inoculation, viral exposure, and fixed timing between medication and viral inoculation. As opposed to traditional randomized controlled clinical trials, a CHIM allows us to investigate efficacy of intranasal mAb

independent of virus circulation, short half-life, medication adherence, and viral exposure. The primary aim of the current proof-of-concept study is to assess the prevention of experimental RSV infection with intranasal palivizumab in healthy adults.

Methods

Study design and participants

We conducted a single-centre, randomized, placebo-controlled, double-blind controlled human infection study in the Netherlands between October and December, 2023 (EudraCT number 2020-004137-21). Individuals responding to local adverts were screened for the study after providing informed consent. Eligible healthy volunteers aged 18-55 years old were selected according to protocol-defined inclusion and exclusion criteria (see study protocol in Supplementary Information). In short, the exclusion criteria were: living with children under 3, older adults over 65 years old, or any household member with a significant immunodeficiency; medical conditions associated with increased risk of viral respiratory complications; current cold or nasal congestion; current use of nasal medication; history of nasal surgery; or pregnancy.

Randomisation and masking

Participants were randomized 1:1 using the Castor electronic data capture (EDC) randomization tool in non-stratified blocks of 2 or 4 to receive intranasal palivizumab[10] or placebo. Group allocation was sent to the trial pharmacy who prepared the allocated treatment vial per participant. Study medication and placebo were identically packaged and indistinguishable by sight or smell. Participants, people giving the intervention, those assessing the outcomes, and those analysing the data were all blinded.

Procedures

Participants received 100 µL of 1 mg/mL palivizumab or placebo per nostril. One hour later, participants were inoculated intranasally with 4 $\log_{10}$ plaque-forming-units (PFUs) of RSV-A Memphis-37b challenge virus.[11] After inoculation participants home-quarantined for a maximum of 10 days with daily home visits by research personnel. Samples were taken on all study days: predose (day 0), daily on 1-10 days post-infection (DPI), and on 14 and 25 DPI (**Supplementary Figure 1**).

Nasal wash samples were obtained by introducing 5 mL of 0.9% saline into each nostril using a syringe attached to a nasal olive. After 10 washes in and out of the cavity, the fluid was recovered and kept on ice until laboratory processing.

Participants self-reported their symptoms once daily on all study days using an established participant-reported symptom score (Study Protocol in Supplementary Information).[29] Additionally, participants recorded their own oral temperature four times daily. Lung function was assessed once daily on all study days using portable devices (NuvoAir Air Next v1.0).

Spirometry was conducted by well-trained research staff, who selected the best manoeuvre from three correctly performed tests. Safety data were collected through self-reporting of local and systemic (serious) adverse events (for definitions see protocol in Supplementary Information). Study personnel asked about adverse events daily as part of a checklist for home visits.

Drug dose

We determined the dose based on best knowledge available from clinical studies of trough levels upon therapeutic efficacy, which were used for intramuscular dose determination for current market approval. Serum trough concentrations are minimally 30 µg/mL and ideally greater than 40 µg/mL (as a margin of safety for person-to-person variability) for clinical efficacy.[30] IgG levels are 30-70x lower in the nose than in serum after intramuscular mAb injection.[4] Consequently, a protective dose of palivizumab on the upper airways may be presumed to be 30-70x less than serum concentration or 0.57-1.33 µg/mL for therapeutic efficacy. The nasal epithelial lining fluid is estimated to be 800 µl per nostril.[31] Thus, in order to achieve a minimum trough concentration of 1.33 µg/mL in 800 µL, 1.1 µg per nostril is needed as a minimal protective dose. In this study we administered a single dose of 0.1 mg palivizumab per nostril, easily above the minimal threshold needed for therapeutic efficacy (at least 94x higher than ideal trough concentration).

Outcomes

The primary outcome was the area under the curve (AUC) for RSV viral RNA in nasal-wash samples quantified using an RT-qPCR assay.[32] Cycle threshold (Ct) values below 45 were considered to be RSV positive. Productive RSV infection was defined as positive RSV detections by RT-qPCR on two consecutive days at least two DPI during quarantine. The RSV viral load (RNA copies/mL) was calculated from the Ct values using a calibration curve derived from an electron microscope counted RSV standard. Two samples had low internal controls but were included due to expected minimal underestimation of the already low Ct values.

The secondary outcomes were all exploratory and included daily participant-reported upper respiratory tract (URT) and lower respiratory tract (LRT) symptom scores 2-14 DPI, the AUC of viral culture in nasal washes of 2-14 DPI, the lung function (specifically, FEV1 as percent of predicted over time), and safety.

Symptom scores were self-reported by participants: For each symptom, a score of 0 (absent) to 3 (severe) was assigned. Both URT and LRT symptoms were recorded to calculate a symptom score per day. RSV positive participants were considered symptomatic if two or more of the following were present: a cumulative clinical symptom score of 14 or greater over a six day period, presence of nasal discharge on three or more days over the six-day period, and/or a subjective impression of a cold observed by study staff (see the SAP for detailed descriptions of criteria).

Lung function was assessed as forced expiratory volume in 1 second (FEV1), predicted FEV1, forced vital capacity (FVC), predicted FVC and FEV1/FVC-ratio. The predicted values of FEV1 and FVC were calculated according to the Global Lung Function Initiative standards.[33] All lung function results were reviewed afterwards and, if necessary, the best manoeuvre per session was re-selected. A selection of the data was checked by a lung function specialist as quality control of the procedure. Lung function procedures were graded using an grading system adapted from the American Thoracic Society/European Respiratory Society[34] using the three best manoeuvres; measurements with poor quality (two tests with repeatability larger than 0.25L or only one test result) were excluded from analysis.

Nasal-wash samples were cultured using a tissue culture half infectious dose (TCID50) assay as a proxy of infectious viral load. Samples underwent quadruplicate 10-fold dilution series in DMEM supplemented with Normocin (100 µg/mL) and 1% fetal bovine serum for 10 dilutions to infect monolayers of Hep-2 cells (ATCC CCL-23). Cells were checked daily for CPE and end-point titers were evaluated as the 50% tissue culture infective dose per mL after 7-10 days using the Spearman-Karber method.[35] The AUC of viral culture in nasal washes of 2-14 DPI quantified as TCID50/mL were compared between treatment groups.

Safety and potential (serious) adverse events (AEs) were assessed daily. Respiratory symptoms were presumed to represent virus infection consequent to challenge and were not additionally captured as adverse events. Participants' household members with symptoms of respiratory tract infection were tested for RSV (ID NOW, Abbott) if the criteria for a potential transmission event were met: symptoms started ≥6 DPI and the respective study participant had tested positive for RSV at 7 DPI.

Statistical analysis

Statistical analysis was performed (D.C.) per the statistical analysis plan (available online) using R 4.3.2 and SAS Enterprise Guide 8.2. Sample size calculations are available in the study protocol. All participants were inoculated with RSV-A M37b regardless of previously receiving intranasal palivizumab or placebo, so all participants were included as the analysed population. The primary and secondary endpoints were prospectively defined and compared across treatment groups. No correction for multiplicity was made in testing the primary hypothesis since there was only one primary endpoint. In testing the secondary equivalence hypotheses, no correction for multiplicity was applied since these inferences were considered exploratory. RT-qPCR values that were undetectable in nasal-wash samples were assigned half of the limit of detection in copies/mL (390 copies/mL; detection limit 780 copies/mL). The viral load AUC from day 2-14 was calculated for each participant and the Mann-Whitney U Test was used to compare the AUC distributions between the two groups. URT and LRT symptom scores from day 2-14 were analysed with linear mixed models using treatment group, time of recording the symptoms, quadratic effect of the time of recording, interaction between treatment group, and time and interaction between treatment group and time squared as fixed effects, and intercept as random effect. A mixed effects repeated

measures model was used to analyse the predicted FEV1 of 2-14 DPI with the mean of FEV1%predicted at day 0 and 1 as baseline variable (to minimize any learning effect) and treatment group, time of measurement, and quadratic effect of time of measurement as fixed effects, and intercept as random effect. In order to evaluate whether there was a difference in the trajectory over time of the symptoms and lung function scores between placebo and palivizumab, the models with and without interaction effects were compared using the likelihood ratio test. Missing symptom data were imputed as having no symptoms and missing lung function data was not imputed. Risk difference in safety between intervention and placebo groups could not be calculated due to low numbers of events. The trial was registered in the EU clinical trial register under trial registration number 2020-004137-21 on August 14, 2021.

Study approval

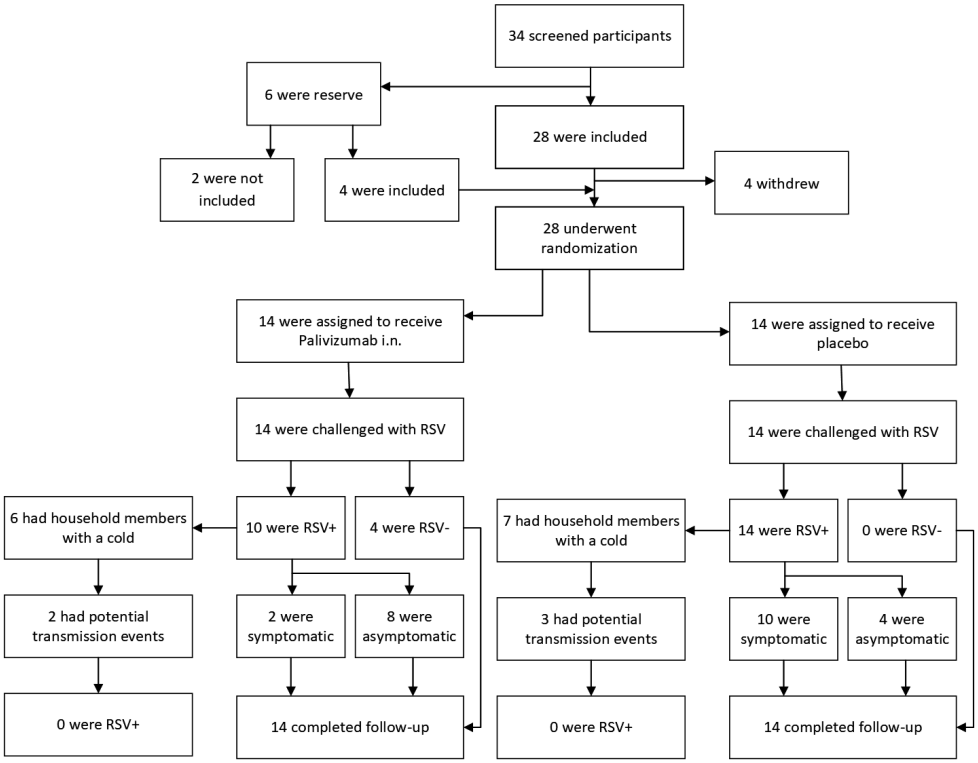
The study protocol was approved by the institutional review board of the University Medical Center Utrecht, the Netherlands (NL78591.041.21) and is available online. In the phased study design, we first carried out an inpatient RSV CHIM to establish safety and, when safety criteria were met, we progressed to the outpatient CHIM described here. All participants provided written informed consent, and the trial was conducted according to the principles of the current revision of the Declaration of Helsinki 2008 and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines for Good Clinical Practice.

Results

In this CHIM, 28 participants underwent randomization between October 30 and December 22, 2023 (**Figure 1**). Of those, 14 received 100 µL of 1 mg/mL palivizumab and 14 received 100 µL of placebo per nostril in a double-blind manner similar to the phase 2b trial in infants. One hour later, all 28 participants were inoculated with 4 $\log_{10}$ plaque-forming-units (PFUs) of RSV-A Memphis-37b challenge virus[11] and all completed the 25 day follow-up (**Supplementary Figure 1**). The baseline characteristics of the participants were similar between groups (**Table 1**).

To investigate whether intranasal palivizumab could prevent RSV infection, the RSV viral load was measured in RNA copies/mL. Intranasal palivizumab had no effect on reducing the area under the curve of viral load on days 2-14 after challenge compared to placebo (palivizumab median 8.24 (interquartile range (IQR) 5.7-10.29) vs placebo median 9.57 (IQR 7.77-9.98), p = 0.395; **Figure 2** and **Supplementary Figure 2**), despite lack of infection in four participants from the palivizumab group. The inoculum was undetectable in nasal washes of RSV negative participants after 3 days (**Supplementary Figure 3A**) and viral load appeared lower in asymptomatic RSV positive participants than in symptomatic participants (not statistically tested; **Supplementary Figure 3B**). Low amounts of infectious RSV as measured by TCID50 assay were found in nasal washes from three participants on 1-3 days (6/364 total nasal washes, 13 time points of 28 participants) whereas the other washes were negative.

Figure 1. Participant flow-chart



All 34 potential participants were screened of whom 28 were selected for inclusion and six were added to a reserve pool of eligible participants to fill-in when selected participants were no longer eligible (i.e. had cold symptoms, n = 1) or withdrew before randomization (n = 3). All randomized participants completed follow-up, defined as completed sample and data collection up to the last study day (25 days post inoculation). Potential transmission was defined as contact between an RSV positive participant and their household member, who reported that symptoms started < 7 days after the participant was inoculated. Abbreviations: RSV +, RSV positive; RSV -, RSV negative.

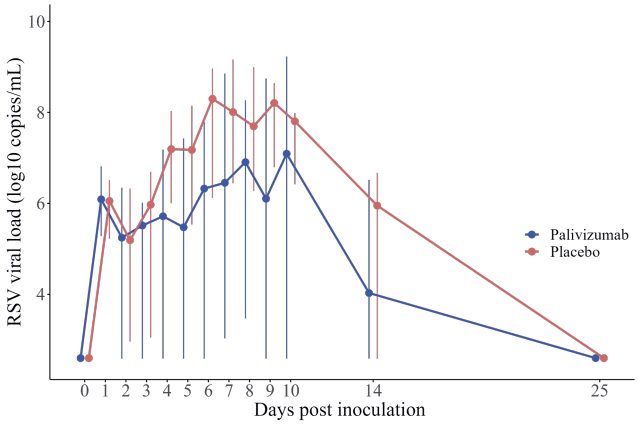
The trajectory of URT symptom scores over time differed statistically between the treatment groups (likelihood ratio test, p=0.03; **Figure 3A**). The median peak in URT symptoms in the all participants of the palivizumab group was 1.5 points lower than the median peak for the entire placebo group (2 vs 3.5 out of a maximum score of 24; **Figure 3B**). As expected in healthy adults, we did not see LRT involvement after RSV challenge: no differences in LRT symptoms were observed between treatment groups (likelihood ratio test, p=0.07, **Supplementary Figure 4A**) and median total LRT symptom score was 0 at all time-points in both groups (**Supplementary Figure 4B**). None of the participants developed a fever, as measured by a temperature of 38°C or higher, at any point during the entire study period.

Table 1. Participant baseline characteristics.

	Palivizumab n = 14	**Placebo n = 14**
Sex		
Male	5 (35.7%)	5 (35.7%)
Female	9 (64.3%)	9 (64.3%)
Age (years)	23 (20-54)	23 (19-53)
Education – postgraduate	10 (71.4%)	10 (71.4%)
Atopy*		
None	10 (71.4%)	7 (50.0%)
Asthma	0 (0%)	1 (7.1%)
Eczema	5 (35.7%)	2 (14.3%)
Allergies	7 (50.0%)	6 (42.9%)
LRTI in the last year	0 (0%)	0 (0%)
Chronic illness	0 (0%)	0 (0%)
Smoking history		
Never	11 (78.6%)	11 (78.6%)
Past	0 (0%)	0 (0%)
Current	3 (21.4%)	3 (21.4%)

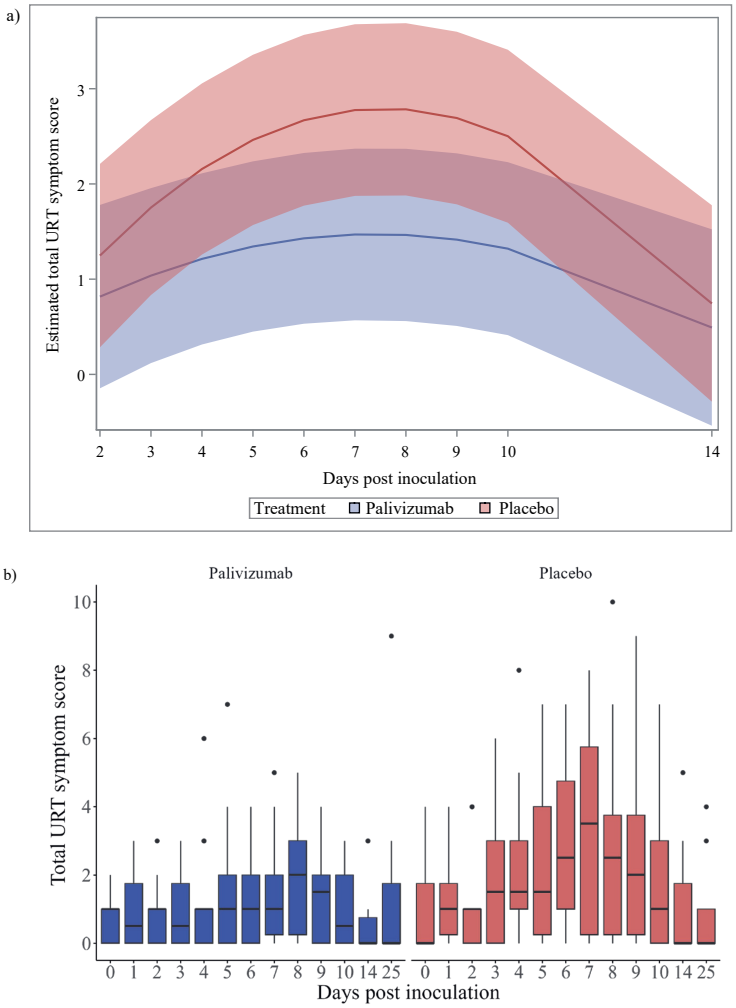
Data are presented in n (%) or median (range). *Atopy denotes participants with one or more atopic conditions, including asthma, eczema and any allergies. LRTI, lower respiratory tract infection.

Figure 2. Efficacy of i.n. palivizumab to reduce RSV viral load after viral challenge.



RSV viral copies/mL (median + IQR) over time per treatment group after RSV challenge as determined by RT-qPCR assay (n=14 per group). The area under the curve from days 2-14 does not show a significant difference between groups. i.n., intranasal; IQR, interquartile range; RT-qPCR, real-time quantitative polymerase-chain-reaction.

Figure 3. Upper respiratory tract symptoms after RSV challenge



a) Mean estimates of regression model for URT symptom score on days 2-14 after challenge per treatment group (line with 95%CI in shaded area). A statistically significant difference in the trajectory over time of the symptom score between placebo and palivizumab groups is observed (likelihood ratio test, p = 0.03). Linear mixed model using treatment group, time of recording the symptoms, quadratic effect of the time of recording, interaction between treatment group, interaction between treatment group and time squared as fixed effects, and intercept as random effect. b) Boxplot of daily URT symptom score over time per treatment group. The boxes in the plots represent the interquartile range (IQR), with the median marked by a line inside the box. The whiskers extend from the box to the minimum and maximum values. Outliers are depicted as individual points beyond the whiskers. CI, confidence interval; URT, upper respiratory tract.

After participants were inoculated with the challenge virus, 86% (24/28) was infected with RSV while four participants in the palivizumab group were not infected. Of these RSV infections, half (12/24) were symptomatic infections: 20.0% (2/10) of participants in the palivizumab group had symptomatic infections compared to 71.4% (10/14) in the placebo group (Relative Risk Reduction (RRR) = 72.0%).

Lung function did not change over time for the intervention group compared to the placebo group. There was no difference in the trajectory over time of the forced expiratory volume in the first second (FEV1) %predicted between placebo and palivizumab (likelihood ratio test, p=0.28; **Figure 4**). The FEV1 %predicted and the forced vital capacity (FVC) %predicted were similar between groups (**Supplementary Figures 5A and 5B**). The FEV1/FVC ratio remained stable in the palivizumab group while the small reduction observed in the placebo group returned to baseline level after 14 days (**Supplementary Figure 5C**).

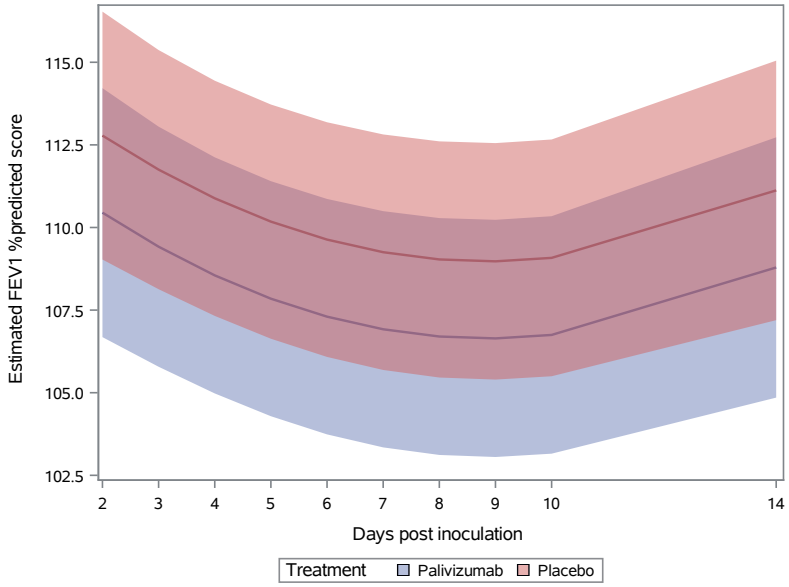
Within 25 days after immunization, three participants reported three adverse events (AEs): two in the placebo group and one in the palivizumab group (**Table 2**). The two adverse events in the placebo group took place during baseline sampling before administration of treatment or inoculum, and were not considered to be related to the study agent: one participant vomited during blood draw and the other participant had a vasovagal response during blood draw with spontaneous recovery. The participant in the palivizumab group with an adverse event had a nose bleed after sneezing during sampling on study day 9. None of the AEs resulted in withdrawal from the study. No immediate local or systemic reactions (within 1 hour after administration) and no serious adverse events were reported. Over the entire course of the study, there were 5 potential transmission events to household members (**Figure 1**), however all household members tested negative for RSV (5/5).

