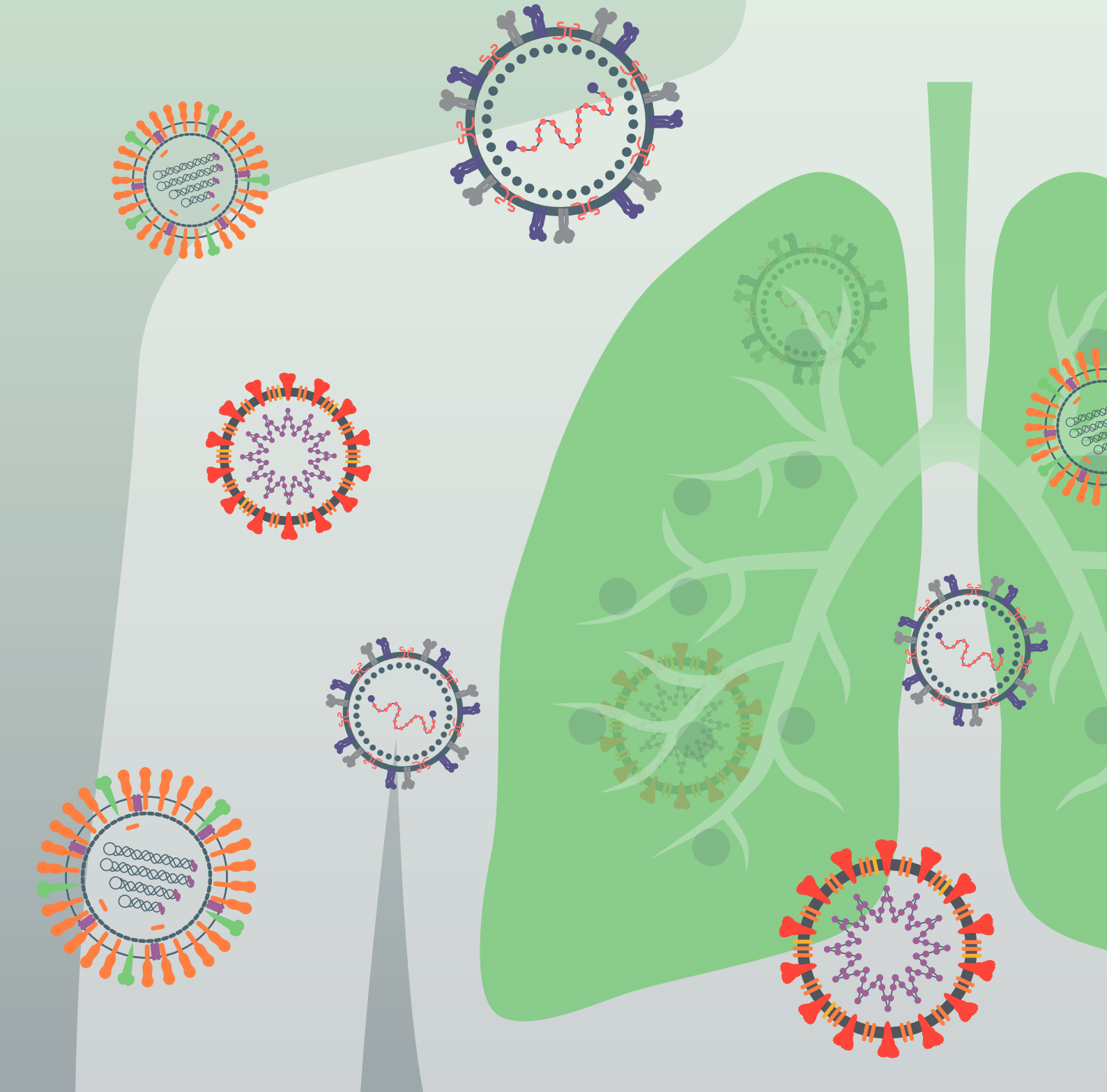
FEBRUARY 2024

Volume 3, Issue 1

VACCINE INSIGHTS

SPOTLIGHT ON
Respiratory diseases



CONTENTS

SPOTLIGHT: RESPIRATORY DISEASES

Spotlight

Respiratory diseases

Latest articles

VACCINE INSIGHTS

RESPIRATORY DISEASES

SPOTLIGHT

EXPERT INSIGHT

RSV vaccines: a new hope but the virus might strike back

John Tregoning, Haoyuan Li, Chubicka Thomas, Ziyin Wang, and Lucy G Mosscrop

Respiratory syncytial virus (RSV) was discovered in 1956—that it took nearly 70 years to develop working vaccines, speaks to the challenges posed by this virus (by contrast, a SARS-CoV-2 vaccine took 11 months). There are, like the proverbial London buses, now three RSV vaccines at once—two for use in the elderly (Arexvy and Abrysvo™) and one as a maternal vaccine (Abrysvo). In parallel, there is a new monoclonal antibody, nirsevimab. Both the vaccines and the antibody target the RSV-F protein. The current challenge is how to best implement these preventative measures to maximize their impact and reduce the menace of this important viral pathogen. In this review, we will give an overview of the antibodies and the vaccines and look at the immunological, viral, economic, and societal challenges that might arise in the next few years to avoid the return of the virus.

Vaccine Insights 2024; 3(1), 47–64

DOI: 10.18609/vac.2024.010

INTRODUCTION

Respiratory syncytial virus (RSV) is a major respiratory pathogen causing an estimated 33 million episodes of acute lower respiratory infection in 2019 and accounting for 2% of deaths in children under 5 years old [1]. It also has a large clinical burden in older adults with an estimated 1.5 million episodes in over 65-year-olds and 214,000 hospitalizations in high-income countries in 2015 [1]. This burden of disease is even more significant in low- and middle-income countries (LMIC), accounting for 97% of RSV-attributable deaths. The financial burden of RSV is high as there is no specific treatment against RSV

infection once acquired, with treatment largely through supportive management. 65% of this €4.82 billion economic burden falls on LMIC [2]. Prevention of severe RSV infections through immunization has therefore become a global health priority.

Despite the clear need for preventative measures against RSV infection, progress has been slow. The gap between virus isolation and vaccine development for RSV was longer than for polio, influenza, chickenpox, measles, or Ebola [3]. This was partly due to the legacy of the formalin-inactivated vaccine trial of 1969, in which infants were vaccinated with RSV that had been grown in cell culture and inactivated with formalin. The FI-RSV vaccine was initially well tolerated but on exposure to natural RSV infection, particularly in the younger children with no prior exposure to RSV, vaccine-enhanced respiratory disease was seen. This led to 80% of the children being hospitalized and ultimately the death of two infants in the trial [4–7]. Understanding what went wrong with this vaccine trial was vital for RSV vaccine development to continue. Many factors have also been speculated to play a role in the failure of the FI-RSV vaccine [8], including immune complexes [9], the presence of carbonyl groups [10], and a T helper 2 bias [11].

RSV vaccine efforts have been refocused since the FI-RSV tragedy, with developments in understanding of the RSV fusion (RSV-F) protein structure underpinning most new vaccine candidates [12]. RSV-F is considered a major antigenic target for preventative interventions due to its essential role in the viral life cycle and relative genetic stability. RSV-F is expressed as an envelope glycoprotein on the outside of the virion and is responsible for the fusion of viral and host cell membranes, allowing entry to the cell; blocking RSV-F prevents viral entry. Critically, RSV-F is a meta-stable protein, with two forms, pre- and post-fusion [13]. It has six antigenic sites (numbered sites Ø through V); of these, sites I, II, and IV are found on both pre- and post-fusion forms, and Ø, III, and V only on the pre-fusion form [14]. One important strand in the development of new vaccines and antibodies was differences observed in the structure of RSV-F in the formalin-inactivated vaccine, which was found to be predominately in the poorly neutralizing, post-F conformation [15]. Understanding the structure of RSV-F has contributed to the development of both vaccines and antibodies.

ANTIBODIES

In the absence of vaccines, using monoclonal antibodies as passive immunization has proven successful for the most at-risk pediatric populations, with palivizumab (Synagis®) licensed for use in vulnerable infants in the UK since 1999 and the USA since 1998. Palivizumab is a humanized murine monoclonal antibody targeting antigenic site II on the F protein. Palivizumab was first demonstrated to be effective across two large studies [16,17] and has since shown efficacy in practice too. Importantly, use of palivizumab has been shown to reduce post-bronchiolitic wheeze [18]. Because it is unmodified, palivizumab has a short half-life (equivalent of human IgG), and therefore must be administered monthly throughout the RSV season. The dosing regimen contributes to the main drawback of palivizumab—the high cost—which makes it prohibitive for low-income countries where the disease burden is highest. It is currently only used for the highest-risk infants (in the UK that includes infants born preterm with chronic lung disease, infants with congenital heart disease, infants requiring long-term ventilation, and various other co-morbidities) [19].

The short half-life of palivizumab has led to the development of alternative approaches. One of the major advances in monoclonal antibodies has been the incorporation of the YTE (M252Y/S254T/T256E) mutation in the Fc region, which has been shown to extend serum half-life fourfold [20]. Several clinical trials have been performed with new monoclonals, but not all trials have been successful. A successor to palivizumab, motavizumab, was derived by affinity maturation,

selecting for improved binding—it differs from palivizumab in 13 amino acids with a greater neutralizing activity [21]. However, trials were discontinued in 2010, with some concerns about cutaneous adverse effects and limited benefit compared to palivizumab [22]. Regeneron also dropped its monoclonal antibody to RSV (suptavumab) in 2017, after it failed to meet the primary endpoint in a trial for infants. The failure of suptavumab was due to two spontaneous mutations arising in the F protein of circulating RSV-B strains, leading to complete resistance to the antibody [23].

Fortunately, the development of anti-RSV monoclonal antibodies has been a multi-pronged approach and there were multiple other candidates in contention (Table 1). The most established of these is AstraZeneca/Sanofi's Beyfortus® (nirsevimab) which was licensed in Europe on October 31, 2022 [24]. Beyfortus targets antigenic site Ø which is present only on the pre-fusion conformation of F, leading to stronger neutralizing activity compared to palivizumab. It has been specifically engineered for increased stability and has a longer half-life, meaning one dose can protect an infant for the entire RSV season. In the initial Phase 2b trial, the incidence of medically attended lower respiratory tract infection caused by RSV was reduced by 70.1% compared to placebo (NCT02878330) and in the subsequent Phase 3 MELODY trial, incidence was reduced by 74.5% compared to placebo. Following these trials, which met their primary endpoint, there was a further pre-specified pooled analysis of the data, demonstrating an efficacy of 79.5% for infants born at term or preterm and entering their first season [25]. The MEDLEY trial (Phase 2/3, NCT03959488) was carried out to assess safety and tolerability in preterm infants and infants with congenital heart disease and/or chronic lung disease of prematurity, with infants in their first season receiving either palivizumab or Beyfortus. Beyfortus was well tolerated in these groups, with serum levels comparable to those seen in the Phase 3 MELODY trial, giving some assurance that there will be similar protection in these groups compared to healthy term and late preterm infants [26]. Taken together, these trials demonstrate the protection that Beyfortus can provide infants in their first RSV season whether they are preterm, healthy late-term and term, or have high-risk co-morbidities.

In the HARMONIE trial, Beyfortus was tested in healthy infants in settings approximating real-world settings and showed very promising results, with a reduction in RSV-related hospitalizations in the season in which the RSV was administered of 83% in treated (infants <12 months) compared to placebo, and minimal side effects [27]. These positive outcomes have been mirrored post-Beyfortus licensure with nirsevimab being used clinically for the first time in Galicia, Spain for the 2023/2024 RSV season [28]. The preliminary results coming from this work suggest a flattening of RSV-hospitalization rate compared to previous seasons (rolling data in [29]).

Another antibody, clesrovimab, has been developed by Merck. It is also a half-life-extended anti-RSV-F monoclonal, but it targets antigenic site IV (present on both pre-F and post-F). Clesrovimab is still in Phase 3 clinical trials (NCT04938830) and a Phase 1b/2a study (NCT04086472) has been carried out in healthy adults demonstrating tolerability with no serious adverse events. A model-based meta-analysis looking at RSV serum neutralizing activity and clinical endpoints predicted 75% efficacy for the prevention of medically attended lower respiratory tract RSV infection in infants [30] but whether this will be the case remains to be seen. There are also RSV-F-targeting monoclonals by Trinomab Biotechnology, TNM001, currently in Phase 1b/2a clinical trials (NCT05630573), and a site Ø-targeting antibody, RSM01, supported by the Gates Foundation, which has completed Phase 1 [31].

MATERNAL VACCINES

Another way of providing passive immune protection to infants is maternal immunization.

TABLE 1

Anti-RSV monoclonal antibodies that have progressed to at least Phase 3 trials.

	mAb (market name)	Product description	Target site	Half-life extended? (Y/N)	Efficacy	Escape mutants	Comments
Approved for clinical use	Palivizumab (Synagis®)	Murine humanized monoclonal IgG1	II	N	55% reduction in RSV-related hospitalizations [17]	K272Q, K272E, K272N, K272M, K272T, S275L, S275F [72]	Limited application due to cost and monthly dosage regimen
	Nirsevimab (Beyfortus™)	IgG human monoclonal antibody	Ø	Y	83% reduction in RSV-related hospitalizations [27]	K68N, K68E, N201S, N201T, N208Y, N208D, N208S [109,110]	In use in Galicia Spain as of March 2023 [26]
In clinical trials	Clesrovimab	IgG1 human monoclonal antibody	IV	Y	Predicted 74.2% efficacy for prevention of medically attended lower respiratory tract RSV infection [30]	N/A	Large Phase 3 clinical trial set to complete 2026 (NCT04938830)
Withdrawn	Suptavumab	IgG human monoclonal antibody	V	Y	No clinical benefit in RSV-related hospitalization in Phase 3 trial [23]	L172Q, S173L in RSV-B [23]	Withdrawn in 2017 due to failure to meet primary end point [23]
	Motavizumab	Humanized monoclonal IgG	II	Y	26% relative reduction in RSV hospitalization compared to palivizumab [22]	K272E [72]	Withdrawn due to adverse effects (skin reactions) and questions regarding improved efficacy [22]

TABLE 2

RSV vaccines that have progressed to Phase 3 clinical trials.

	Vaccine name	Type	Adjuvant	Efficacy	Adverse events
Maternal vaccine					
Licensed	Pfizer: Abrysvo™	Bivalent pre-F protein (RSV-A and B)	No	81.8% efficacy against severe illness after 90 days (Phase 3 trial) [38]	Rates of adverse events low and similar between vaccine and placebo group
	GSK: RSVPreF3	Pre-F protein	No	Studies were halted across three Phase 3 trials due to 6.8% preterm births in vaccine arm compared to 4.9% in placebo [33]	Increased risk of preterm birth seen in those vaccinated
	Novavax	RSV-F nanoparticle	Yes: alum	39.4% efficacy against medically significant, RSV-associated lower respiratory tract infection but primary endpoint success criteria not met [34]	Well-tolerated with rates of adverse events similar between vaccine and placebo group
Older adult vaccine					
Licensed	Pfizer: Abryvso	Pre-F protein	No	67% efficacy against RSV-LRTD with two or more symptoms, 86% efficacy in RSV-LRTD with three or more symptoms and 62% efficacy for RSV-associated acute respiratory illness [51]	Incidence of local reactions was higher with vaccine (12%) than with placebo (7%); the incidences of systemic events were similar (27% and 26%, respectively)
	GSK: Arexvy	Pre-F protein (RSVPreF3 subunit)	Yes: AS01E	83% against RSV-lower respiratory tract disease and 94% against severe RSV-LRTD [44]	Vaccine more reactogenic than placebo but adverse events transient and mild-moderate. Incidence of severe events similar in vaccine and placebo group
Discontinued	Bavarian Nordic: MVA BN RSV	MVA recombinant vector vaccine	No	59% efficacy in preventing at least 2 pre-defined LRTD symptoms but only 42.9% for at least 3 and so did not meet primary end point [59]	No serious adverse events, injection site pain higher in vaccine group
	Janssen: Ad26.RSV.pre	Adenovirus recombinant vector vaccine with pre-F	Pre-F protein adjuvant	80% efficacy for reduction of lower respiratory tract infection in a Phase 3 [56]	Local and systemic adverse events were higher in the vaccine group than in placebo (local, 37.9% vs 8.4%; systemic, 41.4% vs 16.4%). Most reactions mild to moderate
In trials	Moderna: mRNA-1345	mRNA encoding for membrane-anchored pre-F	No	84% efficacy against lower respiratory tract disease [60]	Higher incidence of local and systemic adverse reactions in vaccine group compared to placebo. Most reactions mild to moderate

During pregnancy, maternal-derived antibodies are actively transported through the placenta from mother to fetus, which offers passive immunity for infants up to 6 months against infection [32]. This has proved highly effective for other pathogens such as influenza, pertussis, and tetanus, and is also being proposed for group B streptococcus [33].