Discussion

Even under optimally controlled trial conditions using unique study design of a CHIM, intranasal mAbs do not prevent respiratory infections in healthy adults. Intranasal prophylaxis had a minor effect on reducing the URT symptoms during RSV infection with limited clinical relevance (1.5 point reduction on a 24-point symptom scale).

The observed lack of effect in this study may be explained by several non-exclusive mechanisms. First, viral load might not be the most suitable indicator of disease severity in adults. Clesrovimab, a mAb against RSV, showed non-significant dose-dependent reduction in nasal viral load in a CHIM when administering high doses in adults (100-900 mg IV).[12] Second, the minimal threshold needed for protective efficacy was previously calculated using trough concentrations observed in infants.[10] As the registered mAbs are dosed to prevent hospitalization in infants rather than reduce viral load in adults, the dosing in the current study might have been too low to prevent mild disease in adults. Third, IgA might be more protective than IgG considering the larger role for IgA in mucosal immunity. Potentially, IgG is degraded or inactivated in the mucosa due to proteolysis, reduced

Figure 4. Lung function after RSV challenge



Mean estimates of regression model for FEV1 %predicted on days 2-14 after challenge per treatment group (line with 95% CI in shaded area). No difference in the trajectory over time of the lung function between placebo and palivizumab is observed. A mixed effects repeated measures model with the mean of FEV1%predicted at day 0 and 1 as baseline variable and treatment group, time of measurement, and quadratic effect of time of measurement as fixed effects, and intercept as random effect. FEV1, forced expiratory volume in the first second.

Table 2. Summary of adverse events.

	Palivizumab (n = 14)	**Placebo (n = 14)**
Adverse events	1 (7.1%)	2 (14.3%)
Participants with AEs	1 (7.1%)	2 (14.3%)
Participants with grade 1 AEs	1 (7.1%)	2 (14.3%)
Participants with serious AEs	0 (0%)	0 (0%)
Participants with ≥1 AE possibly related to trial regimen	1 (7.1%)	2 (14.3%)
Participants with ≥1 AE possibly related to RSV challenge	1 (7.1%)	0 (0%)

Data are in n (%). Number of participants who experienced adverse events during study follow-up as self-reported, when asked about any adverse events as part of daily checklist during household visits or as observed by study personnel. Symptoms included in the symptom diary were presumed to represent virus infection consequent to RSV challenge and not regarded as AE unless they met the definition of an AE (Supplementary Materials). Grading scale: Grade 1 = mild (awareness of a symptom but the symptom is easily tolerated); grade 2 = moderate (discomfort enough to cause interference with usual activity); grade 3 = severe (incapacitating; unable to perform usual activities; requires absenteeism from work or bed rest). AE, adverse event.

receptor binding, or the induction of anti-drug-antibody induction. Fourth, although half-life of intranasal mAbs has been found to last at least 6-24 hours,[13,14] a shorter half-life could have contributed to suboptimal drug availability at time of RSV inoculation. The drug availability may also be improved with an aerosol nebulizer rather than nose drops.[15] Fifth, the administered palivizumab might not be sufficient to neutralize the inoculated viral dose, which is likely higher than during natural infection. Lastly, the role of Fc-mediated antibody effector functions remain largely unknown in the context of intranasal immunity. Therefore, the mechanism of action in the mucosa is considered to be limited to neutralization while systemic mAbs are complemented by antibody-effector functions destroying virus-infected cells.[16,17] In summary, the lack of efficacy in this trial may be due to several factors including (1) inability of mAbs to protect against mild disease, (2) insufficient dosing, (3) the potential superiority of IgA, (3) rapid IgG degradation or inactivation, (4) limited drug availability, (5) high inoculated viral doses, and (6) lack of Fc-mediated antibody effector functions in the mucosa.

In the context of highly active clinical development of mucosal mAbs (Supplementary Table 1), this study is the first trial in humans under optimal conditions, which shows no efficacy. Lack of efficacy in the phase 2b trial with intranasal palivizumab may be explained by low virus circulation, lack of virus exposure, and potential low drug adherence during the Covid-19 pandemic.[10] The SARS-CoV-2 broadly neutralizing antibody F61 nasal spray showed 72% efficacy against SAR-CoV2 infection for seven days using daily dosing, but no efficacy after a single dose. The dose used (24 mg) is likely too high to be cost effective.[18] Moreover, the trial has serious methodological concerns: (1) small sample size with a drop-out rate as high as 47%, high withdrawal rates, and no reason for withdrawal given, (2) no objective adherence data, (3) only 45% of the target sample size completing the study; (4) no clinical trial registration of the real-world study; and (5) short follow-up time of one week. No other efficacy results have been published.

The lack of protection in humans stands in contrast to preclinical work in animal models and we consider several reasons for this discrepancy. Mice, hamster, and rabbits are consistently protected against infection with respiratory viruses such as influenza, SARS-CoV-2, RSV, or MERS when administered a dose (range 0.005-50 mg/kg) relatively higher than in the human studies (Supplementary Table 1). While the rodents have a smaller nasal cavity than humans,[19,20] they were dosed with relatively larger volumes compared with humans thereby allowing the mAbs to reach further down the respiratory tract into the lungs.[21-23] The higher relative dose per body weight, larger epithelial coverage, and potential lower respiratory tract involvement in rodents could explain the observed discrepancy between animal models and human trials. Humanized mAbs have an abnormally high affinity for mouse and rat neonatal Fc receptor (FcRn), resulting in an artificially prolonged persistence in rodent models[24] making the model inadequate for preclinical i.n. mAb studies. Lastly, immunological history, microbiome composition, and natural disease history differs between species, which limits the translational value of rodent models to implementation in humans.[25] In summary,

several mechanisms may explain the inconsistency in efficacy of intranasal mAbs between animal models and human trials.

The strengths of our study are the controlled and optimal conditions for studying (prevention of) RSV infection using intranasal palivizumab, bypassing the relevant limitations of the phase 2b trial.[10] We achieved high infection rates without any safety issues. This was the first ever RSV CHIM in an outpatient setting, in which participants home-quarantined to reduce financial, logistical, and participant burden. We show outpatient RSV CHIM is safe as we measured no transmission of the virus to household members when participants adhere to WHO droplet isolation measures.

The outpatient setting of the trial also came with some limitations, such as the need for sample transportation, leading to a median time of 1 hour and 8 minutes (range 5 minutes - 3.5 hours; palivizumab group median 01:05 hours and placebo 01:10 hours) between sample collection and processing. We did not test the impact of this time variation on sample quality, potentially resulting in false-negative viral culture, although it is to be expected that freeze/thaw cycles have a bigger impact. Second, the outpatient setting in combination with the timing of the trial at the beginning of the respiratory season could have increased the chance to co-infections (one participant had a laboratory-confirmed SARS-CoV-2 infection at 25 dpi). Although we did not perform laboratory confirmation of co-infections, we expect the impact thereof to be limited as the self-reported progression of URT symptoms over time was as expected. One RSV-positive participant developed an RSV viral load only on 7-10 dpi, suggesting later (re-)exposure, which may represent a secondary infection caused by a household member who was also included in the trial. Third, the viral load found in all participants was high compared to previous RSV CHIMs although direct comparison is difficult due to different laboratory assays used.[12,26-28] Lastly, the trial was conducted in an adult population which is not representative of the target infant population. Adults typically experience mild cold-like RSV infections while infants are more likely to suffer from more severe disease.

Although promising preclinical data support the efficacy of intranasal mAbs to prevent respiratory infections, we find that these results cannot be replicated in humans. Therefore, intranasal mAbs are highly unlikely to be a feasible and affordable method of protection against respiratory infection without high and frequent dosing.

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Supplement

Supplementary files can be downloaded from:



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RSM01, an extended half-life RSV monoclonal antibody with a high barrier to resistance

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Abstract

Respiratory syncytial virus (RSV) causes substantial infant morbidity and mortality, particularly in low- and middle-income countries (LMICs). RSM01 is a long-acting RSV monoclonal antibody (mAb) in development for LMICs. In vitro generation of RSM01 resistant mutants has been unsuccessful. We systematically assessed the in vitro resistance barrier of RSM01 and the susceptibility of a panel of global contemporary strains to RSM01. Monoclonal antibody resistant mutants (MARMs) were generated by serially passaging triple plaque-purified RSV-A2 and RSV-B1 under pressure from RSM01, nirsevimab, or palivizumab, followed by phenotypic and genotypic characterisation. Moderate resistance was defined as > 3-fold and high resistance as > 30-fold increase in IC_{50} compared with parental virus. The susceptibility of contemporary global clinical strains to RSM01 was tested in a neutralisation assay. RSV-A and B strains developed high resistance to palivizumab and nirsevimab, while RSM01 pressure induced moderate resistance. No MARMs demonstrated a fitness advantage. RSM01 potently neutralised global contemporary strains. In conclusion, in vitro RSM01 resistance was infrequent and moderate and RSM01 had broad activity against circulating strains. The confirmation of the high barrier to resistance of RSM01 is encouraging and provides an alternative should resistance emerge with widespread use of current licensed anti-RSV mAbs.

Introduction

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections (LRTI) among infants and young children and a major contributor to global child mortality.[1,2] While new immunisation products have the potential to substantially improve infant health, access to monoclonal antibody (mAbs) remains highly inequitable.[3,4] The mAb nirsevimab5 has been implemented almost exclusively in high-income countries (HICs),[6] despite more than 97% of the RSV-associated infant mortality occurring in low- and middle-income countries (LMICs).[1] Ensuring a durable, affordable, and resistance-resilient RSV mAb is therefore essential to achieving global health equity.

Current and developmental mAbs neutralise RSV by binding the fusion (F) protein and blocking viral entry into host cells. RSM01 is a fully human IgG1 mAb with half-life extending YTE-mutation targeting antigenic site Ø on the prefusion (preF) conformation of the RSV F protein.[7] It demonstrates potent neutralization of diverse RSV-A and RSV-B isolates in vitro, prophylactic efficacy in animal models, a favourable non-clinical pharmacology profile, and was well tolerated in healthy adults volunteers in a phase 1 clinical study.[8] The development program explicitly prioritizes manufacturing efficiency and affordability, positioning RSM01 as a potential single-dose, season-long RSV prevention strategy tailored for LMIC settings.

RSM01 and nirsevimab bind overlapping epitopes within antigenic site Ø,[8,9] whereas clesrovimab targets site IV on both pre- and post-fusion state of the F protein (**Fig. S1**).[10] Together, these mAbs are replacing palivizumab, which targets site II and has a restricted indication and requires multiple costly injections per season.[11] As the global use of long-acting mAbs increases, understanding their vulnerability to viral escape is critical. Like other RNA viruses, RSV can generate monoclonal antibody-resistant mutants (MARMs) under selective mAb pressure, as seen in in vitro and animal studies.[8,12-18] Resistance-associated substitutions are rarely observed in circulating strains (~1%)[17,19-21] and often impose viral fitness costs.[14,16,17] To date, in vivo resistance in infants immunised with palivizumab or nirsevimab remains uncommon[22,23] and breakthrough infections are often linked to suboptimal dosing.[24,25] Although some resistance-associated mutations have been reported, mainly in RSV-B,[23,25] circulating viruses have largely remained susceptible,[23] and target epitopes are highly conserved.[18,21] The long-term effectiveness of antibody-based prevention depends on the barrier to resistance of each product, however, because nirsevimab has only recently been introduced, the clinical significance of resistance cannot yet be fully assessed.

High-barrier-to-resistance mAbs (those requiring multiple or fitness-impairing mutations for escape) are particularly important in settings where genomic surveillance is limited and timely product replacement is challenging and sometimes economically inviable. Evaluating resistance robustness during development is therefore a critical component of sustainable RSV immunoprophylaxis. Previous studies failed to generate escape mutants against RSM01 or its parental antibody, while MARMs were readily obtained for palivizumab and nirsevimab;[8] however, more stringent experimental design and quality control are required to validate these findings.

Here, we systematically assess the resistance barrier of the LMIC-focused candidate RSM01 relative to existing RSV mAbs by characterizing in vitro–selected escape mutants, their effects on viral fitness, the prevalence of associated mutations in circulating RSV sequences. We also present the susceptibility of newly isolated contemporary global strains to RSM01. Together, these data provide an integrated assessment of the resistance potential of RSM01 and its suitability for durable global RSV prevention.

Results

Selection and characterisation of RSV MARMs

In vitro generation of MARMs

In order to understand potential immune escape, we generated MARMs for RSV-A2 and B1. ATCC master stocks were triple plaque purified (TPP) into parental strains (**Fig. S2**). ATCC RSV-A2 master stock contained a minor variant N262Y mutation in comparison with the master stock consensus sequence (allele frequency (AF) = 31%) in the palivizumab epitope, while 36% of the RSV-A2 parental strain contained the mutation (**Table 1**). ATCC RSV-B1 master stock contained a K65E mutation, which was lost during plaque purification. TPP parental strains showed comparable growth curves to the ATCC master stocks (**Fig. S3**). Parental strains were serially passaged under mAb pressure for 10 passages under various in vitro culture conditions (**Fig. 1A-B** and **S2**). We display experimental conditions previously tested to generate in vitro MARMs against RSM01 (**Fig. 1C**) alongside the experimental conditions tested in this study to show a full overview of the potential of RSM01 to generate MARMs in vitro.[8]

Identification and characterisation of MARMs

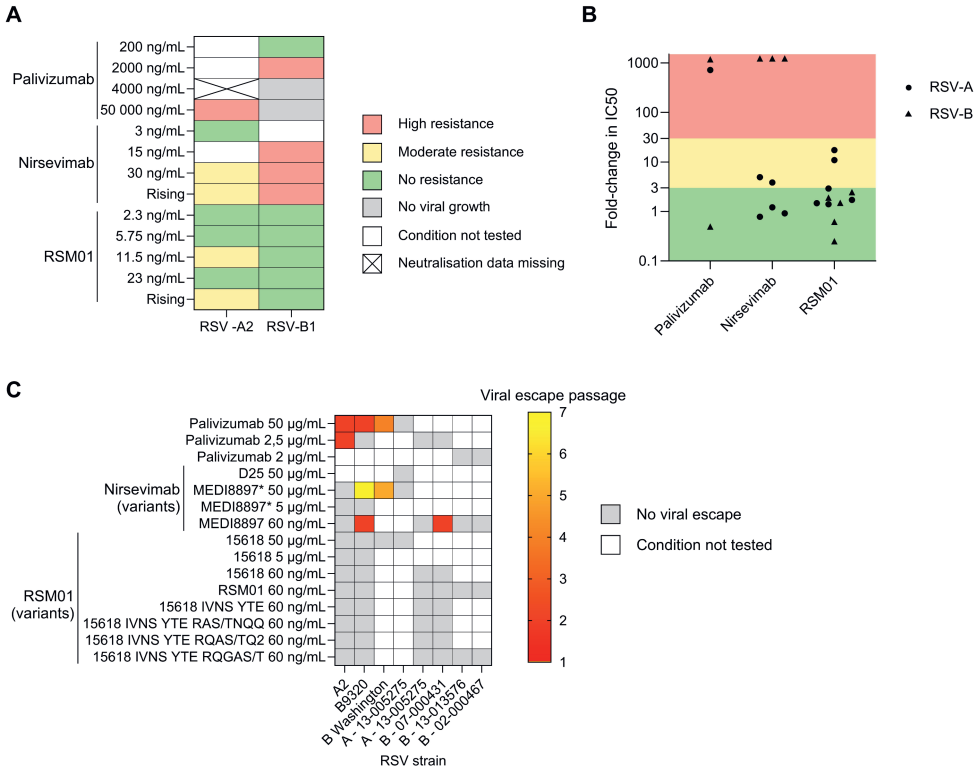
All virus strains cultured under mAb pressure at passage 10 as well as strains showing high cytopathic effects (CPE) under rising mAb pressure at earlier passages, were systematically screened for at least 3-fold increase in half inhibitory concentration (IC_{50}) compared with parental strains. Selected MARMs were further characterised by neutralisation and sequencing to align functional with genotypical changes (**Table 1** and **Fig. 1A-B**). Additionally, RSV-B1 grown under RSM01 pressure resulted in a nirsevimab MARM with a known mutation in the nirsevimab epitope (K65E) and was also further characterised. Virus controls, cultured without mAb pressure, showed a N262Y mutation as the consensus strain in four RSV-A2 controls at passage 10 and no mutations occurred in F protein for one RSV-B1 control at passage 12. MARMs were named after the mAb used to generate the MARM and numbered.

Table 1. Genotypic and neutralisation changes in selected MARMs

	Virus name	Subtype	mAb pressure (concentration in ng/mL)	Passage	Amino acid substitution		Mean IC50 (ng/mL)	Fold-change to parental strain
					Consensus strain	Minor variant (AF)		
Virus stocks	A2 ATCC Master stock	RSV-A2	None	ATCC+2	None	N262Y (31%)	··	··
	A2 TPP Parental	RSV-A2	None	ATCC+7	None	N262Y (36%)	RSM01 20.3 Nirsevimab 31.3 Palivizumab 3460.8	1
	A2 virus controls[a]	RSV-A2	None	Parental+10	N262Y	None	··	··
	B1 ATCC Master stock	RSV-B1	None	ATCC+1	K65E	None	··	··
	B1 TPP Parental	RSV-B1	None	ATCC+9	None	None	RSM01 42.8 Nirsevimab 101.2 Palivizumab 2116.0	1
	B1 virus control	RSV-B1	None	Parental+12	None	None	··	··
RSM01 MARMs	RSM-1	RSV-A2	Fixed RSM01 (11.5)	Parental+12	I206T/N262Y	None	352.1	17.3
	RSM-2	RSV-A2	Rising RSM01 (46)	Parental+12	I206T/N262Y	None	220.9	10.9
	RSM-3	RSV-B1	Rising RSM01 (230)	Parental+15	N216H[b]	None	26.5	0.6
Nirsevimab MARMs	NIR-1	RSV-A2	Fixed nirsevimab (30)	Parental+12	D194N/N262Y	None	120.4	3.8
	NIR-2	RSV-A2	Rising nirsevimab (96)	Parental+9	N63K/N262Y	None	154.5	4.9
	NIR-3	RSV-B1	Fixed nirsevimab (15)	Parental+12	K65E	None	>124,500	>1,230.2
	NIR-4	RSV-B1	Fixed nirsevimab (30)	Parental+12	K65E	N63D (17%)	>124,500	>1,230.2
	NIR-5	RSV-B1	Rising nirsevimab (1,500)	Parental+12	K65E	None	>124,500	>1,230.2
	RSM-4	RSV-B1	Fixed RSM01 (23)	Parental+12	K65E	None	>124,500	>1,230.2
Palivizumab MARMs	PAL-1	RSV-A2	Fixed palivizumab (50,000)	Parental+12	N262Y	None	>2,500,000	>722.4
	PAL-2	RSV-B1	Fixed palivizumab (2,000)	Parental+13	K272I	None	>2,500,000	>1,181.5

ATCC RSV-A2 and RSV-B1 master stocks were triple plaque purified to generate parental strains, which were serially passaged under mAb pressure. Mab concentrations were either fixed for 10 passages or increased based on CPE. Parental strains served as references for MARM sequence analysis; minor variants were reported at 5-40% allele frequency with ≥200× read depth. Rising mAb represent the concentration at MARMs characterisation. aAll four virus controls showed the same mutation. b No mutation was observed in NGS after viral propagation. An N216H substitution observed by Sanger sequencing in two passages (p8, p13) was absent in reverse reads and an intermediate passage (p10), indicating it was not stably fixed. AF, allele frequency; IC_{50}, half-maximum inhibitory concentration; mAb, monoclonal antibody; MARMs, monoclonal antibody resistant mutants; RSV, respiratory syncytial virus; TPP, triple plaque purified; ··, not available.

Figure 1. Generation of RSV mAb resistant mutants



A. RSV-A2 and B1 mAb resistant mutants (MARMs) were generated in the present study by 10 serial passages under mAb pressure. Mab concentrations (fixed or rising) are presented on the y-axis and RSV strains on x-axis. Neutralisation data for RSV-A2 cultured under 4000 ng/mL palivizumab is missing (cross). **B.** All potential MARMs with >3 fold increase in IC_{50} compared with parental strains as determined in a neutralisation assay were further neutralised according to principles of mass violation. Green, yellow, and red banding represent varying degrees of resistance (no, moderate, and high resistance, respectively) in terms of IC_{50} fold change compared with parental strains. Mean IC_{50} values from two independent assays are presented. **C.** Generation of RSV MARMs in an independent laboratory (Arsanis Biosciences GmbH) using various laboratory and clinical strains at various concentrations of mAb.[8] Several variants of nirsevimab (MEDI8897) were used, where MEDI8897 represents nirsevimab without YTE mutation and D25 is the parental antibody. RSM01 variants included parent antibody 15618 IVNS YTE and other 15618 variants. Viral escape was defined as CPE as observed under light microscopy, confirmed by F protein sequencing. No viral escape (grey) was observed for RSM01 and variants, while viral escape was readily observed for both palivizumab and nirsevimab during serial passage (red-yellow scale, where red signifies early passages and yellow at later passages). CPE, cytopathic effects; IC_{50}, half-maximum inhibitory concentration; mAb, monoclonal antibody; MARMs, monoclonal antibody resistant mutants; RSV, respiratory syncytial virus.