Three maternal RSV vaccines have progressed to Phase 3 clinical trials—an adjuvanted RSV-F-nanoparticle (Novavax), and two pre-F protein vaccines (from Pfizer and GSK) (Table 2). In the first 90 days of life, the RSV-F nanoparticle was immunogenic and had a 39% vaccine efficacy based on RSV-associated medically significant lower respiratory tract infections, but did not quite meet the pre-specified endpoint; the study authors suggest it was underpowered due to a lower than expected attack rate of RSV in the infants [34].

The GSK pre-F vaccine (RSVPreF3) was seen to be immunogenic in pregnant women in a Phase 2 study, inducing a 12.7–14.9-fold increase in antibody titer [35]; there were no pregnancy or neonatal adverse events in the Phase 2 trial. This vaccine went forward into three Phase 3 clinical studies (NCT04605159, NCT04980391, and NCT05229068). These studies were stopped in February 2022 due to an increased risk of preterm birth in the vaccine arm, particularly in NCT04605159. GSK reported 6.8% preterm births in the vaccine arm compared to 4.9% in the placebo arm, with a higher rate of preterm births in study centers in LMIC [36]. Why this occurred needs to be determined—it has not been seen for other maternal vaccines, which are safe for both mother and baby. Questions include whether it is an acute reaction to the vaccine or a cross-reactive adaptive response affecting the pregnancy. Understanding the timing of preterm birth relative to vaccination will help address whether it is an acute reaction. In terms of adaptive immunity, since this increased preterm birth effect is not seen for other maternal vaccines, it may be related to the immune response against the F protein. But it should be noted that adult women already have pre-existing levels of RSV antibody—it is not a neoantigen. Other factors include the timing of the trial, performed during the COVID-19 pandemic, and whether SARS-CoV-2 infection had an effect. Likewise, understanding why there was a higher frequency of preterm birth in LMIC is important.

In parallel, Pfizer developed a bivalent RSV pre-F vaccine for use in pregnancy (RSVpreF—trade name Abrysvo™), containing equal amounts of pre-F from both A and B RSV strains. In a Phase 2b trial, the vaccine was immunogenic, and interim analysis had an observed efficacy of 84.7% against medically attended RSV lower respiratory tract illness [37]. In an interim analysis of a Phase 3 trial, vaccine efficacy against severe illness was 81.8% after 90 days and 69.4% within 180 days [38]. However, the vaccine did not meet the success criterion for the other primary endpoint (medically attended RSV-associated lower respiratory tract illness); the reported vaccine efficacy was 57.1% (99.5% CI) but the success criteria of a lower boundary of CI <20% was not met (lower CI was 14.7%). The interim analysis reported no statistically significant differences in the rates of preterm birth. However, there was insufficient data to rule out an effect on preterm birth, leading the US FDA to recommend the vaccine for pregnant persons between 32 and 36 weeks gestation [39], whereas the EMA recommendation is 24–36 weeks gestation (the range studied in the Pfizer trial). The reasons for differences in safety outcomes between the two companies' pre-F vaccines needs further evaluation as they are very similar [36]. The Pfizer vaccine was licensed by the FDA in August 2023 [40], and the UK Joint Committee on Vaccination and Immunization (JCVI) recommended it for use in the UK in September 2023 [41]. Ongoing Phase 4 monitoring will be vital, with a focus on preterm birth, especially in LMIC.

VACCINES FOR THE ELDERLY

As well as causing disease in early life, RSV causes severe respiratory illness in older adults and is estimated to be responsible for 214,000 acute lower respiratory infection hospitalizations per year in adults aged 65 or above in industrialized countries [42]. In 2023, two vaccines that target pre-F, Arexvy and Abrysvo (Table 2), were licensed for people aged 60 years and above [43].

Arexvy

Arexvy is a subunit vaccine developed by GSK that targets RSV-related lower respiratory tract disease (LRTD) in elderly people 60 years or older. It contains the RSVPreF3 subunit, a pre-F protein from the RSV-A2 strain, and the adjuvant AS01E. Arexvy is safe and can significantly reduce RSV-related respiratory infections. Efficacy was 83% against RSV-lower respiratory tract disease and 94% against severe RSV-LRTD [44] and common adverse effects included injection site pain (61%) and fatigue (34%). A 3-year follow-up study is ongoing [45], to evaluate immunity duration and safety of repeated vaccination (NCT04732871).

Arexvy received FDA approval for RSV-LRTD in adults over 60 on May 3, 2023, which made it the first approved RSV vaccine [46]. This was followed by approval by the EMA on June 6, 2023 [47], the UK Medicines and Healthcare Products Regulatory Agency (MHRA) on July 10, 2023 [48], Health Canada in August 2023 [49], and the Ministry of Health, Labour and Welfare (MHLW) of Japan in September 2023 [50].

Abrysvo

Abrysvo is a bivalent pre-F subunit vaccine developed by Pfizer, which contains stabilized pre-F from RSV-A and RSV-B subtypes that also targets RSV-LRTD in elderly people 60 years or older (it is the same vaccine as the maternal one). In trials in the elderly, the vaccine showed 67% efficacy against RSV-LRTD with two or more symptoms, 86% efficacy in RSV-LRTD with three or more symptoms, and 62% efficacy for RSV-associated acute respiratory illness [51]; common adverse effects included injection site pain and fatigue. The vaccine was approved by the FDA on May 31, 2023 [52], the EMA in August 23, 2023 [53], and MHRA on November 23, 2023 [54]. Making it available in the USA, European Union, and UK for individuals 60 years or older.

In the UK (at the time of writing—February 2024), the JCVI is advising an RSV vaccination program for elderly adults aged 75 and above [55], with no preference between the approved RSV vaccines due to comparable efficacy and the absence of direct comparison. The main difference between the two vaccines is the inclusion of the AS01E adjuvant in Arexvy, but whether this will affect the longevity of the immune response remains to be seen.

Three other vaccine approaches have reached Phase 3 trials, two were discontinued. Janssen Pharmaceutical Companies developed a replication-defective adenovirus 26 to encode stabilized pre-F called Ad26.RSV.preF, combined with pre-F protein. This had an 80% efficacy for reduction of lower respiratory tract infection in a Phase 3 trial [56] and was also protective in a human challenge model [57], but was discontinued in March 2023. A modified vaccinia Ankara (MVA)-based vaccine expressing multiple antigens (F, N, M2–1, and G) was also protective in a human challenge model [58], but was discontinued because the Phase 3 study failed to meet the primary objective [59]. The remaining vaccine in play is an mRNA vaccine developed by Moderna, encoding a membrane-anchored pre-F called mRNA-1345. This vaccine has a preliminary efficacy of 84% against lower respiratory tract disease [60] and the common adverse effects were fatigue, headache, myalgia, and arthralgia. In a follow-up study with a median follow-up duration of 8.6 months, the VE was 63%

[61]. Moderna filed a global regulatory submission in July 2023 [62]. mRNA vaccines are also under evaluation in pregnant women (NCT06143046).

IMPLEMENTATION CHALLENGES

Having the tools to prevent RSV is only the beginning in reducing the burden of disease. There are several challenges facing the successful rollout of preventative medicines for RSV, including economic, viral, immunological, and societal challenges.

Economic factors

The largest burden of severe disease and death from RSV falls on low-income countries, particularly in early life [63]. Therefore, equitable rollout of vaccines and antibodies should focus upon these countries. The total healthcare budget per capita for low-income countries is estimated at $17 and for middle-income countries at $90 [64]. Alternative funding systems, such as Gavi, have helped to make up the shortfall and modeling suggests that a maternal RSV vaccine with 60% efficacy could avert 123,700–177,700 deaths in the Gavi-supported countries [65]. The cost–effectiveness of RSV vaccines for the elderly, where the burden of disease, particularly in low-income countries, may be underreported [66], will also need to be evaluated. These cost–effectiveness calculations and the willingness of different agencies to pay for them will impact on whether maternal or elderly vaccines are introduced into low-income settings.

A similar approach will need to be taken for monoclonal antibodies, which have historically been more expensive than vaccines. At the time of writing, the price per dose of nirsevimab in the USA was $495 per 100 mg dose according to data from the American Academy of Pediatrics (accessed December 2023 [67]). Modeling from Canada using CAD$50,000 per quality-adjusted life year (QALY) gained suggests nirsevimab is cost-effective at $290 per dose [68]. UK modeling (using a QALY of GB£20,000) suggests the price point would be £63 per dose (approximately CAD$107 based on an exchange rate of 2:1 at the time of writing) [69]. Different healthcare systems will have ongoing negotiations with providers of vaccines and antibodies. The prices of monoclonal manufacturing are coming down rapidly, with a rabies monoclonal priced at $20 a dose [70]. However, estimates suggest that a product would need to be less than US$4 per dose to be cost-effective [71]. Alternate suppliers or antibodies may lead to the reduction in price needed to achieve global cost–effectiveness.

Virological factors

A critical factor for the success or failure of both antibodies and vaccines for RSV will be virus evolution and escape. All licensed products to date target the RSV-F protein. RSV-F is more conserved than the other RSV surface glycoprotein, G, but RSV can still evolve to escape monoclonal antibodies, as has been seen for palivizumab [72]. Importantly it only took two amino acid substitutions in RSV B strains to cause the failure of suptavumab [23]. Before the introduction of nirsevimab (between 1956 and 2021), its binding site has been highly conserved, but potential resistance mutants are already circulating at a low frequency (<1%) [73]. The risk is that the antibody will select for escape mutations or drive new ones, and thereby reduce its efficacy. An alternative approach is to apply a cocktail of monoclonals, but this will impact cost–effectiveness. Even then, based on experience with COVID monoclonals, respiratory viruses can still evolve to escape a cocktail of antibodies [74]. Because vaccines induce a more polyclonal response, they are potentially less susceptible to viral escape. However, use in elderly adults who may also be immunocompromised might accelerate viral mutation. The cause of viral escape, whether it is natural immunity, vaccine, or antibody, ultimately doesn't matter if

the virus does change, rendering treatments ineffective. There is a need for surveillance of circulating RSV genomic variability and the WHO has established a program that will be invaluable in ongoing efforts to control RSV [75,76]. The recent development of a robust and sensitive amplicon-based whole-genome sequencing assay should help these efforts [77].

A second challenge is viral replacement. This is a theoretical risk and relates to the idea that infection with one respiratory virus may inhibit concurrent infection with another. The COVID-19 pandemic definitely led to changes in patterns of respiratory viral spread [78], which was most likely due to non-pharmaceutical interventions [79], but may have had an element of SARS-CoV-2 displacing other viruses. A similar pattern was seen during the H1N1 pandemic in 2009, when the RSV season was delayed in France [80]. There is also a suggestion that RSV infection suppresses rhinovirus infections [81]. One proposed mechanism for this is that the first viral infection induces an interferon response that can then inhibit subsequent infections [82]. Delaying or removing RSV may open the way for other respiratory infections, particularly human metapneumovirus and rhinovirus, so surveillance for these other agents will be important. But it should also be noted that co-infection or at least co-carriage of respiratory viruses is common, so this may not be an issue.

Immunological factors

An important question is how long will immunity to RSV last? The commonly held view is that the immune memory to RSV is short-lived and that re-infection is common. This received view is based on a relatively small number of primary studies. One of the most cited studies supporting the idea of RSV re-infection followed volunteers who had had natural RSV infection and were deliberately challenged with RSV multiple times afterward. There are some caveats with this study, the challenge virus had been passaged 20 times through three different cell lines, two of which were bovine origin [83], which could have induced some mutations into the virus, especially the bovine cell line. Infection was defined as a rise in antibody titer—under this definition 11 of the 15 subjects were 're-infected' and 50% of them had three or more increases in antibody; whether this counts as an infection or just the recall immune response to the virus is unclear. There was some detectable virus, but by later infectious challenges, the duration of shedding was 1 day, which could potentially be residual inoculum. The infections were either asymptomatic or mild. Anti-F titers were higher in those who were not infected, but since infection was defined as a fourfold increase in titer, there may be a mathematical issue—an increase might not have been seen because antibody titer was already at peak.

There is other evidence to support RSV re-infection. In a surveillance study using PCR, re-infections were detected in 23 out of 55 infants a year after their first infection; interestingly, this did not correlate with T cell levels to RSV [84]. This supports data from earlier longitudinal studies looking for recoverable virus and change in antibody response—one that followed children over a 10-year period [85] and another that followed them for 5 years [86]. A re-infection rate of 0.25% was observed in a much larger study that looked at an insurance database, but it used International Classification of Diseases codes rather than direct measurement of infection [87]. Another study inferred re-infection by the detection of RSV in adults in the US Marine Corps—the argument being that if there was RSV in an adult it was a re-infection due to the ubiquity of childhood infection [88]. Apart from the PCR study, none of these studies would have looked at RSV genome sequence to determine whether re-infection was with the exact same strain or there were subtle shifts.

Taken together, these studies suggest that RSV can re-infect. It is unlikely to be unique

in this respect. Waning immunity is also likely to facilitate repeat infections of other respiratory viruses, though they are likely to be less severe on subsequent infection. If respiratory viruses are looked for in large cohorts using sensitive molecular tools, they are likely to be found in asymptomatic individuals. During the COVID pandemic, re-infection was observed with the same strain of SARS-CoV-2 virus [89], though most re-infections were with different strains. There is also evidence for re-infection with the same strain of influenza during an outbreak on the isolated island community of Tristan da Cunha in 1971 [90].

The question is what does waning immunity mean for the protection offered by vaccines? In infants there is a similar issue for both maternal vaccination and monoclonal antibody strategies—neither of them induce an active immune response in the infant so there is a linear decline in antibody over time. The timing of maternal vaccination will be important to maximize the protection given [91]. One challenge for maternal vaccination is that premature babies are born before the gestational age of 32–36 weeks that is recommended by the US FDA for maternal vaccination, so will not get any additional antibody from the mother. This is problematic as premature babies are at the highest risk of severe disease; however, a monoclonal antibody could be used to backfill the immune gap. A theoretical concern is that when antibody falls below a certain threshold it may no longer neutralize the virus but instead potentiate its infectivity. This has been postulated to be one of the reasons why the FI-RSV vaccine failed, but is unlikely in the case of vaccination with pre-F. A related issue is whether antibody-mediated protection prevents infection or reduces severity of disease after infection. Data from the nirsevimab clinical trials indicate that antibody recipients seroconverted to post-F (i.e., the epitope not recognized by nirsevimab) during their first year of life, suggesting that they were still infected with RSV, but did not develop disease [92].