RSM01-MARMs

RSM01 pressure generated 3 MARMs out of 10 conditions (30% escape frequency; **Fig 1B**). Out of all RSM01 MARMs, 2/3 showed moderate resistance. RSM-1 and -2, RSV-A2 cultured under fixed 11.5 ng/mL RSM01 and under rising RSM01 concentration (up to 46 ng/mL, see Materials and Methods) (**Table 1**), showed the same mutations (I206T/N262Y) and moderate resistance (10.9 and 17.3-fold increase in IC_{50}). Moreover, RSM-3 was observed

as a MARM during culture as it showed >10% CPE under high RSM01 pressure (230 ng/mL). However, no genotypical or functional resistance was observed after viral propagation (0.6-fold change in IC_{50}), potentially due to a loss in mutation during propagation or due to missed mutations outside the F-protein. Sanger sequencing of earlier passages did not reveal consistent consensus-level mutations across passages: an N216H mutation was detected in the forward read of two passages (passage 8 and 13) but was absent in the corresponding reverse reads and in intermediate passage 10, potentially due to an unstable mutation.

Nirsevimab-MARMs

Nirsevimab pressure induced resistance in 5/6 (83%) growth conditions and 3/5 MARMs (60%) showed high resistance and 2/5 (40%) showed moderate resistance (**Fig. 1B-C** and **Table 1**). Two RSV-A2 MARMs, NIR-1, with the D194N mutation neighbouring the nirsevimab epitope, and NIR-2 (N63K mutation) both showed moderate resistance (3.8 and 4.9-fold increase in IC_{50}, respectively). NIR-3 to NIR-5, RSV-B1 cultured under three concentrations of nirsevimab, all showed the same K65E mutation in the nirsevimab binding site, which was associated with complete neutralisation escape (>1230 fold increase in IC_{50}). One of these isolates (NIR-5) showed an additional minor variant at position N63D (AF=17%), with an uncertain effect on neutralisation as the K65E MARM showed complete escape. Notably, a MARM cultured under RSM01 pressure (RSM-4) with the same K65E mutation was still susceptible to RSM01 but showed escape against nirsevimab (>1230 fold increase in IC_{50}).

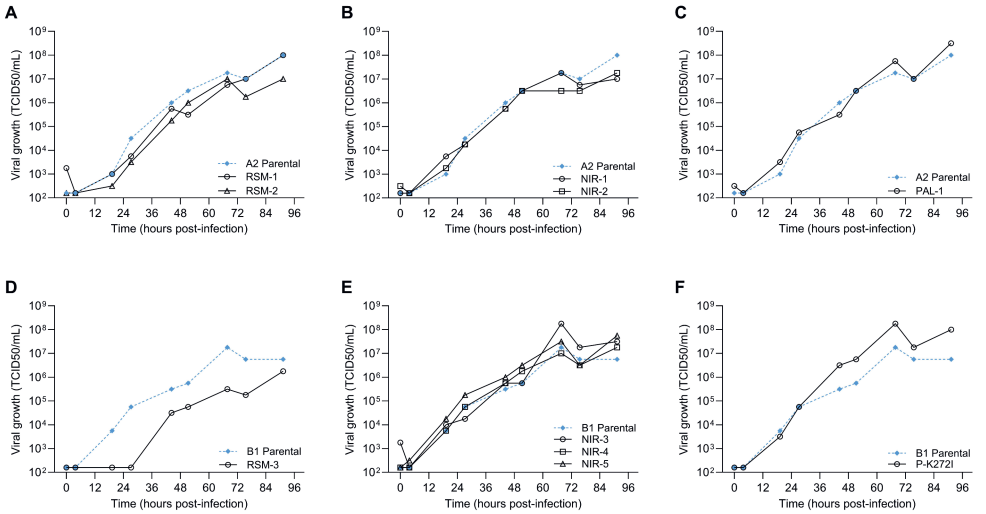
Palivizumab-MARMs

Under palivizumab pressure, 2 out of 5 growth conditions (40%) resulted in escape; both MARMs showed high resistance (**Fig. 1B-C** and **Table 1**). The RSV-A2 MARM (PAL-1) cultured under fixed 50 µg/mL palivizumab showed a N262Y mutation in the consensus, which conferred complete neutralisation escape (>722-fold increase in IC_{50}). RSV-B1 cultured under fixed 2000 ng/mL palivizumab showed a K272I mutation (PAL-2), also associated with complete palivizumab escape (>1181-fold increase in IC_{50}).

MARMs growth kinetics

To determine the impact of antibody escape mutations on viral growth, we characterised the growth kinetics of TPP parental RSV-A2 or B1 and their derived MARMs by inoculating HEp-2 cells with an MOI of 0.01. The cultured supernatant was sampled twice daily for four days and viral growth was assessed using a $TCID_{50}$ assay. Parental RSV-A2 and derived MARMs show similar growth kinetics and peak titres (**Fig. 2A-C**). RSM-3 had slower in vitro growth and lower viral titres compared to the parental strain (**Fig. 2D**). The B1 nirsevimab-MARMs showed similar viral growth to the parental strain (**Fig. 2E**). Viral growth kinetics of RSM-4 are missing as it was initially not identified as a resistant mutant to RSM01 but showed genotypic and phenotypic resistance to nirsevimab in cross-resistance testing. Overall, viral resistance conferred no advantage or fitness costs as the parental strains and derived RSM01 or nirsevimab-MARMs exhibited comparable or slower growth kinetics and similar peak titres.

Figure 2. Viral growth kinetics of MARMs on HEp-2 cells



Growth kinetics of parental TPP RSV-A2 and B1 and the derived MARMs. HEp-2 cells were inoculated at an MOI of 0.01 $TCID_{50}$/cell. The cultured supernatant was sampled twice daily for four days and viral growth was assessed using a $TCID_{50}$ assay. RSV-A2 MARMs (**A-C**) and RSV-B1 MARMs (**D-F**) show similar growth to their parental TPP strains, with the exception of RSM-3 (**D**), which shows delayed and lower titre growth. MARMs, monoclonal antibody resistant mutants; MOI, multiplicity of infection; RSV, respiratory syncytial virus; $TCID_{50}$, half-maximum tissue culture infective dose.

Cross resistance of MARMs to other RSV mAbs

All MARMs could be rescued by at least one other mAb. Three RSM01 MARMs (RSM-1 to RSM-3) could still be neutralised by nirsevimab and clesrovimab (**Fig. 3**). RSM-4 was not resistant to RSM01 or clesrovimab, but was highly resistant (>1230 fold-change) to nirsevimab despite culture under RSM01 pressure. All nirsevimab MARMs could be rescued by RSM01 and/or clesrovimab.

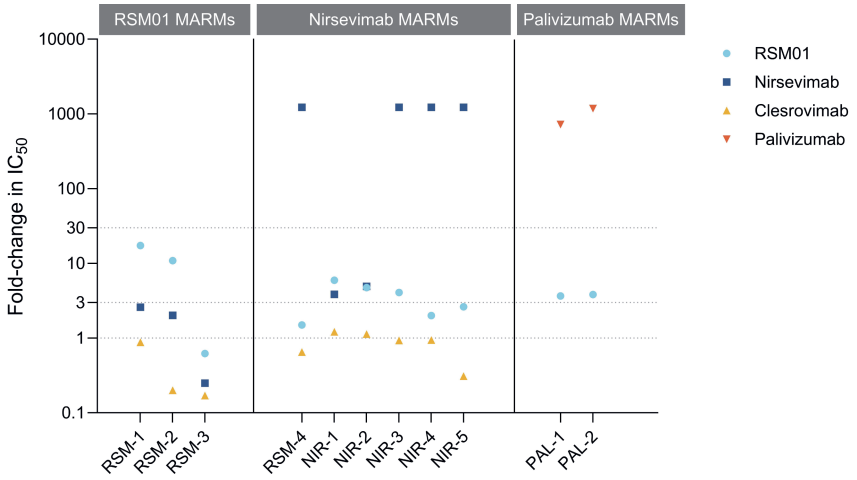
Prevalence of resistance-associated mutations in circulating strains

To investigate the occurrence of MARMs in currently circulating RSV strains, we compared the seven amino acid substitutions identified in the 11 in vitro MARMs to a global GISAID database (**Table 2**). All polymorphisms occurred at low frequencies (ranging between 0.04 and 0.16%) in current global strains. The I206T mutation associated with moderate resistance against RSM01 (and nirsevimab) occurred at a frequency of 0.16%. Notably, K272I, which arose in a palivizumab in vitro RSV-B MARM, was detected exclusively in RSV-A clinical strains.

Amino acid variability in mAb binding sites by Shannon entropy analysis

Shannon entropy analysis was performed to assess diversity on the surface F glycoprotein. The F protein of the RSV-A isolates showed 27 sites with high entropy (H > 0.1) (**Table S2**),

Figure 3. Cross-resistance of MARMs against other RSV mAbs



In vitro neutralisation of MARMs by other available RSV mAbs. Potency is expressed as fold-change in IC_{50} compared with the parental strain per mAb. IC_{50}, half-maximum inhibitory concentration; mAb, monoclonal antibody; MARMs, monoclonal antibody resistant mutants; RSV, respiratory syncytial virus.

Table 2. Natural occurrence of polymorphisms identified in MARMs

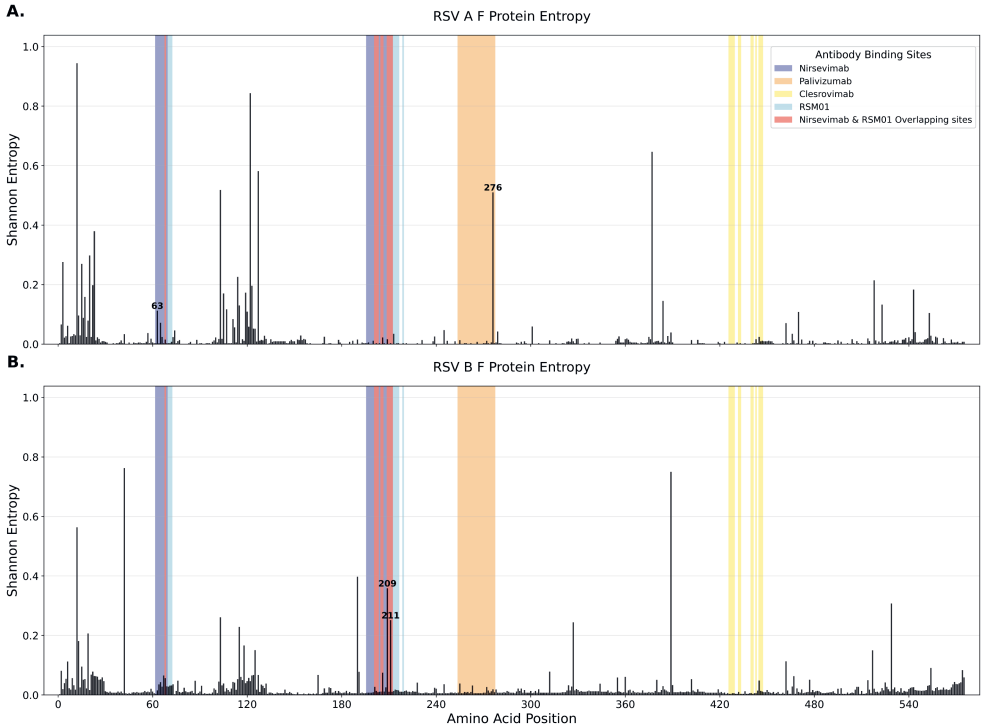
	RSV-A		RSV-B	
Mutation	**Sequences with mutation/Total**	**Frequency (%)**	**Sequences with mutation/Total**	**Frequency (%)**
N63D	0/5037	0	2/5120	0.04
N63K	0/5037	0	0/5120	0
K65E	0/5037	0	2/5120	0.04
D194N	0/5037	0	0/5120	0
I206T	8/5037	0.16	0/5120	0
N216H	0/5037	0	1/5120	0.02
N262Y	0/5037	0	0/5120	0
K272I	8/5037	0.16	0/5120	0

Whole genome sequences were extracted from GISAID database (May 5, 2023 to Dec 1, 2025). MARMs, monoclonal antibody resistant mutants; RSV, respiratory syncytial virus

of which two are located within epitopes: the nirsevimab binding site at residue 63 (H=0.11) and the palivizumab binding site at residue 276 (H=0.59) (**Fig. 4A**). RSV-B isolates displayed 17 highly variable positions, two of which are located in overlapping RSM01/nirsevimab binding sites (**Table S3** and **Fig. 4B**).

The RSM01 binding site was largely conserved across both RSV subtypes, with the majority of positions showing H < 0.1 (**Fig. 4**). However, within the region where RSM01 and nirsevimab binding sites overlap, residues 209 (H=0.35) and 211 (H=0.25) exhibited elevated polymorphisms among circulating RSV-B strains (**Fig. 4B**). Additionally, in RSV-A the nirsevimab binding site showed increased entropy at residue 63 (H=0.11) and the palivizumab binding site displayed higher entropy at residue 276 (H=0.51) (**Fig. 4A**).

Figure 4. Shannon entropy across amino acids positions in RSV F-protein sequences



The RSV whole genomes were downloaded from GISAID (May 5, 2023, up to Dec 5, 2025) with 5,037 RSV-A and 5,120 RSV-B F gene sequences. Shannon entropy was estimated based on each amino-acid position across the F-protein and proportion of amino acids found at a given residue for all genomes for both RSV-A (A) and RSV-B (B). The F protein structure is color-coded to indicate mAb binding sites (nirsevimab in dark blue, RSM01 in light blue, overlap between nirsevimab and RSM01 in salmon, palivizumab in orange, and clesrovimab in yellow). High entropy residues (H0.1) in mAb binding sites are annotated.

Susceptibility of contemporary global strains to RSM01

RSM01 was further evaluated for its neutralizing activity against a panel of contemporary strains in a neutralisation assay. In total, 31 out of 46 isolates (14 RSV-A and 17 RSV-B) were successfully cultured on HEp-2 cells with sufficiently high titres for the neutralisation assay (14 from South-Africa, 11 from Argentina, and 6 from the Netherlands).

All clinical isolates were characterised for subtype-specific diversity patterns. For RSV-A, seven distinct sublineages were identified: A.D.1.9 (n=5), A.D.3 (n=4), A.D.3.1 (n=1), A.D.3.3 (n=1), A.D.4.1 (n=1), A.D.5.1 (n=1), and A.D.5.2 (n=2) (**Fig. 5A**). RSV-B was characterised into three sublineages: B.D.E.1 (n=12), B.D.E.1.1 (n=4), and B.D.E.1.7 (n=2) (**Fig. 5B**). Phylogenetic trees were constructed from whole-genome sequences to assess the diversity of the panel for RSV-A and RSV-B contemporary strains from Argentina, South Africa, and the Netherlands, along with globally representative sequences from six geographic regions spanning 1950–2025 (**Fig. S4**). For both RSV-A and RSV-B, when compared with global

context data, most study samples clustered with high statistical support (ultrafast bootstrap, UFBoot 95%) within lineage-specific clades that circulated globally in the post-pandemic period (2023–2025) except one Argentinian strain (VRSV1046), which falls within the pre-pandemic cluster (**Fig. 5A-B**).

RSM01 potently neutralised the RSV-A strains with IC_{50} ranging between 22.7-99.5 ng/mL (geometric mean 58.3 ng/mL) and the RSV-B strains with a range of 49.8-178.8 ng/mL (geometric mean 114.4 ng/mL) (**Fig. 5C**). Our IC_{50} was reproducible (inter-operator precision 31% coefficient of variation (CV), inter-assay precision 27% CV) and we used an internal control and quality control criteria for every neutralisation assay to assess validity of results.

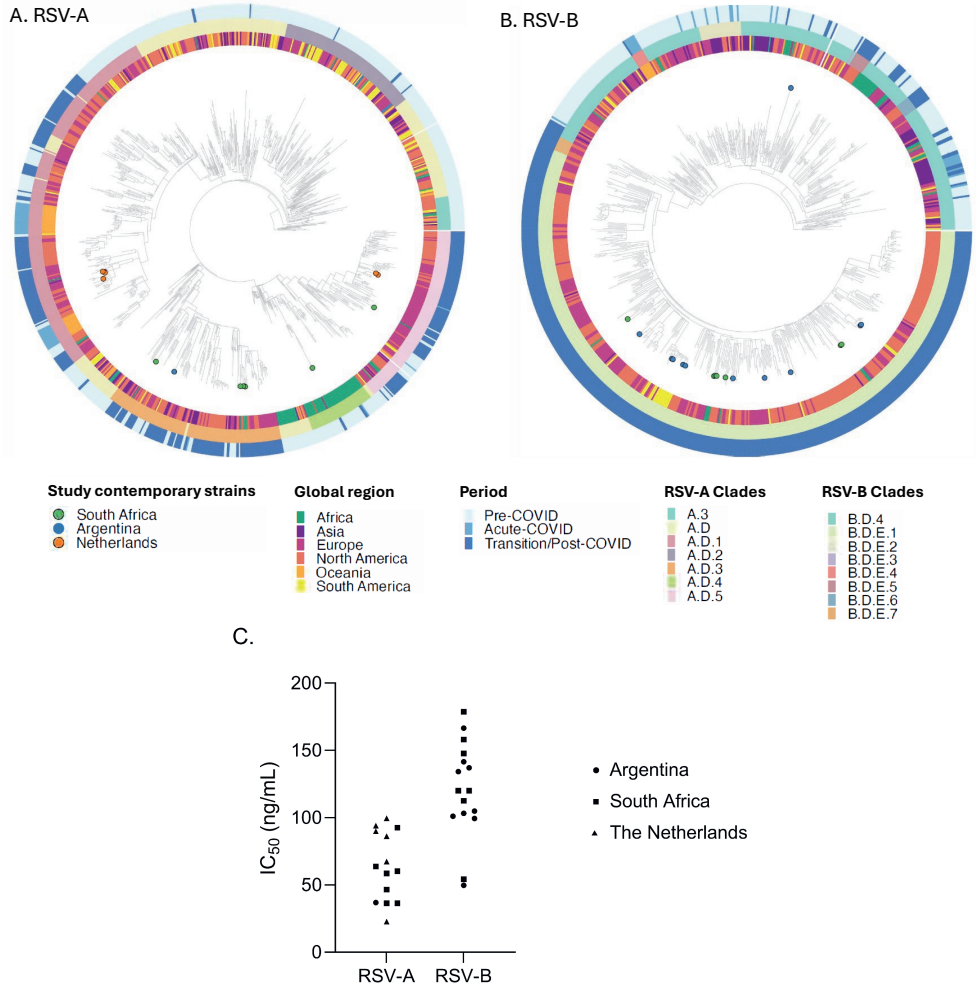
All of the RSV-B strains carried the S211N mutation without direct association with reduced potency of RSM01 (**Table S4**). One RSV-B strain carrying an additional R209Q mutation, which is associated with reduced nirsevimab potency, had an IC_{50} of 141.5 ng/mL for RSM01. A South African RSV-A strain carrying two mutations in nirsevimab and palivizumab binding sites (K65R and S276N, respectively) showed no cross-resistance against these mAbs (**Table S4**). These data confirm that RSM01 is potent against a broad panel of contemporary RSV clinical isolates.

RSM01 binding to preF

AlphaFold 3[26] predicted that RSM01 binds to the membrane-distal apex of the RSV preF protein, with an interface predicted template modeling score (ipTM) of 0.84, indicative of a high-confidence model (**Fig. 6A**). The interaction is mediated almost entirely by the CDRH3, which adopts a β-hairpin conformation and forms a three-stranded β-sheet with RSV F residues 210–213 (**Fig. 6B**). This interaction primarily engages mainchain atoms, and only few contacts with sidechains on RSV F were observed; a notable exception is RSV F lysine 209, which forms two salt bridges with aspartate and glutamate residues in the CDRH3. Observed viral mutations include I206T, which lies underneath the CDRH3, and N216H, which lies outside the direct binding interface but may alter helix capping and influence local dynamics.

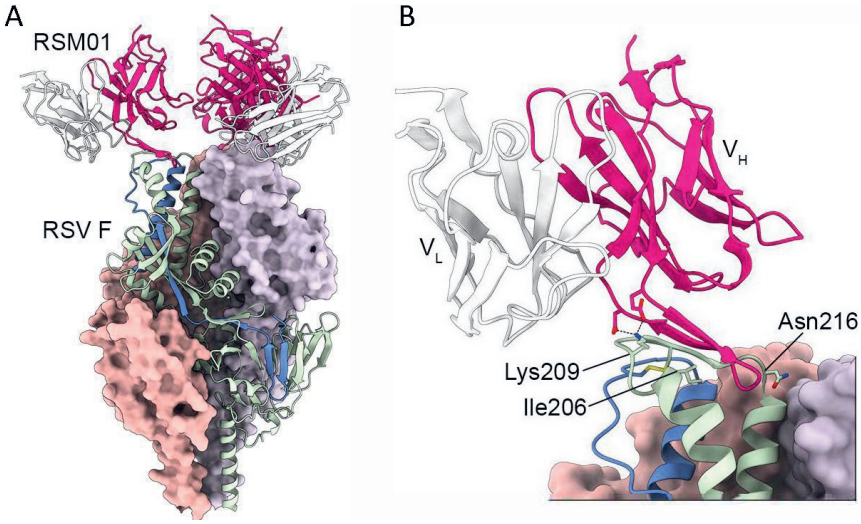
The predicted RSM01 binding mode is similar to that observed for antibody AM22,[27] and the targeted region on RSV F corresponds to a metastable segment that contains a conserved disulfide bond (Cys69-Cys212) and undergoes early conformational changes during fusion. The lack of extensive sidechain interactions on RSV F and the metastability of this region may reduce the likelihood of selecting for escape variants that do not have a fitness cost.

Figure 5. Neutralisation of a diverse panel of global contemporary clinical isolates



The clinical isolates used to evaluate RSM01 neutralisation activity were displayed as a phylogenetic tree to evaluate sequence diversity. **A+B.** Sequences of 14/17 RSV-A and B isolates are marked as coloured dots on clades extracted from a phylogenetic tree of 1,686 RSV-A and 1,650 RSV-B genome sequences representing seven geographic regions representing the time-window from 2010 and 2013, respectively. Pre-pandemic (before 2020), pandemic (2020–2022), and post-pandemic (2023–2025) periods are represented in the outer ring. **C.** RSM01 was assessed for its ability to neutralise RSV clinical isolates using a neutralisation assay. A panel of 30 RSV clinical isolates (14 RSV-A and 16 RSV-B) containing amino acid changes in the F fusion glycoprotein were tested. IC_{50} was calculated and represents the concentration of antibody required for a 50% reduction in the RSV infectivity. IC_{50}, half-maximum inhibitory concentration; RSV, respiratory syncytial virus.

Figure 6. Binding of RSM01 to RSV preF as predicted by AlphaFold



RSM01 binding to the RSV preF protein structure was modeled using AlphaFold. The complex was visualized in UCSF ChimeraX (**A**), highlighting key residues, including viral substitutions at positions 206 and 216 (**B**).

Discussion

In this study, we demonstrate that RSM01 has a high in vitro barrier to resistance, confirming previous findings.[8] Under selective pressure, highly resistant MARMs emerged frequently with palivizumab and nirsevimab, whereas no highly resistant mutants and only low rates of moderate resistance were observed with RSM01. RSM01-selected mutants displayed moderate resistance (10–17-fold IC_{50} increase), likely driven by I206T/N262Y substitutions, compared with markedly higher resistance for nirsevimab- and palivizumab-selected mutants (>1230-fold and >722-fold, respectively). No MARMs showed enhanced growth and one RSM01 mutant exhibited impaired growth. All resistant variants remained susceptible to at least one alternative mAb. A global panel of contemporary strains was susceptible to neutralisation by RSM01.

Previous studies also failed to generate RSM01-resistant mutants, while nirsevimab and palivizumab-MARMs were readily obtained:[8] whereas palivizumab- and nirsevimab-MARMs emerged within 2–7 passages, RSM01-MARMs could not be generated in 11 experiments with 8 different RSV strains, suggesting a higher barrier to resistance (**Fig. 1C**). Contrasting with previous methodology, this study used triple plaque-purified parental RSV strains, viral controls passaged without mAb pressure, systematically generated and identified MARMs, and characterised the MARMs using both functional and sequence data, with virus inoculum that did not violate the principle of mass action. The difficulty in generating RSM01 escape variants suggests that tolerated RSV F substitutions in the epitope are insufficient for escape without substantial fitness costs.

To assess the robustness of RSM01 against globally circulating RSV strains, we assembled a panel of contemporary isolates from 2023–2024 collected across South Africa, Argentina, and the Netherlands. This panel extends previous data,[8] which included older strains (2002–2017) from a single location (Utrecht, Netherlands). Testing contemporary, geographically diverse isolates is particularly important given potential shifts in RSV strain circulation and antigenicity following the COVID-19 pandemic, which may have altered susceptibility patterns. Preliminary analyses indicate that RSV-B isolates exhibit higher IC_{50}s than RSV-A, potentially an artefact of a relatively small sample size or reflecting intrinsic differences in neutralisation sensitivity between subtypes.

We observed higher IC_{50} values for RSM01 against clinical RSV strains (median 99.4 ng/mL) compared with previous work (median 1.8 ng/mL),[8] which most likely reflects methodological differences between neutralisation assays, such as multiplicity of infection (MOI) and read-out. Similarly, laboratory RSV-A2 strain against RSM01, nirsevimab, and palivizumab showed an median fold-change in IC_{50} of 16.2 (range 11.8-28.4) between the laboratories. Absolute IC_{50} values vary substantially across laboratories due to differences in reagents, cell density, viral input, overlay, incubation conditions, and readout systems, limiting direct cross-study comparisons. To enable robust interpretation, we therefore assessed resistance of in vitro MARMs using fold-changes in neutralisation relative to reference strains within the same experimental setting.

Several mechanisms may explain why RSM01 exhibited a higher barrier to resistance in vitro. RSM01 is predicted to engage a highly conserved, backbone-focused epitope at the apex of RSV F, making it largely insensitive to sidechain substitutions that often disrupt other mAbs like nirsevimab. This region is metastable and functionally critical, so mutations that reduce binding likely incur substantial fitness costs by impairing fusion or protein stability. Additionally, RSM01's functional angle of approach may restrict escape: antibodies requiring multi-residue or conformational changes to evade binding tend to have higher resistance barriers, as seen for RSV, HIV, and influenza.[28] Together, targeting a conserved, structurally constrained epitope, imposing fitness penalties for mutation, and limiting simple single-point escape pathways likely contribute to RSM01's observed resistance robustness. Deep mutational scanning could further define which substitutions are tolerated without fitness cost, providing insight into the potential durability of this neutralising antibody.

Some resistance-associated mutations identified in this study have been described previously, but some are novel. For RSM01, we observed a previously described I206T substitution[29] within the shared nirsevimab/RSM01 epitope, which functionally affected RSM01 more than nirsevimab. Prior studies reported alternative substitutions at this residue with minimal impact on nirsevimab unless combined with additional changes such as Q209R or N201T.[9,21,24,25] A novel D194N mutation near the nirsevimab binding site conferred only moderate nirsevimab resistance. We also detected a novel N63K substitution with minimal loss of susceptibility to nirsevimab, similar to other substitutions at this site (N63S clinically;

N63T/S *in vitro*) causing minimal IC_{50} increases.[9,23] Nirsevimab resistance associated with K65E, reported in both laboratory and clinical strains, disrupts antibody binding via electrostatic repulsion.[23] Well characterised palivizumab escape mutations N262Y and N272N (> 350-fold increases in IC_{50}) were confirmed.[16,30] Collectively, these findings show that laboratory-selected MARMs can identify critical preF residues, and alignment with clinical data helps distinguish tolerated mutations from those with potential real-world escape.

These findings have important implications for the global resilience of RSV mAb deployment. In many LMICs, limited genomic surveillance may delay detection of emerging resistance, allowing low-barrier to resistance mAbs to lose effectiveness and worsen health inequities. High-barrier mAbs like RSM01 offer greater resilience, reducing the risk of resistance-driven prophylaxis failure. Moreover, RSM01's high barrier to resistance provides flexibility for future clinical strategies: it could be combined with other mAbs in bi-specific or cocktail formulations to further limit escape, or potentially used as a salvage prophylaxis in cases resistance renders another mAb ineffective. The specific resistance mutations identified here can also guide targeted surveillance.

This study has several limitations. First, the susceptibility panel included a limited number of contemporary RSV strains, though sourced from three global regions, providing at least partial geographic representation and diversity in a contemporary phylogenetic tree. Second, our genetic analysis was limited by small sample sizes, restricting robust virus population-level statistical comparisons. Non-systematic global surveillance data may have reduced the precision of our assessment of F protein diversity and lineage dynamics over time. Third, plaque-selection of polyclonal selected MARMs potentially caused a loss of MARMs. Preliminary functional data showed no differences in IC_{50} between various plaques of the same polyclonal culture, which led to the practical decision to limit the sequencing of one plaque per MARM. Finally, MARM selection used laboratory-adapted strains rather than recent clinical isolates, which may not capture the full genetic diversity or fitness of circulating viruses and thereby limit the translation of findings to the clinic. However, previous use of clinical strains did not generate MARMs against RMS01 either.[8] Future studies should validate these findings using a broad panel of contemporary strains in a primary respiratory human cell model to assess resistance in a more physiologically relevant model than immortalized cell lines.

A major strength of this study is the comprehensive panel of RSV mAbs, including licensed and developmental antibodies, enabling direct comparison of resistance profiles. All viral strains were triple plaque purified to ensure genetic homogeneity, and virus controls passaged without mAb showed no mutations, confirming that resistance emerged under selective pressure. A systematic ten-passage protocol, combined with functional data and NGS sequencing, allowed robust identification of resistance-associated mutations, including minor variants. Mass violation studies were performed to assure the neutralisation curves were robust and reproducible. By integrating neutralisation data with global surveillance

sequences, we provide functional context to circulating RSV diversity. Detection of multiple co-circulating RSV-A and RSV-B sublineages, alongside their neutralisation phenotypes, improves understanding of temporal patterns and the potential impact of emerging mAb selective pressures.

RSM01 exhibits a high barrier to resistance with rare, moderate, and occasionally fitness-impairing escape mutations. Mapping these resistance pathways highlights key prefusion F residues and informs surveillance strategies. Developing resistance-resilient RSV mAbs is critical to achieving durable, equitable global protection.

Materials and methods

Cells, viruses and monoclonal antibodies

HEp-2 cells, RSV-A, and RSV-B were originally obtained from American Type Culture Collection (ATCC). HEp-2 cells (ATCC CCL-23) were passaged in DMEM (Gibco, high glucose, Glutamax, pyruvate, 31966-021) supplemented with 5% fetal bovine serum and pen/strep. Master stocks of RSV-A2 (ATCC VR-1540) and RSV-B1 (B WV/14617/85, VR-1400) were triple plaque purified in HEp-2 cells and propagated for a total of 5 and 8 passages, respectively, before use as parental strains. Four mAbs were evaluated (Table S1): RSM01 (Gates Medical Research Institute), Palivizumab (Synagis®), research-grade Nirsevimab (MedChemExpress, HY-P99756), and research-grade Clesrovimab (MedChemExpress, HY-P99804).

Generation of monoclonal antibody-resistant mutants

MARMs were generated by serially passaging virus in the presence of palivizumab, nirsevimab, or RSM01. Previous escape mutant selection studies led by AB were performed at Arsanis Biosciences GmbH (Oct 2017 – Nov 2018) according to previously published methodology.8 In brief, MARMs were selected by serially passaging laboratory strains and clinical isolates on HEp-2 cells in the presence of fixed concentrations of RSM01 (and variants) or comparator mAbs (nirsevimab (variants) and palivizumab) for a maximum of eight consecutive rounds. Viral populations showing cytopathic effects (CPE) were harvested and F-protein sequences analysed to identify resistance-associated mutations. The growth conditions associated with escape mutations in this earlier work informed the modified selection protocol used in the current study with as main differences that parental strains were triple plaque purified, mAb concentrations were either fixed or rising, and virus controls without mAb pressure.

In the present study performed at the UMC Utrecht, TPP parental strains were mixed with 1-200x published half-maximum inhibitory concentration (IC_{50}; **Table S1**) of mAb and pre-incubated for 30 minutes at 37°C and 5% CO_2. For simplicity, an IC_{50} of 3 ng/mL was used in nirsevimab concentration calculations during MARM production based on 2 RSV-A lab strains[8] and an IC_{50} of 200 ng/mL was used for palivizumab based on lab and clinical strains.[8,17] For RSM01, the IC_{50} was set at 2.3 ng/mL based on 2 RSV-A and 2 RSV-B lab strains from previous work by the Gates Medical Research Institute.[8] HEp-2 cells in 6-well plates

were then infected at multiplicity of infection (MOI) of 0.0133-0.00133 plaque-forming units (PFU)/cell. Controls included uninfected cells and virus passaged without antibody pressure. After 1 hour at 37°C and 5% CO_2, inoculum was removed and fresh medium with appropriate mAb concentrations was added to each well followed by 3-5 day incubation under continuous mAb pressure. The presence of CPE was monitored daily using light microscopy to determine timing of passage, aiming for 40-80% CPE. The contents of each well were harvested by cell scraping and centrifuged at 800g for 5 minutes to remove cell debris. An aliquot of the supernatant was diluted 1:1 with 50% sucrose in dPBS (sterile filter) as a cryo-protectant, snap-frozen, and stored at –80°C. The remaining supernatant was pre-incubated with fixed or rising concentrations of mAbs and then used to infect HEp-2 cells in the subsequent passage based on the degree of CPE (undiluted virus for no CPE, 1:100–1:1000 virus dilution for low CPE (<50% CPE), and 1:1000 to 1:10.000 virus dilution for high CPE (>50% CPE)). For passaging under rising mAb pressure, virus from the well showing optimal replication (i.e. high CPE) was selected at each passage and subsequently cultured under three mAb concentrations, including the previous and higher concentrations. Concentration increments were empirically adjusted based on viral growth to progressively increase selective pressure. This process was repeated for a total of ten sequential passages. Potential MARMs, i.e. all virus strains cultured for 10 passages under mAb pressure as well as strains showing high CPE under rising mAb pressure at earlier passages, were single plaque purified by infecting HEp-2 cells in 10-fold serial dilutions overlaid with 0.5% agarose to restrict viral spread via supernatant. After 3-5 days, two or three individual plaques were picked and propagated separately under continuous mAb pressure when >10% CPE were visible. After titration, potential MARMs were screened with a neutralisation assay at an MOI of 0.1. If the IC_{50} of a potential MARM was > 3-fold higher than the IC_{50} of the parental strain, it was further characterised for neutralisation capacity after performing mass violation, cross-resistance, RNA mutations, and viral growth assays.

Tissue culture half infectious dose ($TCID_{50}$) assay

Viral titres were assessed using a tissue culture half infectious dose ($TCID_{50}$) assay as previously described.[31] In brief, RSV strains underwent quadruplicate 10-fold dilution series in DMEM supplemented with antibiotics and 5% foetal bovine serum for 10 dilutions to infect monolayers of Hep-2 cells. Cells were checked daily for CPE and end-point titres were evaluated as the 50% tissue culture infective dose per mL after 7-10 days using the Spearman-Kärber method.[32]

Neutralisation assay

A validated neutralisation assay was adapted to quantify RSV replication by expression of F protein by enzyme-linked immunosorbent (ELISA) assay.[33] Three-fold serially diluted mAbs were incubated on 96 well-plates with equal volume of RSV for 1 hour at 37°C before adding to a monolayer of HEp-2 cells. After 2 hours, inoculum was replaced by fresh medium with 2.5% FCS and cells were cultured for 3 days at 37°C and 5% CO_2. Cells were fixed with ice

cold 80% acetone in PBS for 15 minutes at 4°C before blocking with 1% BSA in PBS for 1 hour at room temperature. The primary antibody (mouse anti-RSV; Merck Millipore MAB8262) was added 1:2500 for 1 hour and the secondary antibody (goat anti-mouse IgG-HRP; Dako P0447) was added 1:4000 for 1 hour followed by TMB (BioLegend 421101) until colour development was stopped with 1M H2SO4 after 15 minutes. The absorbance was measured at 450-570 nm (Spectramax M series, Company Molecular Devices). The neutralising titre (IC_{50}) was quantified using a log (inhibitor) vs response 4-parameter nonlinear regression curve in GraphPad Prism version 10.6.1 (GraphPad Software Inc., San Diego, CA, USA). An internal control virus neutralised by nirsevimab was taken along on every plate to monitor variation between assays. Other quality control criteria were copied from a previously validated neutralisation assay.[33]

To assess the law of mass violation (the IC_{50} value obtained in response to a given antibody concentration will remain constant across a range of virus titres[34]) and ensure reproducible IC_{50} data, we performed neutralisation assays with RSV strains at a range of three MOIs.[34,35] All potential MARMs and contemporary strains were neutralised at an MOI of 2, 0.5 and 0.1, where an MOI of 2 is expected to violate mass action. If an MOI of 2 could not be obtained due to low viral titres, we assessed the highest MOI possible. The principles of mass action were considered violated if the IC_{50} between the highest and lowest MOI differed more than 2-fold. If strains were resistant against one mAb, another mAb was used to determine mass violation. IC_{50} data of the parental strains were obtained by three operators and the MARM IC_{50} data were obtained in replicate by one operator (n=2). IC_{50} data of contemporary strains were obtained by one operator in a single replicate.

Moderate resistance was defined as IC_{50} > 3-fold compared to the parental strain and high resistance > 30-fold IC_{50} compared to the parental strain. Cross-resistance was measured using the neutralization assay against other mAbs.

Virus growth kinetics

Growth kinetics were performed by inoculating HEp-2 cells with RSV parental strains or MARM variants at an MOI of 0.01 $TCID_{50}$/cell. The culture supernatant was collected twice daily for four days post-inoculation, and viral titres were determined by a $TCID_{50}$ assay.[31] Titres below the lower limit of detection (LLOD) were imputed as half the LLOD (158 $TCID_{50}$/mL). Viral growth curves of MARM variants within 1 log deviation of the parental strain were considered as similar growth.

Contemporary global RSV strains

A panel of 46 newly isolated contemporary global RSV clinical isolates was selected to assess susceptibility to RSM01: 20 strains from South-Africa and 20 from Argentina were prospectively obtained between January and August 2024. Additionally, 6 strains from The Netherlands sampled during Nov-Dec 2023 were randomly selected from the RSV-STRAIN study. Nasopharyngeal swabs were obtained from infants within 48 hours (South-Africa)

or 5 days (Argentina and The Netherlands) of hospital admission with PCR-confirmed RSV-associated LRTI and stored at ≤-70°C in universal transport media before shipment on dry ice. If strains were genotyped at time of sample selection, we selected equal amounts of RSV-A and RSV-B strains. Viral stocks were propagated on HEp-2 cells at 37°C and 5% CO2 in 1 passage and subsequently sequenced and neutralised by RSM01. One outlier (VRSV1051, RSV-B) with IC_{50} of 337.5 ng/mL was removed from analysis as the neutralisation curve did not meet quality control criteria.

Sanger sequencing of RSV F-protein

Subtype-specific primer pairs were used to sequence the F-protein. Viral RNA was extracted using Purelink viral RNA/DNA mini kit (Thermo Fisher). 20 µL of RSV samples were isolated according to manufacturer's protocol and eluted in 50 µL RNase-free water. RT-PCR was performed on 1 µL of RNA sample to produce amplicons of approximately 1000 basepairs from each of the five primer pairs. Thermal cycling protocol was performed on a T100 Thermal Cycler (Bio-Rad) and consisted of a cDNA step at 55°C for 30 min and pre-denaturation at 98°C for 2 min. For amplification 40 cycles of denaturation 98°C for 15 s, annealing 55°C for 15 s, extension 72°C for 1 min, followed by one cycle 72°C for 5 min for final extension. The desired PCR fragments were selected by DNA gel extraction (1% agarose gel). These fragments were further purified by GeneJET Gel Extraction Kit (Thermo Fisher, K0692), according to manufacturer's protocol, and eluted in 50 µL elution buffer. Samples were sent to Macrogen for Sanger EZ sequencing with sequence primers. Snapgene version 8.0.3. was used to analyse the sequencing data. For data analysis, sequences were aligned with triple-plaque purified parental RSV-A2 and RSV-B1 sequences.

Next generation whole genome sequencing

Nucleic acid amplification

Whole genome sequencing of generated MARMs and global contemporary strains was performed using Illumina NovaSeq X Plus platform, adapted from previously described.[36] Following RSV subtyping, subtype-specific primer pairs were used to reverse transcribe and amplify four overlapping genome fragments using the SuperScript IV One-Step RT-PCR System (Invitrogen, CA, USA) in a 9800 Fast thermal cycler (Applied Biosystems). These four fragments collectively span the complete RSV genome, encompassing all viral genes. Primer sequences incorporated degenerate bases at positions of genetic variability between RSV-A and RSV-B strains where necessary.[36] The RT-PCR cycling conditions consisted of reverse transcription at 55 °C for 10 min and initial denaturation at 98 °C for 2 min, followed by 40 cycles of 98 °C for 10 s, 61 °C for 10 s, and 72 °C for 3 min.