The impact of waning antibody will depend on what the major driver of RSV disease is. If in infants, it is an airways/plumbing issue, and severity is due to smaller airways, then moving infection to later in life should reduce disease severity. But 6 months of age is not a magic cut-off—there is a substantial burden of hospitalizations and deaths in older children up to age 5 [1], so top-up immunization will be important. Whilst an mRNA vaccine is possible and currently in late-phase clinical trials, we believe infant boosting is likely to take the form of a live-attenuated vaccine if the problems of balancing infectivity and immunogenicity can be achieved. New candidates are relatively advanced: RSV/ΔNS2/Δ1313/I1314L lacks the *NS2* gene and has temperature-sensitive mutations in other parts of the genome, and RSV/276 lacks the *M2-2* gene. Both have been trialed in children aged 6–24 months, and were infectious and well tolerated, leading to a four-fold rise in serum neutralizing titers in 60% (RSV/ΔNS2/Δ1313/I1314L) and 92% (RSV/276) of vaccinees [93]. An alternative parainfluenza virus type 5 (PIV5)-vectored vaccine has recently completed Phase 1 clinical trials in adults [94]. Mucosally delivered vaccines are likely to have the benefit of inducing resident memory T cells, which have been shown to be protective in pre-clinical models [95], but whether infants make resident memory T cells needs further investigation [96].

In the elderly who are antigenically experienced with RSV, any RSV vaccine is effectively a booster, but there will be complex natural history of infection that may affect the quality of response, with the potential to expand poorly protective epitopes due to original antigenic sin [97]. T cells might also play a protective role in the elderly—a recent study showed that functional T cell responses were associated with asymptomatic infection [98]. In the elderly, it is likely that there will need to be booster vaccinations to keep antibody levels high. In a Phase 2a study of

RSVPreF3-AS01E (now Arexvy), boosting at 2 months had no effect on antibody titer, but a subsequent re-boost at 20 months led to a twofold rise in levels [99].

In general, more research is needed to identify correlates of protection for RSV [100], these will help with bridging studies for future vaccines and determining the optimum schedule for boosters. The most likely candidate is neutralizing antibody in blood, but RSV-F binding antibody and nasal IgA both have potential. Human infection challenge studies, both historic [101] and recent [102], indicate that the presence of antibody contributes to protection but in and of itself is not enough to prevent infection. Whether the same correlates of protection can be used for infants and the elderly is also an important question.

Societal factors

A final challenge is getting people to use the vaccines, especially pregnant women in the context of the safety signal relating to preterm birth, which halted the GSK trial. In a survey sample comprised of 315 respondents, 70.2% showed willingness to get the RSV vaccine, whereas 15.2% resisted, and 14.6% were hesitant [103]. As only 67.6% of all respondents had heard of RSV before the study, it was inevitable that RSV vaccine acceptance was associated with concerns about potential risks to themselves or their fetuses. In a separate study with 427 participants in Italy, only 45.9% were willing to be vaccinated during pregnancy, and this was more likely among those with a university degree [104]. The likelihood of vaccination by parents was higher for RSV than COVID-19 or influenza [105]. Another study indicated a relatively low awareness of RSV in UK midwives [106], which is of particular concern as they are likely to be the ones recommending vaccination. Future promotion efforts should focus on enhancing RSV vaccine education at the community level to address safety concerns, as well as improving communication strategies to effectively promote the benefits of the vaccine. Interestingly, uptake rates of nirsevimab antibody have been very high, suggesting there may be different levels of acceptance for the different strategies [107].

CONCLUSION

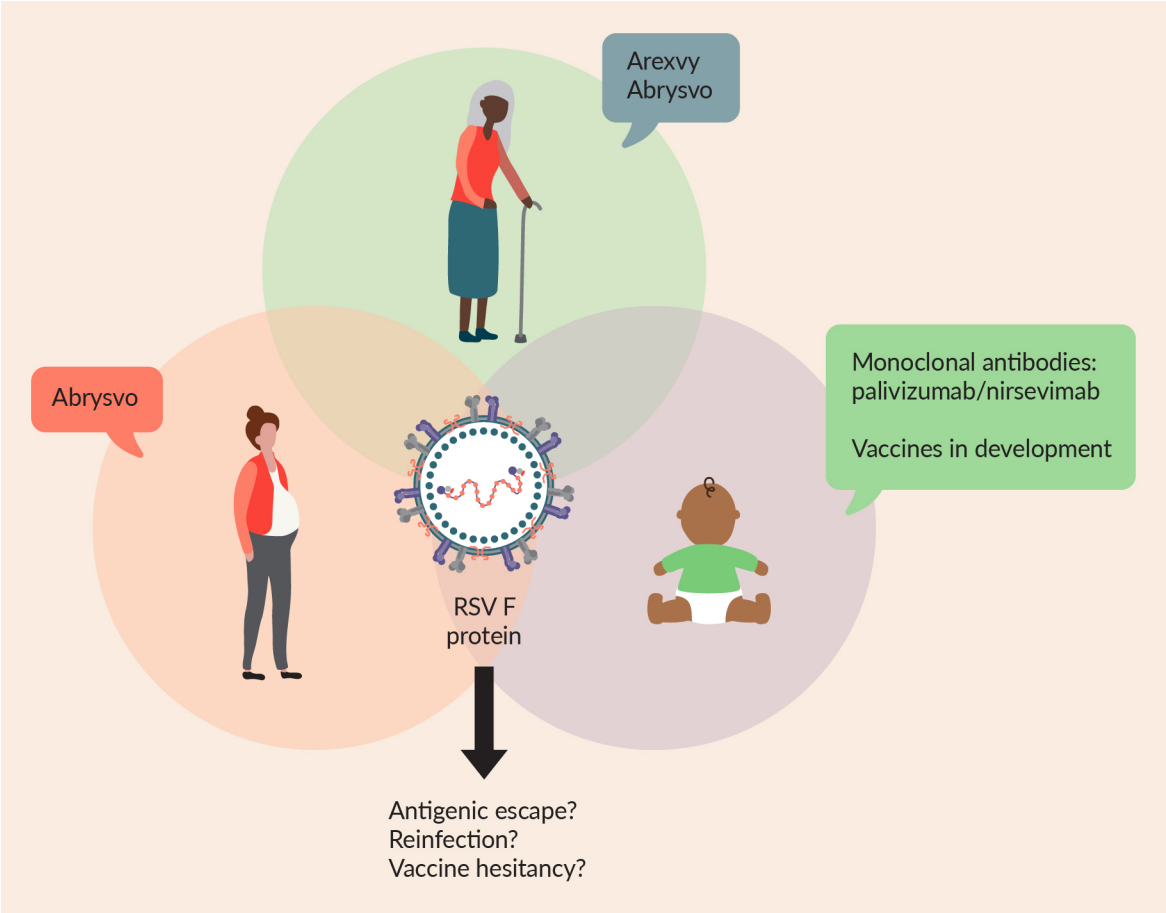
It is a remarkable achievement that after nearly 70 years with no vaccine, there are now three vaccines for RSV, approved within the space of 1 year. This is complemented by the licensure of one antibody and another on the horizon. They will undoubtedly have a major impact on the burden of disease. A modeling study suggests that vaccines could avert 2.4 million infections in the UK alone in a low-transmission scenario and slightly fewer (2 million) in a high-transmission scenario [108], which could translate into 12,000 fewer hospitalizations. There are several potential hurdles to successful implementation, but these are not insurmountable.

TRANSLATION INSIGHT

After 70 years, there has been a sudden surge of licensed approaches to prevent severe RSV disease. Critically, these are available for both age groups that are susceptible to severe RSV disease, the very young and very old (Figure 1). For infants, there is passive protection in the form of antibodies and maternal vaccination; for the over 60s there are two vaccines. There are a number of implementation issues that need to be addressed to ensure equitable access to these products. For the monoclonal antibodies, the main concerns are viral escape leading to reduction of potency and the cost of product. The cost will have a particular impact in LMIC where the burden of severe disease is greatest. For the maternal vaccine, the main concern is vaccine hesitancy; better communication and further safety studies will be required to improve uptake. For the elderly vaccine, communication of the risk of RSV and the need for vaccination will be

FIGURE 1

Options and possible issues for RSV prevention.



vital. Other areas that will need further development are a working correlate of protection for RSV, enabling future bridging studies and monitoring of replacement of RSV with other winter viruses if the campaigns are successful. Education, sequencing, and monitoring will all be vital in the successful rollout of immunization against RSV.

REFERENCES

1. Li Y, Wang X, Blau DM, *et al.* Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: a systematic analysis. *Lancet* 2022; 399(10340), 2047–64.

2. Zhang S, Akmar LZ, Bailey F, *et al.* Cost of respiratory syncytial virus-associated acute lower respiratory infection management in young children at the regional and global level: a systematic review and meta-analysis. *J. Infect. Dis.* 2020; 222(Suppl. 7), S680–s687.

3. Kumari M, Lu R-M, Li M-C, *et al.* A critical overview of current progress for COVID-19: development of vaccines, antiviral drugs, and therapeutic antibodies. *J. Biomed. Sci.* 2022; 29(1), 68.

4. Fulginiti VA, Eller JJ, Sieber OF, *et al.* Respiratory virus immunization I: a field trial of two inactivated respiratory virus vaccines: an

aqueous trivalent parainfluenza virus vaccine and an alum-precipitated respiratory syncytial virus vaccine. *Am. J. Epidemiol.* 1969; 89, 436–48.

5. Kapikian AZ, Mitchell RH, Chanock RM, *et al.* An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. *Am. J. Epidemiol* 1969; 89(4), 405–21.

6. Kim HW, Canchola JG, Brandt CD, *et al.* Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am. J. Epidemiol.* 1969; 89, 422–34.

7. Chin J, Magoffin RL, Shearer LA, *et al.* Field evaluation of a respiratory syncytial virus vaccine and a trivalent parainfluenza virus vaccine in a pediatric population. *Am. J. Epidemiol.* 1969; 89, 449–63.

8. Openshaw PJ, Tregoning JS. Immune responses and disease enhancement during respiratory syncytial virus infection. *Clin. Microbiol. Rev.* 2005; 18(3), 541–55.

9. Polack FP, Teng MN, Collins L, *et al.* A role for immune complexes in enhanced respiratory syncytial virus disease. *J. Exp. Med.* 2002; 196(6), 859–65.

10. Moghaddam A, Olszewska W, Wang B, *et al.* A potential molecular mechanism for hypersensitivity caused by formalin-inactivated vaccines. *Nat. Med.* 2006; 12(8), 905–7.

11. Graham BS, Henderson GS, Tang YW, *et al.* Priming immunization determines T helper cytokine mRNA expression patterns in lungs of mice challenged with respiratory syncytial virus. *J. Immunol.* 1993; 151, 2032–40.

12. McLellan JS, Chen M, Joyce MG, *et al.* Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus. *Science* 2013; 342(6158), 592–98.

13. McLellan JS, Yang Y, Graham BS, Kwong PD. Structure of respiratory syncytial virus fusion glycoprotein in the postfusion conformation reveals preservation of neutralizing epitopes. *J. Virol.* 2011; 85(15), 7788–96.

14. Flynn JA, Durr E, Swoyer R, *et al.* Stability characterization of a vaccine antigen based on the respiratory syncytial virus fusion glycoprotein. *PLOS ONE* 2016; 11(10), e0164789.

15. Killikelly AM, Kanekiyo M, Graham BS. Pre-fusion F is absent on the surface of formalin-inactivated respiratory syncytial virus. *Sci. Rep.* 2016; 6, 34108.

16. Feltes TF, Cabalka AK, Meissner HC, *et al.* Palivizumab prophylaxis reduces hospitalization due to respiratory syncytial virus in young children with hemodynamically significant congenital heart disease. *J. Pediatr.* 2003; 143(4), 532–40.

17. I-RSVSG. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. *Pediatrics* 1998; 102(3 Pt 1), 531–7.

18. Blanken MO, Rovers MM, Molenaar JM, *et al.* Respiratory syncytial virus and recurrent wheeze in healthy preterm infants. *N. Engl. J. Med.* 2013; 368(19), 1791–9.

19. UKHSA. Respiratory syncytial virus. Chapter 27a. In: *The Green Book*. 2013; GOV.UK.

20. Robbie GJ, Criste R, Dall'acqua WF, *et al.* A novel investigational Fc-modified humanized monoclonal antibody, motavizumab-YTE, has an extended half-life in healthy adults. *Antimicrob. Agents Chemother.* 2013; 57(12), 6147–53.

21. Mejias A, Chavez-Bueno S, Rios AM, *et al.* Comparative effects of two neutralizing anti-respiratory syncytial virus (RSV) monoclonal antibodies in the RSV murine model: time versus potency. *Antimicrob. Agents Chemother.* 2005; 49(11), 4700–7.

22. Carbonell-Estrany X, Simões EA, Dagan R, *et al.* Motavizumab for prophylaxis of respiratory syncytial virus in high-risk children: a noninferiority trial. *Pediatrics* 2010; 125(1), e35-51.

23. Simões EAF, Forleo-Neto E, Geba GP, *et al.* Suptavumab for the prevention of medically attended respiratory syncytial virus infection in preterm infants. *Clin. Infect. Dis.* 2021; 73(11), e4400–e4408.

24. Sanofi. European Commission grants first approval worldwide of Beyfortus® (nirsevimab) for prevention of RSV disease in infants 2022.

25. Hammitt LL, Dagan R, Yuan Y, *et al.* Nirsevimab for prevention of RSV in healthy late-preterm and term infants. *N. Engl. J. Med.* 2022; 386(9), 837–46.

26. Domachowske J, Madhi SA, Simões EAF, *et al.* Safety of nirsevimab for RSV in infants with heart or lung disease or prematurity. *N. Engl. J. Med.* 2022; 386(9), 892–4.

27. Drysdale SB, Cathie K, Flamein F, *et al.* Nirsevimab for prevention of hospitalizations due to RSV in infants. *N. Engl. J. Med.* 2023; 389(26), 2425–35.

28. Martinón-Torres F, Mirás-Carballal S, Durán-Parrondo C. Early lessons from the implementation of universal respiratory syncytial virus prophylaxis in infants with long-acting monoclonal antibodies, Galicia, Spain, September and October 2023. *Eurosurveillance* 2023; 28(49).

29. NIRSE-GAL.

30. Maas BM, Lommerse J, Plock N, *et al.* Forward and reverse translational approaches to predict efficacy of neutralizing respiratory syncytial virus (RSV) antibody prophylaxis. *EBioMedicine* 2021; 73, 103651.

31. Levi M, Watson S, Anderson AB, *et al.* 383. Pharmacokinetics and safety in healthy adults of RSM01, a novel RSV Monoclonal antibody, and population PK modeling to support pediatric development. *Open Forum Infect. Dis.* 2023; 10(Suppl. 2), ofad500.453.

32. Niewiesk S. Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. *Front. Immunol.* 2014; 5, 446.

33. Bjerkhaug AU, Ramalingham S, Mboizi R, *et al.* The immunogenicity and safety of Group B Streptococcal maternal vaccines: a systematic review. *Vaccine* 2024; 42(2), 84–98.

34. Madhi SA, Polack FP, Piedra PA, *et al.* Respiratory syncytial virus vaccination during pregnancy and effects in infants. *N. Engl. J. Med.* 2020; 383(5), 426–39.

35. Bebia Z, Reyes O, Jeanfreau R, *et al.* Safety and immunogenicity of an investigational respiratory syncytial virus vaccine (RSVPreF3) in mothers and their infants: a Phase 2 randomized trial. *J. Infect. Dis.* 2023; 228(3), 299–310.

36. Hristio B. Maternal RSV vaccine: Further analysis is urged on preterm births. *BMJ* 2023; 381, 1021.

37. Simões EAF, Center KJ, Tita ATN, *et al.* Prefusion F protein-based respiratory syncytial virus immunization in pregnancy. *N. Engl. J. Med.* 2022; 386(17), 1615–26.

38. Kampmann B, Madhi SA, Munjal I, *et al.* Bivalent prefusion F vaccine in pregnancy to prevent RSV illness in infants. *N. Engl. J. Med.* 2023; 388(16), 1451–64.