Amplicons were verified by electrophoresis on 1% agarose gels, pooled in equimolar ratios, and purified using the GeneJet PCR Purification Kit (Thermo Fisher Scientific). Purified amplicons were quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

Library preparation and sequencing

Normalized PCR products were used for library preparation with the Nextera XT DNA Library Prep Kit (Illumina) following the manufacturer's protocol. Illumina sequencing adapters and unique barcodes were incorporated during PCR amplification of the fragmented DNA using custom oligonucleotide sequences (Integrated DNA Technologies). Tagged DNA was purified and size-selected using 0.6× volume of AMPure XP reagent (Beckman Coulter) according to the manufacturer's protocol. Purified libraries were quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific), pooled in equimolar amounts, and quality assessed using a 4200 TapeStation System (Agilent, USA). Sequencing was performed on either an Illumina NextSeq 500 platform generating paired-end 150 bp reads, or on MiSeq and NextSeq 2000 platforms and whole genome sequence was obtained as previously described.[36]

Viral population level bioinformatic processing and genome assembly

Raw sequencing reads (FASTQ files) underwent quality filtering using Trimmomatic v0.39, with a sliding window approach applied with a 10-base wide window, trimming when average quality dropped below Q30. Leading and trailing bases below Q30 were removed. This process generated paired and unpaired quality-filtered FASTQ files for downstream analysis. The reference sequence was indexed using SAMtools faidx[37] for subsequent mapping procedures. References used for RSV-A and RSV-B respectively, Sequences EPI_ISL_412866 (hRSV/A/England/397/2017) and EPI_ISL_1653999 (hRSV/B/Australia/VIC-RCH056/2019). Quality-filtered paired-end reads were mapped to the reference genome using Minimap2 v2.26 with the short-read alignment mode. Variant calling was performed using LoFreq.[38] Minor variants were filtered to retain only those with allele frequency (AF) $0.4<AF\geq0.05$ and sequencing depth (DP) ≥200 using BCFtools filter[37] and customized scripts with minMutFinder.[39] Variant annotation in variant call format (VCF) was performed using SnpEff with a custom-built database based on the reference genome[40] and GFF3 annotation file. Samples were aligned to reference sequences using Nextclade align, translated into amino acids, and annotated for mutation profiles using Nextclade v3.18.1.[41] To identify polymorphisms in the F protein, binding regions targeted by RSV mAbs were then screened for mutations. MARMs were named after the mAb used to generate the MARM and numbered (RSM for RSM01, NIR for nirsevimab, and PAL for palivizumab).

Prevalence of resistance-associated mutations and Shannon entropy

To assess the prevalence of observed resistance-associated mutations in circulating strains as well as genetic diversity within RSV-A and RSV-B F-protein sequences at mAb binding sites, RSV genomes deposited in GISAID between May 5, 2023, and December 1, 2025 (post-COVID period) were retrieved on December 12, 2025. Genomic sequences were aligned using Nextclade align and annotated using Nextclade v3.18.1,[41] yielding 5,037 RSV-A and 5,120 RSV-B F-gene sequences.

Shannon entropy (H) was calculated to quantify amino acid diversity at each position within the F protein.[42] Reference strains were excluded from the analysis, and aligned sequences were converted to a character matrix. The frequency of each amino acid at each position was determined, and Shannon entropy (H) was computed according to the following formula:

$H = -\Sigma(p_i \times \log_2(p_i))$

where p_i represents the frequency of amino acid i at a given position. Higher entropy values ($H > 0.1$) indicate greater amino acid variability[43] while lower values reflect conservation. Shannon entropy estimates for RSV-A and RSV-B were visualised using the Matplotlib library (Python).

Phylogenetic reconstruction

To reconstruct phylogenetic relationships among contemporary RSV clinical strains and assess the temporal dynamics of circulating lineages, we obtained complete, high-quality RSV genome sequences spanning 1950 to 2025 from the Nextstrain database[44] via Pathoplexus. Sequences were annotated using Nextclade v3.18.1,[41] yielding a dataset encompassing diverse genomic regions and clades. The final dataset comprised 1,686 RSV-A and 1,650 RSV-B genome sequences representing the seven geographic regions and was structured to capture temporal patterns across three epidemiological periods: pre-pandemic (before 2020), pandemic (2020–2022), and post-pandemic (2023–2025). The maximum-likelihood phylogenetic inference was performed with IQ-TREE v3.01 and branch support was assessed using an ultrafast bootstrap approximation (UFBoot with 1,000 replicates). Final phylogenetic trees with bootstrap support values were visualised using the ggtree R package.[45]

Structural modelling of RSM01-preF complex

The 3D structure of the RSM01 variable heavy and light chains in complex with the RSV F ectodomain was predicted from amino acid sequences in a 3:3:3 ratio using the AlphaFold 3 server accessed on January 23, 2026[46] with default parameters. The highest-confidence model was selected based on interface predicted TM (ipTM)-scores and predicted aligned error metrics. Structural models were visualized using UCSF ChimeraX (version 1.10).[47]

Ethical approval

The study protocols were approved by the Institutional Review Board at Hospital General de Niños Pedro de Elizalde, Argentina (approval number 12863) and the Human Research Ethics Committee at the University of Witwatersrand, South Africa (approval number M170966). The institutional research office of the University Medical Centre Utrecht (Object ID 23U-0590) provided a waiver for the STRAIN study as the Dutch Medical Research Involving Human Subjects Act was not applicable. Written informed consent was obtained from parents or guardians of patients before inclusion in the study.

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Data and materials availability

The genomic sequences generated during this study are available in the open-source Pathoplexus database (accession numbers RSV-A: PP_004HWQJ.1, PP_004HWRG.1, PP_004HWSE.1, PP_004HWTC.1, PP_004HWUA.1, PP_004HWV8.1, PP_004HWW6.1 and PP_004HWX4.1; RSV-B: PP_004HXG0.1, PP_004HXHX.1, PP_004HXJV.1, PP_004HXKT.1, PP_004HXLR.1, PP_004HXMP.1, PP_004HXNM.1, PP_004HXPK.1, PP_004HXQH.1, PP_004HXRF.1, PP_004HXSD.1, PP_004HXTB.1, PP_004HXU9.1, PP_004HXV7.1, PP_004HXW5.1, PP_004HXX3.1, PP_004HXY1.1, PP_004HXZZ.1).

Data sources files and scripts developed for this study are available in a dedicated Github repository: github.com/emanuelegustani/Next-Generation_RSV_Monoclonal-Antibody_with_high_barrier_to_resistance.git

Supplement

Supplementary files can be downloaded from:

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Part V

General discussion





Equity-by-design in RSV immunisation development

The World Health Organization (WHO) defines equity as "the absence of unfair, avoidable, or remediable differences among groups of people, whether those groups are defined socially, economically, demographically, geographically, or by other dimensions of inequality such as sex, gender, ethnicity, disability, or sexual orientation."[1] Achieving equitable impact of RSV immunisation requires that translational science moves beyond product development to a deliberate *equity-by-design* approach that begins with the end in mind: ensuring that innovations are affordable, accessible, acceptable, and adoptable where the burden is greatest. In this final chapter, I will discuss how the current *trickle-down innovation* perpetuates global inequity in RSV burden and access to immunisation, how we applied translational science principles to promote equitable RSV immunisation development, and how inclusion can serve as a foundation for *equity-by-design* in RSV immunisation development.

RSV as a global health and equity challenge

Respiratory syncytial virus (RSV) has gained increasing recognition over the past decade as a major global health concern, acknowledged by organisations such as the World Health Organization (WHO) and the Bill & Melinda Gates Foundation (BMGF). Respiratory infections remain among the leading causes of death in children under five years of age worldwide,[2] and RSV is the single largest contributor to respiratory infection–related mortality in the first year of life beyond the neonatal period.[3] Addressing RSV is therefore essential to achieve the United Nations (UN) Sustainable Development Goal (SDG) 3.2: ending preventable deaths in new-borns and children under five.[4]

Despite RSV being nearly universal (infecting almost all children by the age of two) the ability to survive an RSV infection is not. Known medical risk factors include young age (particularly < 6 months), prematurity, low birth weight, male sex, bronchopulmonary dysplasia, congenital heart disease, and other comorbidities that compromise the ability to control respiratory infections.[5] Yet beyond individual biology, the ability to survive RSV infection depends on access to timely and effective healthcare, specifically to respiratory support in an intensive care setting. This access remains deeply inequitable. More than 95% of RSV-associated hospital admissions and over 97% of the estimated 101 000 annual RSV-attributable deaths in children under five occur in low- and middle-income countries (LMICs).[6] These figures expose not only the disproportionate burden of disease but also the persistent inequities in healthcare infrastructure, diagnostic capacity, and preventive interventions.

This imbalance stands in stark contrast to the UN's guiding principle to Leave No One Behind (LNOB), embedded in both the Sustainable Development Cooperation Framework and the Immunization Agenda 2030, which explicitly calls for prioritising the needs of the most disadvantaged populations and tackling inequities both between and within countries.

RSV thus represents both a biomedical challenge and a social justice call. Reducing its global burden requires not only scientific innovation but also deliberate efforts to ensure that

innovations reach and are usable by the populations who need them most. Translational science, when applied with an equity lens, offers a framework to achieve this goal by addressing inequities throughout the entire research and implementation continuum.

The widening inequity gap: Trickle-down innovation

According to the LNOB framework, access to RSV immunisation (i.e. vaccines or monoclonal antibody (mAb) prophylaxis) should be greatest where the burden of disease and barriers to care are highest. In practice however, the opposite is true: access to immunisation has largely followed a *trickle-down innovation* pattern, in which technologies are developed, licensed, and implemented first in high-income countries (HICs), with delayed or uncertain availability in LMICs. This phenomenon is known as the *inverse equity hypothesis*, which predicts that when health innovations emerge, they are initially adopted by wealthy and connected segments of the population, thereby amplifying rather than reducing inequality.[7] In the case of RSV immunisation, *trickle-down innovation* has led to products that do not comply with WHO preferred product characteristics (e.g. single-dose vials rather than multi-dose),[8] are not tested in populations with prevalent comorbidities, and are neither affordable nor accessible in the majority of the world. The selective access to new immunisation tools further widens the RSV equity gap.

Since its registration in 1998, palivizumab has been available only to a limited number of high-risk children in high-income countries (HICs);[9] 99% of palivizumab sales are in the USA and Europe while 97% of RSV deaths are in LMICs.[10] Assuming a conservative efficacy of 50% for palivizumab against RSV-related deaths, this drug could have prevented approximately 1 million paediatric deaths in the past 20 years on the market if it had been globally accessible.[11] The past decade has seen an acceleration in RSV immunisation development, with more than 30 candidates in the pipeline [**chapter 2**].[12,13] Between 2022 and 2025, one maternal vaccine (RSVpreF) and two long-acting mAbs (nirsevimab and clesrovimab) received market authorisation.

The maternal vaccine RSVpreF, administered during pregnancy, provides passive protection to the infant from birth up to six months of age.[14] Its advantages include immediate protection from birth; however, vaccine efficacy might be reduced in cases of prematurity, malaria, or maternal hypergammaglobulinemia (e.g. due to HIV infection),[15] all of which are more prevalent in LMICs. The mAbs nirsevimab and clesrovimab, administered postnatally, offer protection during the whole RSV season after a single dose,[16-19] offering flexible timing relative to the RSV season and coverage for preterm infants. Yet their high production and thus high procurement costs, as well as the lack of protection between birth and administration, limit their feasibility for widespread use in LMICs.

Despite the disproportionally high disease burden in LMICs, access to new RSV immunization remains concentrated in HICs. Recent product launches have followed a historically familiar pattern of *inverse equity*: rapid approval and introduction in wealthy settings, with multi-

year delays before registration or rollout in LMICs (**Figure 1**). Besides availability, affordability is a key barrier to access,[10] as all currently available products come at prohibitive costs for most LMIC health systems [**chapter 3**]. Gavi, the Vaccine Alliance, has recently committed to including the maternal RSV vaccine in its portfolio, marking a significant milestone for equitable RSV immunisation. This decision reflects global prioritisation of RSV prevention in LMICs and has the potential to enable sustainable financing, coordinated procurement, and accelerated national introduction. By facilitating earlier and more affordable access to maternal RSV vaccination in high-burden settings, Gavi's commitment represents a critical step toward reducing global inequities in RSV-related morbidity and mortality. However, upper-middle-income countries (UMICs) - often facing high RSV burden but ineligible for Gavi support - must self-finance vaccine introduction, likely delaying implementation. Meanwhile, RSV mAbs remain largely inaccessible in LMICs, despite their potential advantages or complementarity with maternal vaccination strategies.

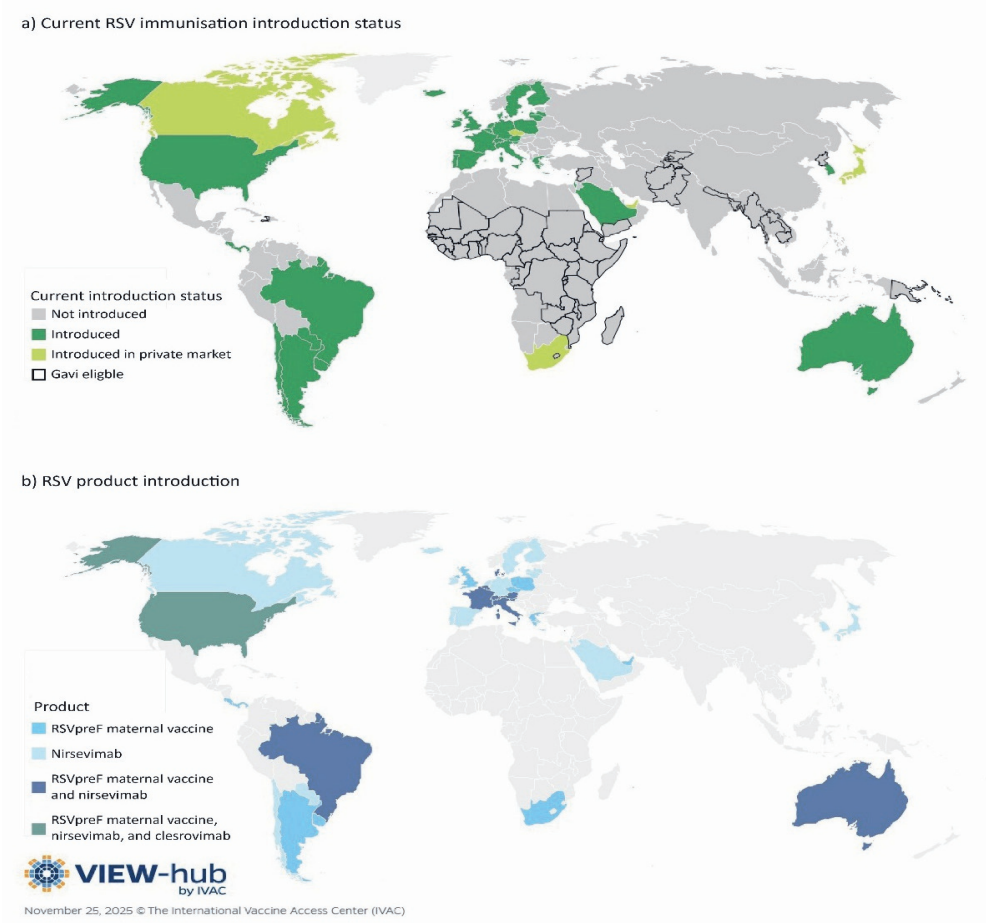
Consequently, RSV immunization is at risk of reinforcing rather than reducing global inequities in child health. The populations most affected by RSV morbidity and mortality remain the least likely to benefit from new biomedical advances. To counter this trend, a shift toward *equity-by-design* is required embedding affordability, local capacity strengthening, and end-user perspectives into each stage of RSV vaccine and mAb development. Translational science offers a practical framework to operationalise this shift by linking discovery, development, and implementation processes to the realities of the populations most in need.

Translational science as a framework for equitable RSV immunisation

To address these inequities, a more deliberate application of translational science principles is needed to ensure that innovations in RSV prevention move efficiently from discovery to delivery. Translational science provides a systematic framework to move scientific discoveries from the laboratory to real-world health impact. It translates observations and needs from the laboratory, clinic, and community into interventions that improve individual and public health: ranging from diagnostics and therapeutics to medical procedures and behavioural strategies.[21] This field generates scientific, operational, financial, and administrative innovations that streamline research processes, making them faster, more efficient, and more impactful (**Box 1**).[21]

In the context of global health, translational science for equity integrates principles of implementation science, health systems research, and social science into every stage of product development and evaluation. This means identifying barriers and facilitators to adoption early in the research process and designing technologies, interventions, and immunisation strategies that remain feasible and impactful within low-resource environments. In this view, equity is not an outcome added after innovation is complete; it is a guiding design parameter embedded throughout the translational spectrum.

Figure 1. Global introduction of RSV immunisation products to protect infants



Three RSV immunisation products, RSVpreF maternal vaccine, nirsevimab, and clesrovimab, have been authorised for use globally since their market approvals from 2022 onwards. **a)** Global RSV immunisation introduction status. As of 25 November 2025, one or more products were introduced in 41 countries, of which four UMICs, zero LMICs, and none of the 54 Gavi-eligible countries (black border). Six of the 41 introductions were in private market, while the other 35 countries introduced RSV products into national immunisation programs. **b)** Global RSV product introduction. Twelve countries introduced more than one product, 11 introduced RSVpreF maternal vaccine, while 18 introduced nirsevimab. Gavi, the Vaccine Alliance; LMICs, low and lower-middle income countries; RSV, respiratory syncytial virus; RSVpreF, respiratory syncytial virus prefusion protein; UMICs, upper-middle income countries. Source: International Vaccine Access Center[20]

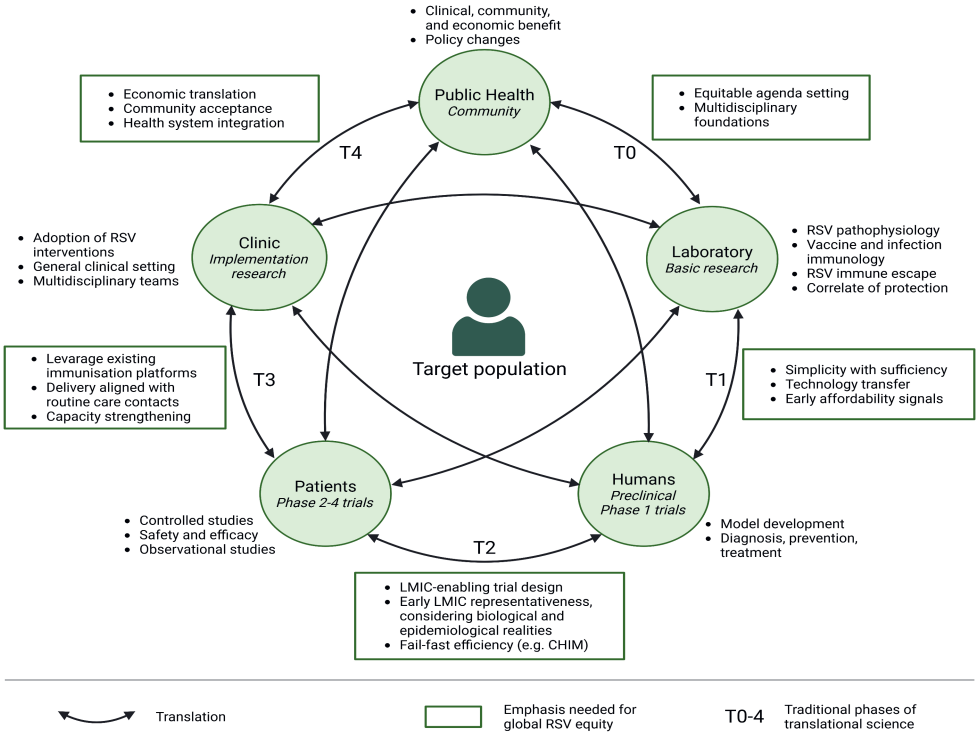
Box 1: Translational science principles

- Prioritise unmet needs: focus on pursuing scientific goals that address unmet scientific needs (e.g. research advances in under-investigated areas) or patient and population health needs.
- Generalisable solutions: innovations should address common and persistent challenges. Approaches may span across multiple projects or initiatives, across diseases or conditions, or target organisational policies, structures, and shared resources.
- Creativity and innovation: leveraging creativity and innovations in research design and implementation, research processes and structures, and the organisational environment can increase the impact of the research.
- Cross-disciplinary team science: Teams with expertise across disciplines, fields, and professions produce research that advances across the translational research continuum.
- Efficiency and speed: implementation of evidence-informed practices and scientific or operational innovations accelerate the pace of translational research.
- Cross-sector partnerships: collaborations across agencies and sectors as well as engagements with patients and communities in research advance the translational progress.
- Bold and rigorous approaches: ambitious research questions addressed with rigorous and robust methods generating reproducible findings contribute to advancing translation.

Adapted from the translational science principles as defined by the National Centre for Advancing Translational Sciences (NCATS).[22]

This approach reframes the translational research spectrum comprising five interrelated stages, each informing and strengthening the others (**Figure 2).** In the following sections, I discuss how we applied these principles across different phases of the translational spectrum. Together, these examples highlight how translational science, when guided by equity, can shorten the distance between discovery and real-world impact and ensure that the benefits of RSV immunisation extend to all populations, not just those with the most resources.

Figure 2. Translational science spectrum in global RSV immunisation equity to protect all infants



The translational science spectrum illustrates the interconnected stages of translational research, ranging from basic discovery (T0) to population-level health impact (T4). These stages are not linear; rather, they form a dynamic, bidirectional system in which insights generated at any point can inform earlier or later phases of development. Patient and community involvement is foundational across all stages, shaping research questions, feasibility, acceptability, and real-world implementation. In this thesis, the spectrum is adapted to highlight key opportunities to embed RSV *equity-by-design*, shown in the green boxes. These elements are critical decision points where early attention can prevent downstream inequities in access, affordability, and effectiveness. Emphasising equity across the spectrum underscores that global health impact cannot be achieved through scientific innovation alone, but requires contextual alignment with the needs, capacities, and priorities of populations in LMICs. This adapted spectrum also illustrates the iterative relationship between laboratory science, clinical research, implementation studies, and policy translation. By integrating equity considerations at each stage, the spectrum promotes a translational pathway in which innovations are designed from the outset to be feasible, acceptable, and adoptable in high-burden settings. The figure was made in Biorender and adapted from the National Center for Advancing Translational Sciences.[23] CHIM, controlled human infection model; LMIC. Low and middle income countries; RSV, respiratory syncytial virus.