39. Fleming-Dutra KE, Jones JM, Roper LE, *et al.* Use of the Pfizer respiratory syncytial virus vaccine during pregnancy for the prevention of respiratory syncytial virus-associated lower respiratory tract disease in infants: recommendations of the Advisory Committee on Immunization Practices—United States, 2023*; MMWR Morb. Mortal. Wkly Rep.* 2023; 72(41), 1115–22.

40. Pfizer. US FDA approves ABRYSVO™, Pfizer's vaccine for the prevention of respiratory syncytial virus (RSV) in infants through active immunization of pregnant individuals 32–36 weeks of gestational age 2023.

41. *JCVI*. Respiratory syncytial virus (RSV) immunisation programme for infants and older adults: JCVI full statement, Sep 11, 2023 DHSC (Editor).

42. Rozenbaum MH, Begier E, Kurosky SK, *et al.* Incidence of respiratory syncytial virus infection in older adults: limitations of current data. *Infect. Dis. Ther.* 2023; 12(6), 1487–504.

43. Ruckwardt TJ. The road to approved vaccines for respiratory syncytial virus. *NPJ Vaccines* 2023; 8(1), 138.

44. Papi A, Ison MG, Langley JM, *et al.* Respiratory syncytial virus prefusion F protein vaccine in older adults. *N. Engl. J. Med.* 2023; 388(7), 595–608.

45. Schwarz TF, Hwang SJ, Ylisastigui P, *et al.* Immunogenicity and safety following one dose of AS01E-adjuvanted respiratory syncytial virus prefusion F protein vaccine in older adults: a phase 3 trial. *J. Infect. Dis.* 2023; jiad546.

46. GSK. US Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices votes to recommend Arexvy for the prevention of RSV disease in adults aged 60 and older with shared clinical decision making 2023.

47. GSK. European Commission authorises GSK's Arexvy, the first respiratory syncytial virus (RSV) vaccine for older adults 2023.

48. GSK. Medicines and Healthcare products Regulatory Agency authorises GSK's Arexvy, the first respiratory syncytial virus (RSV) vaccine for older adults 2023.

49. GSK. GSK's Arexvy, the first respiratory syncytial virus (RSV) vaccine for older adults approved in Canada 2023.

50. GSK. Japan's Ministry of Health, Labour and Welfare approves GSK's Arexvy, the country's first respiratory syncytial virus (RSV) vaccine for older adults 2023.

51. Walsh EE, Pérez Marc G, Zareba AM, *et al.* Efficacy and Safety of a bivalent RSV prefusion F vaccine in older adults. *N. Engl. J. Med.* 2023; 388(16), 1465–77.

52. Pfizer. US FDA approves ABRYSVO™, Pfizer's vaccine for the prevention of respiratory syncytial virus (RSV) in older adults 2023.

53. Pfizer. European Commission approves Pfizer's ABRYSVO™ to help protect infants through maternal immunization and older adults from RSV 2023.

54. Barrie R. MHRA approves GSK's RSV vaccine for older adults in the UK, in *Pharmaceutical Today*. 2023.

55. *DHSC*. Respiratory syncytial virus (RSV) immunisation programme for infants and older adults: JCVI full statement. Sep 11, 2023; DoHaS Care (Editor).

56. Falsey AR, Williams K, Gymnopoulou E, *et al.* Efficacy and safety of an Ad26.RSV.preF-RSV preF protein vaccine in older adults. *N. Engl. J. Med.* 2023; 388(7), 609–620.

57. Sadoff J, De Paepe E, DeVincenzo J, *et al.* Prevention of respiratory syncytial virus infection in healthy adults by a single immunization of Ad26.RSV.preF in a human challenge study. *J. Infect. Dis.* 2022; 226(3), 396–406.

58. Jordan E, Kabir G, Schultz S, *et al.* Reduced respiratory syncytial virus load, symptoms, and infections: a human challenge trial of MVA-BN-RSV vaccine. *J. Infect. Dis.* 2023; 228(8), 999–1011.

59. Bavarian Nordic. Bavarian Nordic provides update on RSV vaccine program 2023.

60. Wilson E, Goswami J, Baqui AH, *et al.* Efficacy and safety of an mRNA-based RSV preF vaccine in older adults. *N. Engl. J. Med.* 2023; 389(24), 2233–44.

61. Moderna. Statement on RSVVW abstract on mRNA-1345 2024.

62. Moderna. Moderna announces global regulatory submissions for its respiratory syncytial virus (RSV) vaccine, MRNA-1345 2023.

63. Fitzpatrick MC, Laufer RS, Baral R, *et al.* Report of the WHO technical consultation on the evaluation of respiratory syncytial virus prevention cost effectiveness in low- and middle-income countries, Apr 7–8, 2022. *Vaccine* 2023; 41(48), 7047–59.

64. Schneider MT, Chang AY, Crosby SW, *et al.* Trends and outcomes in primary health care expenditures in low-income and middle-income countries, 2000–2017. *BMJ Glob. Health* 2021; 6(8).

65. Baral R, Li X, Willem L, *et al.* The impact of maternal RSV vaccine to protect infants in Gavi-supported countries: estimates from two models. *Vaccine* 2020; 38(33), 5139–47.

66. Li Y, Kulkarni D, Begier E, *et al.* Adjusting for case under-ascertainment in estimating RSV hospitalisation burden of older adults in high-income countries: a systematic review and modelling study. *Infect. Dis. Ther.* 2023; 12(4), 1137–49.

67. AACP. Nirsevimab (Beyfortus) product & ordering information 2023.

68. Shoukat A, Abdollahi E, Galvani AP, *et al.* Cost–effectiveness analysis of nirsevimab and maternal RSVpreF vaccine strategies for prevention of respiratory syncytial virus disease among infants in Canada: a simulation study. *Lancet Reg. Health Am.* 2023; 28, 100629.

69. Hodgson D, Koltai M, Krauer F, *et al.* Optimal respiratory syncytial virus intervention programmes using nirsevimab in England and Wales. *Vaccine* 2022; 40(49), 7151–7.

70. *Trust W, IAVI.* Expanding access to monoclonal antibody-based products: a global call to action 2020.

71. Srikantiah P, Klugman KP. New respiratory syncytial virus immunization products in low- and middle-income countries: potential for cost-effective impact on a high burden of disease in young infants. *BMC Med.* 2023; 21(1), 177.

72. Zhu Q, McAuliffe JM, Patel NK, *et al.* Analysis of respiratory syncytial virus preclinical and clinical variants resistant to neutralization by monoclonal antibodies palivizumab and/or motavizumab. *J. Infect.Dis.* 2011; 203(5), 674–82.

73. Wilkins D, Langedijk AC, Lebbink RJ, *et al.* Nirsevimab binding-site conservation in respiratory syncytial virus fusion glycoprotein worldwide between 1956 and 2021: an analysis of observational study sequencing data. *Lancet Infect. Dis.* 2023; 23(7), 856–66.

74. Tuekprakhon A, Nutalai R, Dijokaite-Guraliuc A, *et al.* Antibody escape of SARS-CoV-2 Omicron BA.4 and BA.5 from vaccine and BA.1 serum. *Cell* 2022; 185(14), 2422–2433.e13.

75. Pebody R, Moyes J, Hirve S, *et al.* Approaches to use the WHO respiratory syncytial virus surveillance platform to estimate disease burden. *Influenza Other Respir. Viruses* 2020; 14(6), 615–21.

76. World Health Organization. Progress and future directions of WHO respiratory syncytial virus surveillance—report from the WHO meeting in November 2022. *Wkly Epidemiol. Rec.* 2023; 98(15), 159–63.

77. Talts T, Mosscrop LG, Williams D, *et al.* Robust and sensitive amplicon-based whole-genome sequencing assay of respiratory syncytial virus subtype A and B. *Microbiol. Spectr.* 2024; e0306723.

78. Chow EJ, Uyeki TM, HY Chu, The effects of the COVID-19 pandemic on community respiratory virus activity. *Nat. Rev. Microbiol.* 2023; 21(3), 195–210.