The value of simplicity

Translational laboratory sciences is often viewed as separate from global health research, as the majority of high-end laboratories are located in HICs. Complex technologies, while powerful in high-resource settings, often create barriers to participation and adoption in LMICs. Using simplicity as a guiding principle in experimental laboratory sciences aligns with early phases of the translational science spectrum (T0-T2), where feasibility, adaptability, and knowledge

transferability are key. Approaches such as dried blood spots, lean-and-mean neutralisation assays, and robust technology transfer can enable resource-limited settings to participate meaningfully in research and implementation.

In **chapter 5** of this thesis, we adapt an RSV neutralisation assay for the use of dried blood rather than conventional serum to measure RSV neutralizing antibodies. The adaptation prioritised simplicity over maximal precision to allow easier sampling and measurement in resource-limited settings while maintaining sufficient analytical accuracy for clinical and epidemiological purposes. Dried blood reduces logistical and patient burden in clinical trials, particularly for infants in LMICs and can replace serum in pharmacokinetic, viral neutralisation, and immunogenicity analysis [**chapter 6**], enabling further mAb development in LMIC settings.

To support a technology transfer [**chapter 7**], we used phosphate-buffered saline (PBS) as a simple elution medium and implemented a high-throughput live-virus fluorescence assay to minimise equipment and reagent needs. Our PBS-only method was considered noninferior to the resource-heavy validated method for recovery of RSV PreF-binding antibody. This trade-off between simplicity and precision reflects a broader translational principle: excessive methodological refinement can hinder adoption in resource-limited settings, whereas simpler methods that maintain adequate validity and reliability can achieve broader implementation and real-world impact. In this sense, translational value lies not in maximising precision, but in optimising fitness-for-purpose.

By intentionally lowering technical and logistical barriers, simplicity becomes a driver of equity, which allows LMICs to be more easily included in the global RSV research field. Equitable translational science begins in the laboratory (T0-T1); not only once market approval is granted.

Development of affordable RSV immunisation products

Traditional *trickle-down* mAb development has predominantly targeted HIC markets, resulting in products with high production costs and limited affordability for LMICs. Palivizumab, the first licensed RSV mAb, exemplifies this inequity: despite proven efficacy, its cost has restricted use almost exclusively to HICs. Nirsevimab, although more cost-effective than palivizumab, is still prohibitively expensive for most countries.[24] This pattern exemplifies how conventional *trickle-down* research and development (R&D) pipelines contribute to the well-known "90-10 gap", wherein the majority of global health research investment benefit a minority of the population.[11]

To redress this imbalance, RSV mAb development must adopt an *equity-by-design* approach, in which affordability and accessibility for LMICs are integrated from the earliest stages of product conception (T0-T1). RSM01, an RSV prefusion site Ø monoclonal antibody (mAb) developed by the Gates Medical Research Institute, embodies this paradigm shift. RSM01 was designed with LMIC populations in mind: PK studies were modelled on African infant populations to predict the fraction of infants with serum RSM01 concentrations above the

estimated threshold of protection [**publication not included in this thesis**];[25] dried blood rather than serum was used to facilitate PK and immunogenicity assessments in resource-limited settings [**chapter 6**]; immune escape risk profiling focused on contemporary LMIC strains [**chapter 10**]; and development was guided by a commitment to achieve vaccine-like pricing.[26] Next steps in RSM01 development (i.e. T2, phase 2 and 3 trials) would ideally be conducted in the target population, namely LMICs, with sample analysis performed in Ghana [**chapter 6**].

Our investigator-initiated research on intranasal palivizumab [**chapter 8-9**] further illustrates affordability-focused innovation within the translational spectrum using two principles: (1) the innovation was designed with affordability and feasibility as its core challenge and (2) we applied a fail-fast trial design, which saves resources. First, by targeting the primary site of RSV infection, the nasal mucosa, this strategy aimed to achieve protective efficacy at lower concentrations, thereby reducing production costs and enabling child-friendly administration. Stability testing under conditions reflective of LMIC settings (4°C, ambient, and 37°C) further underscored the commitment to implementation feasibility. Second, following the phase 2b trial's lack of efficacy [**chapter 8**], we conducted a controlled human infection model (CHIM) to identify potential causes for lack of efficacy before further investment. CHIMs embody the translational science principles (**box 1**) by enabling bold, efficient, and early hypothesis testing, allowing ineffective candidates to fail fast,[27] avoiding prolonged and costly clinical development. Despite encouraging preclinical data, our CHIM trial demonstrated that intranasal mAbs are unlikely to be an effective prevention strategy [**chapter 9**]. Ideally, both the phase 2b and CHIM trials would have included the target LMIC population, as immune responses, efficacy, and safety profiles may differ substantially across geographical and demographic contexts (see ethnic sensitivity in timely representative trials).

Together, these examples demonstrate a paradigm shift from *trickle-down innovation* toward inclusive innovation. By integrating specific LMIC needs and contexts into the earliest research stages (T0–T2), *equity-by-design* transforms translational science into a tool for structural change rather than post hoc adaptation.[28]

Begin with the end in mind

Ultimately, the goal of immunisation is to protect people. Yet vaccines cannot achieve impact if they fail to achieve acceptance among communities, healthcare workers, or governments. Translational science therefore extends beyond the laboratory bench and clinical trials to encompass the systems, behaviours, and perceptions that determine real-world uptake. Within the translational spectrum, this corresponds to the later stages (T3–T4), where innovations are translated into practice, policy, and population-level benefit.

Vaccine uptake is influenced by multiple determinants (**Figure 3**), including social and personal processes as well as practical issues. Research that seeks to *begin with the end in mind* must therefore address not only biomedical efficacy but also implementation readiness

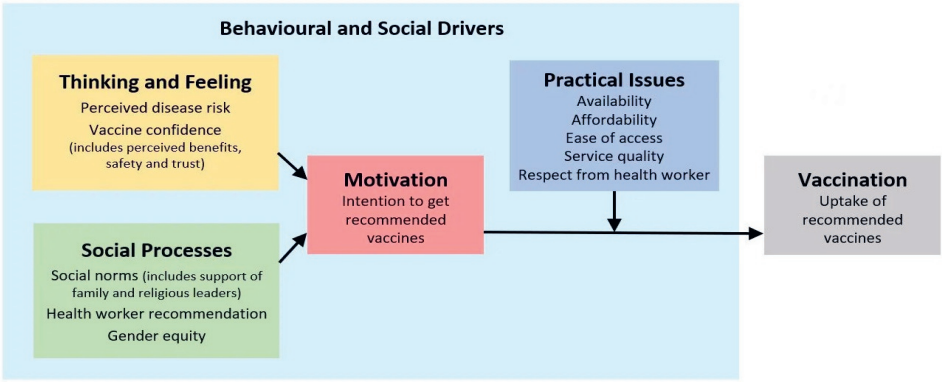
and community alignment. Understanding local disease awareness, risk perception, health system access, equitable uptake, determinants of hesitancy, interventions to promote vaccine confidence,[29] and cultural narratives surrounding vaccination[30-33] is essential to ensure that immunisation strategies are not only available but also acceptable and adoptable.

Therefore, immunisation development should integrate qualitative and interdisciplinary research components, engaging social scientists, anthropologists, and behavioural researchers alongside laboratory and clinical investigators. This approach, applied in Maternal Immunisation Readiness Network in Africa and Asia (MIRNA), provides a more holistic understanding of vaccine acceptance and can identify barriers long before implementation begins [**chapter 4**]. Furthermore, stakeholder mapping and participatory research within vaccine development ensures that the perspectives of policymakers, healthcare workers, and communities inform product design and implementation strategies. Implementation and operational research on perceived needs, benefits, and risks can directly guide policy decisions and shorten the pathway from scientific discovery to equitable public health impact.

Beginning with the end in mind thus represents equity-focused translational science. The *inverse equity effect*[7] of RSV immunisation is predictable but not inevitable: by integrating contextual, behavioural, and operational considerations into the research pipeline, scientific innovation becomes more responsive and resilient. Rather than relying on downstream adaptation, this approach anticipates the sociotechnical systems within which an intervention will operate, ensuring that the path from discovery to delivery is both efficient and equitable.

Together, these lessons demonstrate that equitable RSV immunisation cannot be achieved through scientific innovation alone. The value of simplicity highlights the need for scientific co-ownership by ensuring that methods, assays, and product designs reflect the realities of diverse settings. The development of affordable products illustrates the centrality of economic viability, showing how early decisions on formulation, dosing, and manufacturing determine long-term accessibility. Finally, beginning with the end in mind reinforces the importance of social integration and operational investment, emphasising that uptake, acceptability, and health-system readiness must be integrated from the outset. The insights from this thesis directly inform the following recommendations.

Figure 3. Behavioural and Social Drivers (BeSD) of Vaccination framework



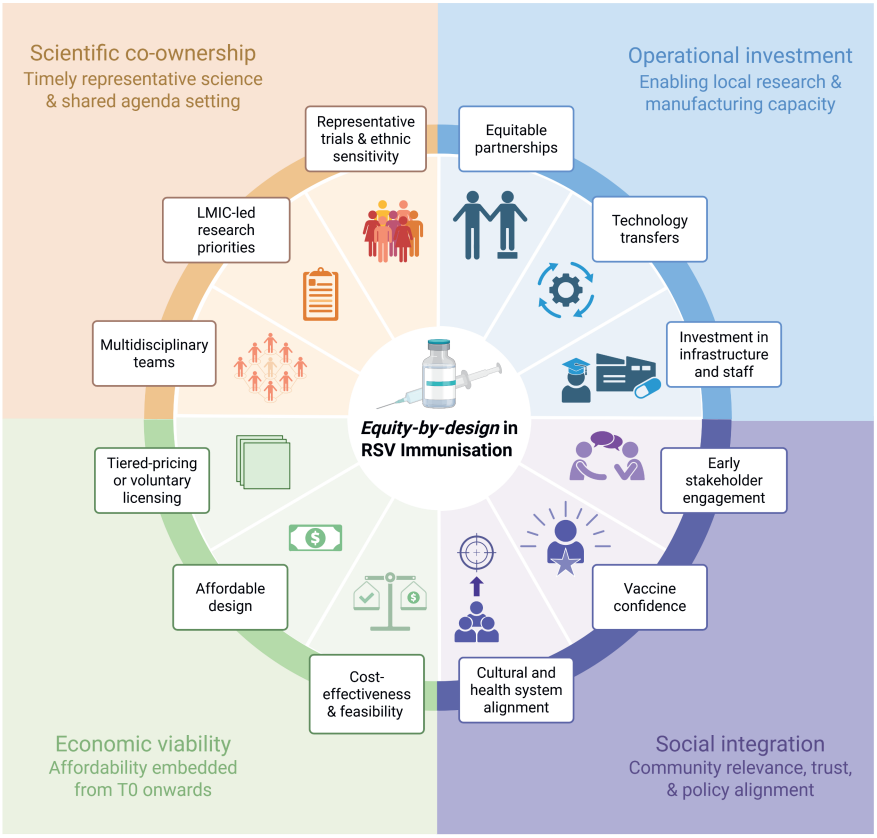
Source: The BeSD working group, World Health Organisation, 2018

Recommendations for *equity-by-design* in RSV immunisation development

Achieving *equity-by-design* in RSV immunisation development requires reframing how we apply translational science to a global health context. Equity cannot emerge from downstream adaptation alone; it must be intentionally embedded in every stage of discovery, development, and implementation. Deliberate integration and empowerment of LMICs across the translational science spectrum ensure that the prioritised research questions reflect community health needs and that innovations can be readily translated into practice, policy, and population-level benefit. Ideally, global health innovation need to move beyond inclusion towards agency - where LMIC researchers, institutions, and communities shape agendas, lead studies, and own solutions. However, given current global disparities in power and resources, meaningful inclusion remains an essential and pragmatic step toward that agency.

Equity must therefore be embedded from the earliest laboratory stages (T0) through clinical development (T1-3) and implementation research (T4), ensuring that innovations are not only scientifically sound but also feasible, affordable, and acceptable in high-burden contexts. Drawing directly from the lessons outlined above, the following recommendations reframe the translational science spectrum around four domains of inclusion that operationalise equity-by-design: scientific co-ownership, operational investment, economic viability, and social integration (**Figure 4**).

Figure 4. Recommendations for *equity-by-design* in RSV immunisation development



Four interdependent domains are required to embed equity-by-design across the RSV immunisation development pathway: scientific co-ownership, operational investment, economic viability, and social integration. The circular layout emphasises that equity is not a linear endpoint but a continuous, iterative process operating across the full translational science spectrum (T0–T4), with feedback between stages. Scientific co-ownership refers to designing research that reflects the genetic, epidemiological, and environmental diversity of global populations while enabling researchers and institutions in LMICs to shape research agendas. This includes early multiregional inclusion in preclinical and clinical studies, population pharmacokinetic approaches to anticipate ethnic sensitivity, and multidisciplinary teams integrating clinical, laboratory, epidemiological, and social science expertise. Operational investment highlights the need for sustained strengthening of local research ecosystems through laboratory infrastructure, regulatory capacity-building, and technology transfer, enabling equitable trial participation and long-term scientific autonomy in line with global strategies such as the WHO Immunization Agenda 2030. Economic viability requires affordability to be addressed from the earliest scientific decisions, including antigen and manufacturing platform selection, formulation stability, and dose-sparing trial designs, complemented by later-phase cost-effectiveness and feasibility assessments to inform equitable pricing and access models. Social integration recognises that population-level impact depends on community trust and policy alignment; engagement with caregivers, healthcare workers, and policymakers, supported by social science methods, ensures acceptability and feasibility within existing health systems. This framework provides a structured approach for embedding equity throughout RSV vaccine and monoclonal antibody development and is applicable to global health innovation more broadly. Figure created with BioRender. LMIC, low- and middle-income countries; WHO, World Health Organisation.

Scientific co-ownership

Scientific co-ownership entails that studies and trials are designed to reflect genetic, epidemiological, and environmental diversity of global populations as well as letting LMICs set the research agenda. As little as 1.2% of global health research funding in 2020 was awarded to LMICs.[34] This thesis provides several examples of how scientific co-ownership can be integrated into translational work. The simplicity-driven assay adaptations in **chapter 5**, which facilitated the technology transfer to Ghana in **chapter 6** directly support LMIC leadership in immunogenicity and PK assessment of future clinical trials and correlate of protection studies. Likewise, the viral strain analyses in **chapter 10** demonstrate how candidate characterisation must reflect contemporary LMIC viral diversity to avoid the Global north–centric bias embedded in *trickle-down innovation* pathways.

Early inclusion of LMIC populations in preclinical and clinical studies allows assessment of immune responses, efficacy, and safety in representative contexts, reducing the need for post-licensure bridging trials. It also ensures that correlates of protection and preferred product characteristics are globally relevant rather than derived solely from HIC populations. To ensure context-specific relevance, multidisciplinary research teams are needed.

Ethnic sensitivity in timely representative trials

The majority of trial data of the approved RSV immunisation comes from HICs: phase 2/3 and phase 3 trials for RSV immunisation in the last five years were run in only 12% of the 113 LMICs included in the 2024 classification and most of those were UMICs.[35] Drug development programs initiated in Europe or North-America have traditionally expanded to include LMIC populations at later developmental phases. Of the new molecular entities approved between 2014 and 2019 by the US Food and Drug Administration (FDA), 10% showed a difference in exposure and/or response based on race/ethnicity or pharmacogenetic factors known to vary in frequency across global populations.[36] For the approvals between 2008 and 2013, this difference was 21%.[36] Human clearance mechanisms and global pharmacogenetic variation emphasise the need for ethnic sensitivity in trial design.[37] Timely participation in multiregional clinical trials is vital to decreasing the risk of late-phase trial failures or the delay in regional approval of drugs (i.e., country-level drug lags), thereby decreasing the associated delay in access to innovative therapies (i.e., access lag).[37] Population PK models, as used in the RSM01 phase 1 trial analysis [**publication not in this thesis**],[25] can be useful in early drug-development to forecast PK well in advance of clinical data availability to get an early read on level of risk for ethnic sensitivity.[37]

Besides ethnic sensitivity in the context of PK, timely representative trials are needed to increase generalisability of trial results. Infants are at higher risk of severe RSV infection when born prematurely5 or exposed to HIV[38], yet mothers with HIV or a history of premature labour were excluded from RSV maternal immunisation trials. Timely inclusion of these prevalent populations into clinical trials prevents late bridging trials and further delays to immunisation access.

The study in **chapter 8** was intended to take place in an LMIC to ensure early inclusion of diverse populations, but the lack of an established LMIC collaborator made this unfeasible. This limitation further underscores the structural barriers to equitable collaboration and highlights why deliberate investment in long-term and mutually beneficial partnerships is essential for achieving truly representative translational research.

Multidisciplinary translational science teams

For RSV immunisation, contextual relevance depends on understanding not only viral biology and immune mechanisms, but also the social, behavioural, and health system factors that determine real-world effectiveness. Multidisciplinary research teams that integrate clinicians, laboratory scientists, social scientists, epidemiologists, and implementation experts are therefore essential to designing innovations that are both scientifically rigorous and socially meaningful. Such collaboration ensures that research questions address local health priorities, study protocols are feasible within existing health systems, and end-user perspectives are considered early in the design process. This approach is exemplified in the MIRNA study described in **chapter 4**, where qualitative research and vaccine-readiness assessments informed the interpretation of immunisation strategies beyond clinical efficacy alone.

To institutionalise this, funding bodies and research institutions should explicitly require and incentivise multidisciplinary team structures as part of grant criteria and trial design. Multidisciplinary stewardship not only strengthens the scientific rationale for new RSV interventions, but also establishes pathways for LMIC agency—an essential step toward a more equitable translational science ecosystem.

Operational investment

The WHO immunisation agenda 2030, a global strategy to leave no one behind, lists needs-based innovations and local capacity strengthening as its research priorities to increase the reach and impact of immunisation programs to all countries and communities.[39] Local capacity strengthening includes investments in laboratory infrastructure, training of scientists and regulatory staff, and early planning for technology transfer and local manufacturing. Equitable transnational partnerships may help overcome the structural and economic challenges faced by LMIC researchers in strengthening (adaptive) clinical trial platforms.[40] The importance of operational investment is reflected in several components of this thesis. **Chapter 6** illustrates this most clearly through the technology transfer to Ghana, where early strengthening of laboratory capacity and personnel enabled locally conducted analyses and increased scientific autonomy. Additional scholarly visits to South Africa, Ghana, and Bangladesh further demonstrate how informal knowledge exchange contributes to capacity strengthening through day-to-day collaboration, shared problem-solving, and mutual learning. By contrast, the studies envisioned in **chapters 8** and **9** could not be conducted in LMIC settings due to insufficient infrastructure and the absence of a research partner,

underscoring how gaps in operational capacity directly impede equitable participation in early translational research. Collectively, these experiences highlight the necessity of embedding sustained capacity-building within translational science partnerships not only to facilitate equitable trial participation, but also long-term scientific autonomy in LMICs.

Economic viability

Economic viability demands that affordability is built into vaccine and mAb development from the earliest translational stages. Translational science can be used strategically to identify and control cost drivers throughout the research pipeline, transforming affordability from a late-stage pricing decision into a guiding principle of R&D. At early development stages (T0-2), scientific and manufacturing choices can include antigen or epitope selection, expression systems, purification strategies, and formulation stability determine production scalability and cost per dose, as well as dose-sparing trial designs, minimising the amount of antibody required while maintaining efficacy. Affordability as a design principle is illustrated throughout the development of the RSM01 mAb [**chapters 5–7** and **10**], where early choices regarding product selection, manufacturing platforms, and PK-informed dose optimisation were explicitly oriented toward achieving vaccine-like pricing. A similar affordability lens guided the intranasal palivizumab programme [**chapters 8–9**], where the hypothesis of increased local protective efficacy was equally important as the possibility to reduce the dose and thus costs. The use of a CHIM allowed the programme to fail fast, which also saved valuable resources and time.

At later stages (T3-4), operational translation ensures that economic inclusion extends beyond production to implementation. Cost-effectiveness studies, feasibility assessments, and local manufacturing partnerships can guide tiered-pricing, technology transfer, biosimilar development, or voluntary licensing models [**chapter 3**].[41,42] Together, these examples show how translational science can be used to identify and manage cost drivers across T0–T4, ensuring that economic viability is built into product design, development, and implementation from the outset.

Social integration

HICs have generated the bulk of research data on RSV immunisation using large grants, while LMICs are left to implement vaccines that may not be contextually optimal without sufficient resources. Social integration recognises that scientific and economic advances will not translate into public health impact without community trust and policy alignment. Effective engagement with local stakeholders (patients, caregivers, healthcare workers, and policymakers) helps tailor communication, identify barriers to uptake, and integrate interventions within existing health systems. Collaboration with social scientists and implementation researchers can ensure cultural relevance, strengthen vaccine confidence, and inform evidence-based adoption strategies. The importance of social integration is embraced in the MIRNA study [**chapter 4**], where co-creation with healthcare providers and

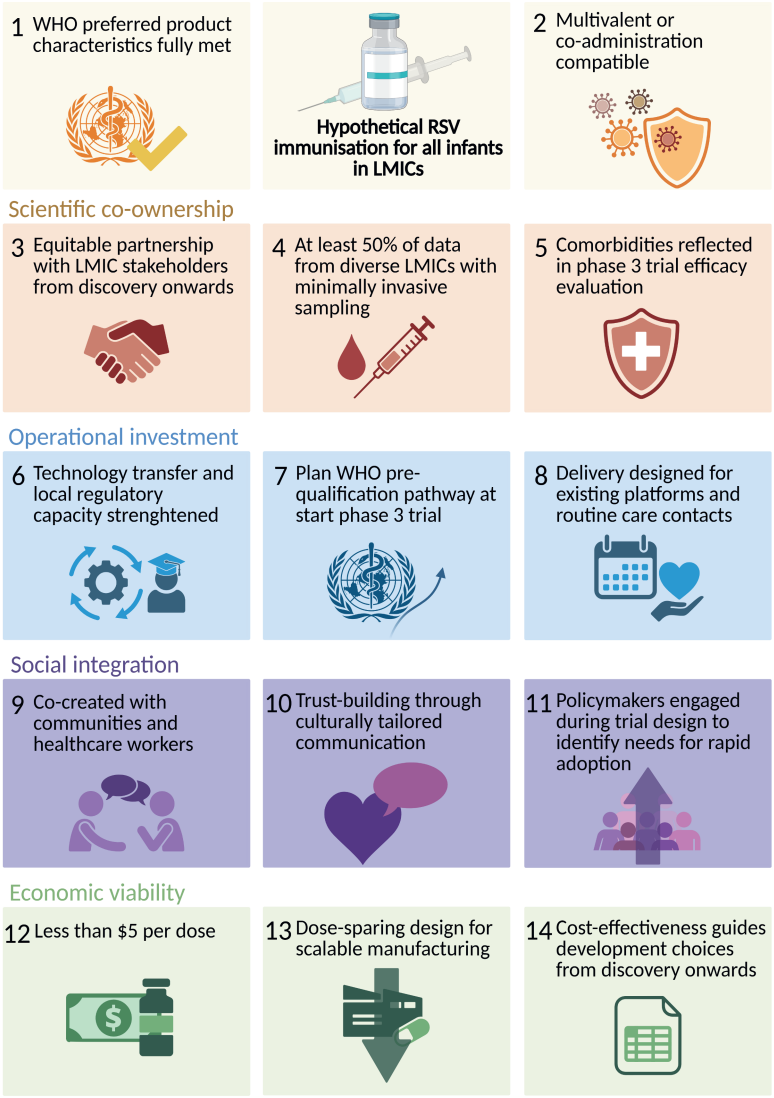
community stakeholders informed context-specific approaches to improve maternal RSV vaccine uptake, demonstrating how social integration strengthens both vaccine confidence and adoption pathways.

Embedding scientific, operational, economic, and social inclusion throughout the translational science process enables an *equity-by-design* approach. The four domains represent a progressive pathway toward LMIC agency. Rather than a reactive model of adaptation, *equity-by-design* ensures that RSV immunisation products are co-created with and for the populations who bear the greatest burden of disease to ensure they are among the first to benefit from vaccines.

Building on these lessons and recommendations, **figure 5** translates the abstract principles of equity-by-design into a concrete, illustrative example of a hypothetical RSV immunisation product developed explicitly with global RSV health equity in mind. Drawing on the WHO preferred product characteristics,[43,44] WHO paediatric drug optimisation for RSV,[45] and the translational choices explored throughout this thesis, the example demonstrates how equity considerations can be embedded across the translational science spectrum. While conceptual, it highlights how the work presented in this thesis contributes to operationalising these principles and shows how deliberate design choices can shape real-world impact.

If implemented, an *equity-by-design* RSV immunisation strategy has the potential to substantially transform RSV prevention in LMICs. By embedding affordability, feasibility, and contextual relevance from the outset, such an approach would enable equitable and timely access to RSV protection, reducing delays between product availability in high-income countries and uptake in high-burden settings. Widespread implementation could prevent a large proportion of severe RSV infections and hospitalisations, thereby lowering infant morbidity and mortality and alleviating pressure on already constrained health systems. Alignment with existing maternal and infant immunisation platforms would support efficient rollout with minimal disruption to routine services and fewer missed opportunities for immunisation. Affordable pricing and early cost-effectiveness considerations would enhance financial sustainability for national immunisation programmes and facilitate rapid scale-up. In parallel, deliberate attention to community trust, acceptability, and health-system fit is expected to increase vaccine confidence and uptake, accelerating translation to population-level impact. Finally, integration of local trial participation and technology transfer would strengthen scientific and laboratory capacity, contributing to sustainable protection through scalable, long-acting products that are compatible with LMIC supply chains.

Figure 5. A hypothetical RSV immunisation product designed according to equity-by-design principles



The 14 tiles represent key *equity-by-design* requirements that a hypothetical RSV immunisation product should fulfill to achieve equitable RSV immunisation. Tiles are colour-coded across four interdependent domains: scientific co-ownership (orange), operational investment (blue), economic viability (green), and social integration (purple), highlighting that equity must be embedded throughout the translational science spectrum. Figure was created using Biorender.

Personal reflections

The span of my PhD (2021-2026) was an exciting time in the RSV vaccine development: in 2022 two novel preventative interventions were market-approved and gradually implemented into national immunisation programs of rich countries. Born, raised, and educated in a high-income setting, I am painfully aware of my position of power and privilege, and I feel a need to contribute to more equitable access to RSV prevention to help reduce any social injustice. I have had life-changing experiences during various research projects in lower resource settings with inspiring local scientists and physicians that simply keep going in their battle to protect young babies from deadly infections. It was a relief to hear in 2025 that Gavi will support RSV immunisation in poorer countries but the access delays and unsustainability of this charitable system still require work. It is heart-breaking to realize that equitable RSV immunisation would have saved one life every five minutes, along with the prevention of 2.7 million severe RSV infections per year; yet this also underscores that a deliberate shift toward *equity-by-design* offers a tangible opportunity to change this trajectory in the years ahead.

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Appendix

English summary

Rationale behind this thesis

Respiratory syncytial virus (RSV) is a leading cause of acute respiratory infections (ARI). Nearly all children are infected with RSV at least once by the age of 2 years, and reinfections occur commonly throughout life. Most infections cause mild upper respiratory tract infection symptoms, commonly referred to as the "common cold". Nonetheless, RSV can cause severe, potentially life-threatening lower respiratory disease in infants, particularly in premature babies and those with comorbidities. If there is a lack of access to adequate healthcare, such as can be the case in low- and lower-middle income countries (LMICs), the outcome can be fatal.

Until recently, palivizumab, a short-acting monoclonal antibody (mAb), had been the only licensed product to prevent RSV infections, with its use limited to high-risk infants due to high costs. In 2022, nirsevimab became the first long-acting mAb approved for RSV prevention for all infants, shortly followed by the first-ever maternal RSV vaccine. In 2025, clesrovimab, another long-acting mAb, was approved for infants. The world health organisation (WHO) has recommended all countries to introduce prevention against RSV either through the maternal vaccine or through mAbs. Countries worldwide are implementing these preventive interventions in their national immunisation programmes. However, implementation is delayed in LMICs due to several logistical and financial barriers as well as the need for additional safety and effectiveness data.

The principle aim of this thesis is to use a multidisciplinary approach to reduce inequitable access to RSV immunisation for infants in LMICs. Five research types on the translational science spectrum were used in the pursuit of global RSV equity:

1. Public health research: to provide an overview of the RSV vaccine pipeline and outline the road to global access
2. Basic research: to develop an assay to measure RSV neutralizing antibodies in dried blood
3. Preclinical research: to evaluate mAb resistant RSV mutants
4. Clinical research: to evaluate novel affordable protective strategies against RSV infection
5. Implementation research: to assess readiness for implementation of maternal immunisation

Findings of this thesis

Over the course of this PhD research (2021-2025), the field of RSV prevention witnessed major progress. With our review in **chapter 2**, we aimed to provide a comprehensive overview of the current landscape of RSV vaccines and mAbs, and first real-world results following immunisation rollout. We conducted an extensive literature review, supplemented with insights from the RSV Vaccines for the World Conference 2024. At the time of writing (June 2024), five RSV immunisation products had reached the market, with 30 more still in development. Following approval, several countries implemented RSV immunisation,

showing promising real-world results including over 80% effectiveness of nirsevimab against RSV-associated hospitalisation. However, access remains limited to high- and upper-middle-income countries, despite the greatest burden of life-threatening disease predominantly existing in LMICs. This inequitable distribution delays vaccine access for those who need it most. Our review outlines urgent next steps to close this "vaccine gap". A critical first step was the World Health Organisation's Strategic Advisory Group of Experts (SAGE) recommendation in September 2024, urging all countries to consider RSV immunisation for infants. However, significant challenges to vaccine access in LMICs remain, including high costs, low RSV awareness, and competing immunisation priorities. To close the global RSV vaccine gap, addressing these barriers should remain an urgent priority shared by multiple stakeholders. In **chapter 3** we propose that public-health-oriented access-to-medicine mechanisms (such as voluntary licensing and technology transfers to local manufacturers) could ensure at-scale access to affordable RSV mAbs in LMICs. The WHO recommendation facilitates the development of a regulatory pathway through WHO prequalification, which might be necessary for biosimilar manufacturers. Supporting regulatory coordination may speed up approval procedures, especially if accompanied by technology transfer maximizing similarity with the originator product. Public sector finance and procurement mechanisms, such as Gavi, the Vaccine Alliance, could play a crucial role in facilitating access and encouraging the adoption of biosimilar products in LMICs that benefit from Gavi funding.

Immunisation development is not finished when registration is completed. Ideally implementation would already be considered during development to be ready for roll-out without any delay after registration. In **chapter 4**, we establish the barriers and facilitators to maternal immunisation in Soweto, South Africa, as experienced by local health care professionals (HCPs) and community representatives. During co-creation workshops, HCPs recommend solutions to the perceived barriers to locally improve maternal immunisation supply and demand. Recommendations included: HCP education on current and new maternal vaccines through in-service training or micro-education online; raised community awareness on vaccine preventable diseases through community outreach in collaboration with community leaders and traditional healers; incentivisation of HCPs and clinics to provide maternal immunisation by regional or national reporting; and improved documentation in updated maternal case records, or ideally roll-out of electronic patient dossiers, to nudge HCPs to give the right vaccine at the right time and ease referrals between professionals.

RSM01, a novel RSV mAb developed by Gates Medical Research Institute for LMICs, was tested in a Phase 1 trial in healthy adults. The key correlate of protection of RSV vaccines and mAbs is virus neutralisation, measured via sera obtained through venipuncture. Dried blood obtained with a finger prick can simplify acquisition, processing, storage, and transport in trials and thereby reduce costs. We developed an RSV neutralisation assay using dried blood samples in **chapter 5** to measure the amount if neutralising antibodies (nAb) after RSM01 administration in the Phase 1 trial. In **chapter 6** we showed that dried blood can replace serum sampling for pharmacokinetic, neutralisation, and immunogenicity bioanalysis (R2

> 0.95). Dried blood sampling could reduce patient burden in clinical trials, particularly for infants in LMICs. We successfully transferred the RSV neutralisation assay to a Gavi-eligible country, thereby strengthening the laboratory research capacity in **chapter 7**. Reliable measurement of RSV nAbs in a Gavi-eligible country and the use of dried blood can contribute to inclusion of LMICs in RSV vaccine development and access.

Chapters 8 and **9** describe the industry-independent development of palivizumab nose-drops for infants. Mucosal administration of mAbs against respiratory pathogens is a promising alternative for systemic administration because lower doses are required for protection. In chapter 8, we investigate the safety and efficacy of daily intranasal mAb prophylaxis with palivizumab on RSV infection in a phase 1 (healthy adults) and phase 2b (healthy preterm infants) randomised controlled trial. Daily palivizumab nose-drops were safe but could not prevent RSV infection in late preterm infants (palivizumab 38.3% vs placebo 23.4%; aOR 2.2, 95% CI 0.7-6.5). There are multiple possible reasons why this phase 2b trial did not show efficacy: short half-life, low virus circulation, lack of virus exposure, or potential low drug adherence, which are inherent to the trial design. A controlled human infection model (CHIM) provides an unparalleled study design to assess efficacy in a controlled setting with fixed dosing, adequate viral inoculation, controlled viral exposure, and fixed timing between medication and viral inoculation. In a double-blind, randomised, placebo-controlled CHIM in healthy adults, we show that intranasal palivizumab has no effect on RSV viral load despite a 72% reduction in symptomatic infections (**chapter 9**). Intranasal mAbs are highly unlikely to be a feasible and cost-effective method of protection against respiratory infections.

In **chapter 10**, we assess the potential risk for emergence of mAb resistant mutants (MARMs) after global mAb use on RSV by simulating suboptimal mAb pressure in a laboratory setting. MARMs were developed for three RSV mAbs: palivizumab, nirsevimab, and RSM01. RSM01 shows a higher barrier to resistance than the other two mAbs. In general, mAb resistance comes at a viral fitness cost, making it unlikely that MARMs will outcompete non-resistant strains in dominant global circulation. Considering resistance is the Achilles heel of viral neutralisation by mAbs, a high-barrier-to-resistance mAb aligns with equitable and sustainable RSV immunisation development according to the *equity-by-design* principles [**chapter 11**].

Discussion of this thesis

RSV remains a major global health challenge, disproportionately affecting infants in LMICs. Yet current RSV immunisation products largely emerge from a *trickle-down innovation* model in which development is driven by high-income contexts and LMIC needs are addressed only after products are finalised - if at all. This thesis applies a translational science lens to demonstrate how equity can be embedded throughout the research and development pathway through deliberate design choices.

Three central lessons emerged. First, beginning with the end in mind underscores that

scientific advances reach their potential only when innovations are acceptable, feasible, and trusted within communities and health systems. Second, simplicity in laboratory and clinical methods can substantially increase the applicability of innovations in resource-limited settings. The use of dried blood spots and simplified neutralisation assays illustrates how early-stage design choices can remove barriers to participation and enable wider scientific engagement. Third, the development of affordable mAbs requires an *equity-by-design* approach, in which affordability and accessibility for LMICs are integrated from the earliest stages of product conception, as seen in the intranasal palivizumab and the RSM01 mAb development programmes.

Building on these lessons, this thesis proposes four core recommendations for reframing translational science toward equitable RSV immunisation:

1. **Scientific co-ownership**: Research agendas must be shaped with, not for, LMIC partners. Early inclusion of diverse populations prevents late-phase delays, improves generalisability, and ensures globally relevant correlates of protection. Multidisciplinary teams, including clinicians, laboratory scientists, social scientists, and implementation experts, are essential to producing context-sensitive and actionable evidence.
2. **Operational investment**: Equitable innovation requires strengthening local capacity through laboratory infrastructure, research and regulatory staff training, and longitudinal technology transfer. Embedding such investments within research partnerships enhances scientific autonomy and enables LMIC-led translational pathways as well as potential local manufacturing of immunisation products or biosimilars.
3. **Economic viability**: Affordability must be designed into products from the earliest stages. Translational science can shape cost drives, for instance through antigen design, manufacturing platforms, or dose-sparing trials. Later-phase translational work, including cost-effectiveness analyses and implementation feasibility assessments, informs equitable pricing models such as tiered pricing, voluntary licensing, or local manufacturing agreements.
4. **Social integration**: Innovations deliver population-level benefit only when aligned with community priorities, health-system realities, and sociocultural contexts. Continuous stakeholder engagement and integration of social science are crucial to strengthening trust, vaccine confidence, and policy adoption.

Together, these recommendations outline a translational framework in which equity is not a downstream consideration but a foundational principle guiding RSV immunisation development from discovery to delivery.

Nederlandse samenvatting

Rationale achter dit proefschrift

Respiratoir syncytieel virus (RSV) is een belangrijke oorzaak van acute respiratoire infecties (ARI). Vrijwel alle kinderen raken vóór hun tweede levensjaar ten minste eenmaal geïnfecteerd, en herinfecties komen veelvuldig voor gedurende het hele leven. De meeste infecties veroorzaken milde klachten van de bovenste luchtwegen, vaak aangeduid als de "verkoudheid". RSV kan echter ernstige, potentieel levensbedreigende lagere luchtweginfecties veroorzaken bij zuigelingen, met name bij te vroeg geboren kinderen en kinderen met comorbiditeiten. Wanneer toegang tot adequate gezondheidszorg ontbreekt, zoals vaak het geval is in lage- en lagere-middeninkomenslanden (LMIC's), kan de uitkomst fataal zijn.

Tot voor kort was palivizumab, een kortwerkend monoklonaal antilichaam (mAb), het enige geregistreerde middel ter preventie van RSV-infecties; vanwege de hoge kosten was het gebruik ervan beperkt tot hoogrisicozuigelingen. In 2022 werd nirsevimab het eerste langwerkende mAb dat werd goedgekeurd voor RSV-preventie bij alle zuigelingen, kort daarop gevolgd door het eerste maternale RSV-vaccin. In 2025 werd clesrovimab, eveneens een langwerkend mAb, goedgekeurd voor gebruik bij zuigelingen. De wereld gezondheidsorganisatie (WHO) adviseert inmiddels dat alle landen RSV-preventie invoeren via een maternale vaccinatiestrategie of via mAbs. Wereldwijd worden deze interventies opgenomen in nationale immunisatieprogramma's. De implementatie in LMIC's blijft echter achter vanwege logistieke en financiële barrières en de behoefte aan aanvullende informatie over veiligheid en effectiviteit.

Het voornaamste doel van dit proefschrift is het gebruik van een multidisciplinaire benadering om ongelijkwaardigheid in toegang tot RSV-immunisatie voor zuigelingen in LMIC's te verminderen. Daartoe zijn vijf typen onderzoek binnen het translationale onderzoek spectrum toegepast:

1. Publiek gezondheidskundig onderzoek: overzicht van de RSV-vaccinontwikkelingspijplijn en de route naar mondiale toegang.
2. Fundamenteel onderzoek: ontwikkeling van een assay voor het meten van RSV-neutraliserende antistoffen in gedroogd bloed.
3. Preklinisch onderzoek: evaluatie van mAb-resistente RSV-mutanten.
4. Klinisch onderzoek: beoordeling van nieuwe, betaalbare beschermingsstrategieën tegen RSV-infectie.
5. Implementatieonderzoek: evaluatie van de gereedheid voor maternale immunisatie.

Bevindingen van dit proefschrift

Tijdens dit promotietraject (2021–2025) vond aanzienlijke vooruitgang plaats in de preventie van RSV. In **hoofdstuk 2** bieden wij een uitgebreid overzicht van het actuele landschap van RSV-vaccins en mAbs, aangevuld met eerste resultaten uit de praktijk na

introductie. Ten tijde van schrijven (juni 2024) waren vijf RSV-immunisatieproducten op de markt en bevonden zich nog 30 in ontwikkeling. De eerste ervaringen tonen veelbelovende effectiviteit, waaronder meer dan 80% bescherming door nirsevimab tegen RSV-gerelateerde ziekenhuisopname. De toegang blijft echter grotendeels beperkt tot hogere-inkomenslanden, terwijl de hoogste ziektelast juist in LMIC's ligt; een duidelijke illustratie van wereldwijde gezondheidsongelijkwaardigheid. Ons overzicht benoemt de noodzakelijke vervolgstappen om deze kloof te dichten. De aanbeveling van de WHO Strategisch Advies Groep van Experts (SAGE) in september 2024 was hierin cruciaal, maar structurele barrières, zoals hoge kosten, lage RSV-bekendheid en concurrerende immunisatieprioriteiten, blijven bestaan. Om de wereldwijde RSV-vaccinkloof te dichten, moet het aanpakken van deze barrières een urgente prioriteit blijven die door meerdere belanghebbenden wordt gedeeld. In **hoofdstuk 3** stellen wij dat publiek-gezondheidsgerichte toegang-tot-medicijnen-mechanismen (zoals vrijwillige licenties en technologieoverdracht aan lokale producenten) essentieel zijn om grootschalige, betaalbare toegang tot RSV-mAbs in LMIC's te realiseren. De WHO-aanbeveling vergemakkelijkt de ontwikkeling van een regelgevingstraject via WHO-prekwalificatie, wat mogelijk noodzakelijk is voor fabrikanten van biosimilars. Ondersteuning van regelgevende coördinatie kan goedkeuringsprocedures versnellen, vooral als dit gepaard gaat met een technologieoverdracht die de gelijkenis met het originele product waarborgt. Daarnaast zijn internationale financierings- en inkoopmechanismen, zoals Gavi, essentieel om toegang tot immunisatie te versnellen en ontwikkeling van biosimilaire producten te stimuleren.

De ontwikkeling van RSV immunisatie is niet voltooid zodra registratie is bereikt. Idealiter wordt reeds tijdens de ontwikkelingsfase rekening gehouden met implementatie, zodat een onmiddellijke uitrol mogelijk is zonder vertraging na registratie. In **hoofdstuk 4** identificeren wij de barrières en faciliterende factoren voor maternale immunisatie in Soweto, Zuid-Afrika, zoals ervaren door lokale zorgverleners en vertegenwoordigers van de gemeenschap. Tijdens co-creatieworkshops deden zorgverleners aanbevelingen om de waargenomen barrières te doorbreken en de lokale beschikbaarheid en vraag naar maternale immunisatie te verbeteren. Aanbevelingen omvatten: scholing van zorgverleners over bestaande en nieuwe maternale vaccins via nascholing of online micro-educatie; het vergroten van het bewustzijn in de samenleving over vaccinatie-voorkombare aandoeningen via voorlichtingsactiviteiten in samenwerking met gemeenschapsleiders en traditionele genezers; het stimuleren van zorgverleners en klinieken om maternale immunisatie te verstrekken door middel van regionale of nationale rapportage; en het verbeteren van documentatie in geactualiseerde maternale patiëntendossiers, of idealiter de invoering van elektronische patiëntendossiers, om zorgverleners te ondersteunen bij het toedienen van het juiste vaccin op het juiste moment en om verwijzingen tussen zorgprofessionals te vergemakkelijken.

RSM01, een nieuw mAb ontwikkeld door het Gates Medical Research Institute voor gebruik in LMIC's, werd onderzocht in een fase 1-studie in gezonde volwassenen. Neutraliserende antistoffen (nAb), de belangrijkste mate van bescherming voor vaccins en mAbs, worden traditioneel gemeten in serum verkregen via venapunctie. Gedroogd bloed verkregen via een

vingerprik kan monsterafname, verwerking, opslag en transport aanzienlijk vereenvoudigen en daarmee kosten reduceren. In **hoofdstuk 5** ontwikkelden wij een RSV-neutralisatieassay voor gedroogd bloed aan de hand van de monsters uit de fase 1 RSM01 studie. In **hoofdstuk 6** tonen wij aan dat gedroogd bloed serum kan vervangen in farmacologisch, neutralisatie en immunogeniciteit bioanalyses (R^2 > 0,95). In **hoofdstuk 7** werd deze assay succesvol overgedragen aan een laboratorium in Ghana, waarmee lokale onderzoekscapaciteit werd versterkt. Betrouwbare meting van RSV-nAb in een land dat in aanmerking komt voor Gavi-steun en het gebruik van gedroogd bloed kunnen bijdragen aan de betrokkenheid van LMIC's bij de ontwikkeling en toegang tot RSV-vaccins.

Hoofdstukken 8 en **9** beschrijven de industrie-onafhankelijke ontwikkeling van palivizumab-neusdruppels voor zuigelingen. Mucosale toediening van mAbs tegen respiratoire pathogenen wordt beschouwd als een veelbelovend alternatief voor systemische toediening, omdat voor bescherming lagere doses vereist zijn. In **hoofdstuk 8** onderzoeken wij de veiligheid en werkzaamheid van dagelijkse intranasale mAb-profylaxe met palivizumab ter preventie van RSV-infectie in een fase 1 (gezonde volwassenen) en een fase 2b gerandomiseerde gecontroleerde studie (gezonde laat-premature zuigelingen). Dagelijkse toediening van palivizumab-neusdruppels bleek veilig, maar voorkwam RSV-infectie bij laat-premature zuigelingen niet (palivizumab 38,3% versus placebo 23,4%; aOR 2,2; 95% BI 0,7–6,5). Er zijn meerdere mogelijke verklaringen waarom in deze fase 2b-studie geen werkzaamheid werd aangetoond: een korte halfwaardetijd, lage viruscirculatie, beperkte blootstelling aan RSV of mogelijk lage therapietrouw, factoren die inherent zijn aan het studieontwerp. Een gecontroleerd humaan infectie model (CHIM) biedt een ongeëvenaarde onderzoeksopzet om werkzaamheid te beoordelen in een streng gecontroleerde omgeving met vaste dosering, adequate virale inoculatie, gecontroleerde virus blootstelling en een vaste interval tussen toediening van het middel en de virale inoculatie. In een dubbelblinde, gerandomiseerde, placebo-gecontroleerde CHIM-studie in gezonde volwassenen tonen wij aan dat intranasale palivizumab geen effect heeft op de RSV-virale titer, ondanks een reductie van 72% in symptomatische infecties [**hoofdstuk 9**]. Intranasale mAbs lijken derhalve zeer onwaarschijnlijk een haalbare en kosteneffectieve methode te vormen voor bescherming tegen respiratoire infecties.

In **hoofdstuk 10** beoordelen wij het potentiële risico op het ontstaan van mAb-resistente mutanten (MARMs) na wereldwijd gebruik van mAbs tegen RSV door suboptimale mAb-druk in een laboratoriumsetting te simuleren. MARMs werden ontwikkeld voor drie RSV-mAbs: palivizumab, nirsevimab en RSM01. RSM01 vertoont een hogere resistentiebarrière dan de twee andere mAbs. In het algemeen gaat mAb-resistentie gepaard met een vermindering van virale fitness, waardoor het onwaarschijnlijk is dat MARMs de dominante wereldwijde circulatie van niet-resistente varianten zullen overtreffen. Aangezien resistentie de achilleshiel vormt van virale neutralisatie door mAbs, sluit een mAb met een hoge resistentiebarrière aan bij een eerlijke en duurzame ontwikkeling van RSV-immunisatie volgens de *equity-by-design* principes [**hoofdstuk 11**].

Discussie van dit proefschrift

RSV vormt een aanhoudende wereldwijde gezondheidsuitdaging, met een onevenredige ziektelast in LMIC's. De ontwikkeling van huidige preventieve producten volgt grotendeels een *trickle-down* benadering, waarbij innovaties primair worden ontworpen voor hoge-inkomenscontexten. Dit proefschrift toont aan hoe het integreren van *equity-by-design* binnen alle fasen van translationeel onderzoek kan bijdragen aan rechtvaardige en duurzame RSV-preventie.

Drie kernlessen komen naar voren. Ten eerste benadrukt het principe begin with the end in mind dat wetenschappelijke vooruitgang pas haar volledige potentieel bereikt wanneer innovaties acceptabel, uitvoerbaar en vertrouwd zijn binnen gemeenschappen en gezondheidssystemen. Ten tweede kan vereenvoudiging van laboratorium- en klinische methoden de toepasbaarheid in LMIC-contexten aanzienlijk vergroten. Het gebruik van gedroogd bloed en vereenvoudigde neutralisatie assays illustreert hoe keuzes in het vroege ontwerpstadium barrières voor deelname kunnen wegnemen en bredere wetenschappelijke betrokkenheid kunnen faciliteren. Ten derde vereist de ontwikkeling van betaalbare mAbs een *equity-by-design* benadering, waarbij betaalbaarheid en toegankelijkheid voor LMIC's vanaf het allereerste begin van productontwikkeling worden geïntegreerd, zoals zichtbaar is in de ontwikkeling van intranasaal palivizumab en RSM01.

Op basis van deze lessen worden vier kernaanbevelingen gedaan om translationeel onderzoek te heroriënteren richting gelijkwaardige RSV-immunisatie:

1. **Wetenschappelijke co-eigenaarschap**: Onderzoeksagenda's moeten worden vormgegeven met, en niet voor, LMIC-partners. Vroege inclusie van diverse populaties voorkomt vertragingen in latere fasen, verhoogt de generaliseerbaarheid en waarborgt dat correlaten van bescherming wereldwijd relevant zijn. Multidisciplinaire teams, waaronder clinici, laboratoriumwetenschappers, sociale wetenschappers en implementatie-experts, zijn essentieel om contextgevoelige en toepasbaar bewijs te genereren.

2. **Operationele investering**: Gelijkwaardige innovatie vereist versterking van lokale capaciteit door laboratoriuminfrastructuur, training van onderzoeks- en regelgevend personeel, en langdurige technologieoverdracht. Het inbedden van dergelijke investeringen binnen onderzoekspartnerschappen bevordert wetenschappelijke autonomie en maakt LMIC-geleide translationeel onderzoek en potentiële lokale productie van immunisatieproducten of biosimilars mogelijk.

3. **Economische haalbaarheid**: Betaalbaarheid moet vanaf de vroegste ontwikkelingsfasen in producten worden ingebouwd. Translationele wetenschap kan kostendrijvers beïnvloeden, onder meer via antigeenontwerp, productieplatforms of dosisbesparende studies. Activiteiten in latere fasen (zoals kosteneffectiviteitsanalyses en implementatie-haalbaarheidsstudies) ondersteunen eerlijke prijsmodellen, waaronder getrapte prijzen, vrijwillige licenties of lokale productieafspraken.

4. **Sociale integratie**: Innovaties leveren pas voordeel op voor de bevolking wanneer zij aansluiten bij maatschappelijke prioriteiten, realiteiten van het gezondheidssysteem en socioculturele contexten. Doorlopende betrokkenheid van belanghebbenden en integratie van sociaalwetenschappelijke inzichten zijn cruciaal voor het versterken van vertrouwen, vaccinacceptatie en beleidsadoptie.

Gezamenlijk schetsen deze aanbevelingen een translationeel kader waarin gelijkwaardigheid geen afgeleid eindpunt is, maar een fundamenteel uitgangspunt dat de ontwikkeling van RSV-immunisatie stuurt van ontdekking tot implementatie.

List of publications

Publications in this thesis

1. **Terstappen J**, Hak SF, Bhan A, et al. The respiratory syncytial virus vaccine and monoclonal antibody landscape: the road to global access. *The Lancet Infectious Diseases* 2024.
2. Zar HJ, Piccolis M, **Terstappen J**, Mazur NI, Gaayeb L, Morin S, Bont L. Access to highly effective long-acting RSV-monoclonal antibodies for children in LMICs—reducing global inequity. *The Lancet Global Health*. 2024; 12(10):e1582-3.
3. **Terstappen J**, Delemarre EM, Versnel A, et al. RSV neutralizing antibodies in dried blood. *The Journal of Infectious Diseases* 2023; jiad543.
4. White JT, **Terstappen J**, Levi M, et al. Replacing serum with dried blood microsampling for pharmacokinetics, viral neutralisation and immunogenicity bioanalysis supporting future paediatric development of RSM01, a candidate respiratory syncytial virus neutralising monoclonal antibody. *BMC Infectious Diseases* 2024; 24(1):1403.
5. Obodai E*, **Terstappen J***, Mensah JY, et al. Proof-of-principle of a technology transfer of a dried blood virus neutralisation assay to a Gavi-eligible country. *BMJ Global Health* 2025; 10(3).
6. Mazur NI*, Löwensteyn YN*, **Terstappen J**, et al. Daily intranasal palivizumab to prevent respiratory syncytial virus infection in healthy preterm infants: a phase 1/2b randomized placebo-controlled trial. *eClinicalMedicine* 2023; 66.
7. **Terstappen J**, Nibbelke EE, Ascough S, et al. Intranasal monoclonal antibodies do not prevent respiratory infection in a randomized, controlled experimental infection trial. *npj Drug Discovery.* 2025 Jul 3;2(1):15.
8. **Terstappen J**, Gustani Buss EC, McLellan JS, et al. RSM01, an extended half-life RSV monoclonal antibody with a high barrier to resistance. *Under revision for Nature Communications. 2026*.

* these authors are joint first author for this work

Other publications

9. Deyer HM, Ithakisiou GN, Spelier S, et al. Small molecule-directed differentiation of submerged-cultured human nasal airway epithelia for respiratory disease modelling. *Cell Reports Medicin* 2026.
10. Hup RJ, Damen JAA, **Terstappen J**, et al. Oral antihypertensive treatment during pregnancy: a systematic review and network meta-analysis. *American Journal of Obstetrics & Gynecology* 2025.
11. Paediatric drug optimization for respiratory syncytial virus: meeting report, April 2025. Geneva: World Health Organization; 2025. https://doi.org/10.2471/B09464.

12. Siegal EZ, Schoevers JMH, **Terstappen J**, et al. Risk analysis for outpatient experimental infection as a pathway for affordable RSV vaccine development. *Npj Vaccines* 2025; 10.1: 70

13. Bonavia A, Levi M, Rouha H, et al. RSM01, a novel respiratory syncytial virus monoclonal antibody: preclinical characterization and results of a first-in-human, randomised clinical trial. *BMC Infectious Diseases* 2024; 24(1):1-8.

14. Mazur NI, **Terstappen J**, Baral R, et al. Respiratory syncytial virus prevention within reach: the vaccine and monoclonal antibody landscape. *The Lancet Infectious Diseases* 2023; 23(1):e2-1.

15. Okhuijsen-Pfeifer C, Sterk AY, Horn IM, **Terstappen J**, Kahn RS, Luykx JJ. Demographic and clinical features as predictors of clozapine response in patients with schizophrenia spectrum disorders: a systematic review and meta-analysis. *Neuroscience & Biobehavioral Reviews* 2020; 111:246-52.

PhD Portfolio

November 2021 – January 2026

Courses

2024	Vaccine confidence: Building trust and managing risks (Antwerp, Belgium)
2024	Concepts of intervention science to global challenges (Heidelberg, Germany)
2023-2025	Teaching Upcoming Leaders in Pediatric Sciences (TULIPS) PhD Curriculum
2023	Basic Course on Regulation and Organization for Clinical Investigators (BROK©)
2023	Utrecht Summer School: Global Health Ethics & Equity
2022-2025	Graduate School of Life Sciences (GSLS) courses: Introductory Biostatistics for Researchers, Responsible Conduct of Research, Your Personal Development during your PhD Track, Qualitative Data Analysis, Adobe InDesign
2022	Infection and Immunity PhD program, GSLS: Advanced Infection Biology
2021	Utrecht Summer School: Global Health PhD & Postdoc writing retreat

Conferences

2026	ReSViNET RSV Vaccines for the World; Rome, Italy (co-chair and poster)
2025	World Society for Pediatric Infectious Disease; Bangkok, Thailand (oral)
2024	ReSViNET RSV Vaccines for the World; Mumbai, India (oral)
2023	European Congress on Tropical Medicine and International Health (ECTMIH); Utrecht, The Netherlands (oral)
2023	Immunogenicity & Bioassay Summit; Washington DC, USA (oral)
2023	European Society for Pediatric Infectious Disease; Lisbon, Portugal (poster)
2023	ReSViNET RSV Vaccines for the World; Lisbon, Portugal (poster)
2022	Nederlandse Vereniging voor Immunologie (NVVI); Noordwijk (poster)
2022	14th Excellence in Pediatrics conference; Amsterdam (oral)
2021	ReSViNET RSV Vaccines for the World; virtual (poster)

Seminars

2025	INFECT-NL; Utrecht, The Netherlands (oral)
2024	McMaster Virtual RSV Coordinators Conference; online (oral)
2024	MEDtalks – RSV Rewind; Hilversum, The Netherlands (talkshow guest)
2023	Virology Education; Lisbon, Portugal (oral)
2021	Nosy knowledge symposium; Utrecht, The Netherlands (moderator)

Grants and Awards

2025	Research Grant to UMCU; Dutch Ministery of Health (€250 000)
2025	Travel Grant; Jenner Foundation (€2500)
2025	Research Grant to UMCU; Dynamics of Youth, Utrecht University (€30 000)
2024	Eric Hack Publication Award; Universiteit Utrecht (€750)

2024	KNAW Ter Meulen Grant (€4600)
2024	Publication Award; ReSViNET Young Researchers Tournament, 1st place (€3000)
2024	Travel Grant; Girard de Mielet van Coehoorn Stichting (€1250)
2023	Research Grant to UMCU; Gates Medical Research Institute (€500 000)
2023	Travel Grant; ReSViNET RSV Vaccines for the World'23 (€750)
2022	Travel Grant; Infection and Immunity GSLS, Universiteit Utrecht (€2000)
2021	KNAW Dr Saal van Zwanenberg Master Thesis Award (€2000)

Teaching

2024	Co-founder mentor program UMCU Connected
2023	Guest Lecture: Mechanisms of Disease (University College Utrecht)
2022-2025	Guest lectures at various peadiatrics or emergency departments in Dutch hospitals

Supervision

2024	Rosalie Groenendijk (Master student of Medicine, Utrecht University) *"Mucoconversion": Mucosal antibody response after experimental RSV infection*
2024	Manon van der Werff (Master student of Medicine, Utrecht University) *The effect of RSV infection on lung function over time during acute infection in healthy adults: a controlled human infection study*
2023	Anouk Versnel (Master student Infection and Immunity, Utrecht University) *Clinical sample analysis*
2023	Isabelle van Lotringen (Selective Utrecht Medical Master student, Utrecht University) *Burden of acute respiratory infections and etiology in children under five living in refugee camps: a systematic review*

Experiences

2025	Visiting scholar (2 months), Wits-VIDA, Johannesburg, South-Africa
2025	Paediatric Drug Optimization (PADO) for RSV advisory board, WHO
2024-2025	Physician in Emergency Medicine, Zuyderland Medisch Centrum, Heerlen, 0.8FTE
2023	Visiting scholar (2 weeks), icddr,b and Cox's Bazar refugee camp, Bangladesh
2023	Visiting scholar (5 weeks), NMIMR, Accra, Ghana

Brilliant failures

No-go for RSV Burden of disease study in Cox's Bazar refugee camp, Bangladesh

Intranasal mAbs do not prevent respiratory infections

Peer Review

Vaccine, The Journal of Infectious Diseases, BMC Medicine, The Lancet, The Lancet Healthy Longevity, Human Vaccines & Immunotherapeutics, NIHR Open Research

A

About the author



"Jonne is a talented early-career physician-researcher with a holistic approach: she is interested in both basic science and its clinical and societal implications. Her ability to seek, receive, and process help, demonstrates self-reflection and thoughtfulness. Jonne's positive and enthusiastic attitude contributes to a constructive and uplifting atmosphere."

- Natalie I. Mazur

Jonne was born on August 17th, 1995 in Maastricht, The Netherlands, where she grew up with her older brother and sister, Bram and Fieke, and her parents, Lou and Jacqueline. After graduating cum laude from secondary school at Sint Maartenscollege in Maastricht in 2013, she moved to Utrecht to study Liberal Arts & Sciences at University College Utrecht (UCU). During her semester abroad at Queen's University, Kingston, Canada, she decided to switch her major from neurosciences & psychology to pre-medicine. She completed minors in neuroscience and art history and her thesis internship in the psychiatry ward of the University Medical Center Utrecht (UMCU). Jonne spent her free time as the captain and team leader of the UCU women's football team at vv Odin, Utrecht.

In 2017, she started the dual master's degree in medicine and research: Selective Utrecht Medical Master (SUMMA). During her rotations in Gelre Ziekenhuis Apeldoorn, she first met residents in Global Health and her passion for the discipline grew. She joined the RSV group for her research internship as it allowed laboratory research in global health. Here she fell in love with science and earned the KNAW Dr Saal van Zwanenberg Master thesis award.

Jonne graduated as a medical doctor in 2021 and started her PhD at the UMCU with support from the Gates Medical Research Institute. During her PhD track, she followed the multidisciplinary training program TULIPS ("Training Upcoming Leaders in Paediatric Science"). In 2024, she interrupted her PhD to work as a non-resident doctor (ANIOS) in the emergency department at Zuyderland MC, Heerlen. She obtained the KNAW Ter Meulen grant to study the implementation of new maternal vaccines in Soweto, South-Africa. For this project, she returned to research full-time after 9 months in the clinic.

Throughout her PhD track, Jonne was involved in equitable projects both close to home and abroad. She supported admissions process of the medical observership program for refugee medical students from Ukraine and co-founded UMCU Connected, a mentorship program between high-school students with a non-Western migration background and healthcare professionals, to make Dutch healthcare more diverse and inclusive. Using a train-the-trainer approach, Jonne was dedicated to strengthening research capacity in Ghana to enable their inclusion in global RSV research.

Jonne lives happily together with Megan in Utrecht, and finds balance through singing loudly, being outdoors, and undertaking "backyard adventures". After completing her PhD, she continues her diverse career by combining residency and research in Global Health.

Dankwoord (Acknowledgements)

De berg is beklommen – en wat was de tocht toch mooi! Ik heb geen enkel moment spijt gehad een PhD te doen en dat wijd ik volledig aan alle prachtige mensen om me heen. Tijdens de zware stukken omhoog klauteren kon ik rekenen op jullie helpende hand en altijd met een lach op ons gezicht. Ik kijk terug op een bijzondere tijd en wil deze laatste pagina's graag gebruiken om de mensen te bedanken die dit promotietraject mede mogelijk hebben gemaakt.

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Het promotieteam

Mijn promotieteam, Louis, Natalie en Eveline, door jullie voelde ik me vanaf dag één thuis. Jullie gaven me de vrijheid en ruimte om me te ontwikkelen naar eigen ingeving en mijn draai te mogen geven aan de gang van zaken, zoals 5 minuten yoga of meditatie aan het begin van de wekelijkse groepsbespreking. Jullie vroegen tot bijna vervelends toe hoe jullie me het beste konden ondersteunen naar mijn droom toekomst en zo hadden jullie de droombaan al voor me gemaakt. Jullie hebben me ondenkbaar veel kansen en inspiratie geboden, waar ik zo dankbaar voor ben. Impact en dagelijks plezier staan op de eerste plaats en ik had me geen fijnere mentoren kunnen wensen.

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kleine kwesties, werk- of privé, ik kon op je aan. We zijn allebei niet zo van strenge hiërarchie wat snel oversloeg op “wie superviseert wie?”. Je hebt me laten zien hoe je ongebruikelijke studies kan uitvoeren met impact, enorme teams kan aansturen met oog voor de persoon, en duidelijk leiding kan geven op een kwetsbare manier. Ik bewonder hoe jij je door niets of niemand laat tegenhouden en al helemaal niet door wet- en regelgeving. “Kun je nog lachen?” werd onze graadmeter voor lastigere tijden (noodzakelijk om te vragen, want we worden altijd zo blij van elkaar dat het anders bijna niet af te lezen is). Je stond me bij wanneer mijn wereld op z’n kop stond en gaf me juist dan even dat onmisbare gevoel van gronding, waar ik kracht uit kon putten. Het voelt alsof ik met een van mijn beste vrienden mag samenwerken en ik hoop dat we dit nog lang mogen doorzetten!

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Paranimfen

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Collega's

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