79. Mosscrop LG, Williams TC, Tregoning JS. Respiratory syncytial virus after the SARS-CoV-2 pandemic—what next? *Nat. Rev. Immunol.* 2022; 22(10), 589–90.

80. Casalegno JS, Ottmann M, Bouscambert-Duchamp M, *et al.* Impact of the 2009 influenza A(H1N1) pandemic wave on the pattern of hibernal respiratory virus epidemics, France, 2009. *Eurosurveillance* 2010; 15(6), 19485.

81. Achten NB, Wu P, Bont L, *et al.* Interference between respiratory syncytial virus and human rhinovirus infection in infancy. *J. Infect. Dis.* 2017; 215(7), 1102–1106.

82. Essaidi-Laziosi M, Geiser J, Huang S, *et al.* Interferon-dependent and respiratory virus-specific interference in dual infections of airway epithelia. *Sci. Rep.* 2020; 10(1), 10246.

83. Hall CB, Walsh EE, Long CE, Schnabel KC. Immunity to and frequency of reinfection with respiratory syncytial virus. *J. Infect. Dis.* 1991; 163(4), 693–8.

84. Bont L, Versteegh J, Swelsen WT, *et al.* Natural reinfection with respiratory syncytial virus does not boost virus-specific T-cell immunity. *Pediatr. Res.* 2002; 52(3), 363–7.

85. Henderson FW, Collier AM, Clyde WAJ, Denny FW. Respiratory-syncytial-virus infections, reinfections and immunity. A prospective, longitudinal study in young children. *N. Engl. J. Med.* 1979; 300, 530–4.

86. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. *Am. J. Dis. Child.* 1986; 140(6), 543–6.

87. Nduaguba SO, Tran PT, Choi Y, Winterstein AG. Respiratory syncytial virus reinfections among infants and young children in the United States, 2011-2019. *PLoS One* 2023; 18(2), e0281555.

88. Johnson KM, Bloom HH, Mufson MA, Chanock RM. Natural reinfection of adults by respiratory syncytial virus. *N. Engl. J. Med.* 1962; 267(2), 68–72.

89. Ren X, Zhou J, Guo J, *et al.* Reinfection in patients with COVID-19, a systematic review. *Glob. Health Res. Policy* 2022; 7(1), 12.

90. Camacho A, Ballesteros S, Graham AL, *et al.* Explaining rapid reinfections in multiple-wave influenza outbreaks: Tristan da Cunha 1971 epidemic as a case study. *Proc. Biol. Sci.* 2011; 278(1725), 3635–43.

91. Zhong Z, Haltalli M, Holder B, *et al.* The impact of timing of maternal influenza immunization on infant antibody levels at birth. *Clin. Exp. Immunol.* 2019; 195(2), 139–52.

92. Wilkins D, Y Yuan, Y Chang, *et al.* Durability of neutralizing RSV antibodies following nirsevimab administration and elicitation of the natural immune response to RSV infection in infants. *Nat. Med.* 2023; 29(5), 1172–9.

93. Cunningham CK, Karron RA, Muresan P, *et al.* Evaluation of recombinant live-attenuated respiratory syncytial virus (RSV) vaccines RSV/ΔNS2/Δ1313/I1314L and RSV/276 in RSV-seronegative children. *J. Infect. Dis.* 2022; 226(12), 2069–78.

94. Spearman P, Jin H, Knopp K, *et al.* Intranasal parainfluenza virus type 5 (PIV5)-vectored RSV vaccine is safe and immunogenic in healthy adults in a Phase 1 clinical study. *Sci. Adv.* 2023; 9(43), eadj7611.

95. Kinnear E, Caproni LJ, Tregoning JS. A comparison of red fluorescent proteins to model DNA vaccine expression by whole animal in vivo imaging. *PLoS One* 2015; 10(6), e0130375.

96. Zens KD, Chen JK, Guyer RS, *et al.* Reduced generation of lung tissue–resident memory T cells during infancy. *J. Exp. Med.* 2017; 214(10), 2915–32.

97. Tripp RA, Power UF. Original antigenic sin and respiratory syncytial virus vaccines. *Vaccines (Basel)* 2019; 7(3), 107.

98. Salaun B, De Smedt J, Vernhes C, *et al.* T cells, more than antibodies, may prevent symptoms developing from respiratory syncytial virus infections in older adults. *Front. Immunol.* 2023; 14, 1260146.

99. Leroux-Roels I, Van Ranst M, Vandermeulen C, *et al.* Safety and immunogenicity of a revaccination with a respiratory syncytial virus prefusion F vaccine in older adults: a Phase 2b study. *J. Infect. Dis.* 2024; 229(2), 355–66.

100. Abu-Raya B, Reicherz F, Lavoie PM. Correlates of protection against respiratory syncytial virus infection in infancy. *Clin. Rev. Allergy Immunol.* 2022; 63(3), 371–80.

101. Kravetz HM, Knight V, Chanock RM, *et al.* Respiratory syncytial virus: III. Production of illness and clinical observations in adult volunteers. *JAMA* 1961; 176,

102. Kherfan T, Sallam M. Prospective attitudes towards respiratory syncytial virus (RSV) vaccination: validation of a survey instrument among young females in Jordan pending vaccine authorization. *Vaccines (Basel)* 2023; 11(8), 1386.

103. Miraglia del Giudice G, Sansone V, Airoma F, *et al.* Respiratory syncytial virus: willingness towards a future vaccine among pregnant women in Italy. *Vaccines* 2023; 11, 1691.

104. Haeder SF. Assessing parental intention to vaccinate against COVID-19, influenza, and RSV in the United States in late 2023. *Vaccine* 2023; 41(50), 7503–14.

105. Wilcox CR, Calvert A, Metz J, *et al.* Attitudes of pregnant women and healthcare professionals toward clinical trials and routine implementation of antenatal vaccination against respiratory syncytial virus: a multicenter questionnaire study. *Pediatr. Infect. Dis. J.* 2019; 38(9), 944–51.

106. Martinón-Torres F, Mirás-Carballal S, Durán-Parrondo C. Early lessons from the implementation of universal respiratory syncytial virus prophylaxis in infants with long-acting monoclonal antibodies, Galicia, Spain, September and October 2023. *Eurosurveillance* 2023; 28(49), 2300606.

107. Du Z, Wang L, Bai Y, *et al.* Mitigation of respiratory syncytial virus epidemics by RSVpreF vaccines after the COVID-19 pandemic in the UK: a modelling study. *Lancet* 2023; 402(Suppl. 1), S39.

108. Zhu Q, Lu B, McTamney P, *et al.* Prevalence and significance of substitutions in the fusion protein of respiratory syncytial virus resulting in neutralization escape from antibody MEDI8897. *J. Infect. Dis.* 2018; 218(4), 572–80.

109. Wilkins D, Langedijk AC, Lebbink RJ, *et al.* Nirsevimab binding-site conservation in respiratory syncytial virus fusion glycoprotein worldwide between 1956 and 2021: an analysis of observational study sequencing data. *Lancet Infect. Dis.* 2023; 23(7), 856–66.

110. Maas BM, Lommerse J, Plock N, *et al.* Forward and reverse translational approaches to predict efficacy of neutralizing respiratory syncytial virus (RSV) antibody prophylaxis. *EBioMedicine* 2021; 73, 103651.

AFFILIATIONS

John Tregoning
Department of Infectious Disease, Imperial College London, UK

Haoyuan Li
Department of Infectious Disease, Imperial College London, UK

Chubicka Thomas
Department of Infectious Disease, Imperial College London, UK

Ziyin Wang
Department of Infectious Disease, Imperial College London, UK

Lucy G Mosscrop
Department of Infectious Disease, Imperial College London, UK

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: The authors thank Thomas Williams for useful feedback. Lucy Mosscrop is supported by an NIHR/ HPRU studentship, jointly funded by Imperial College.

Disclosure and potential conflicts of interest: The authors have no conflicts of interest.

Funding declaration: John Tregoning and Lucy G Mosscrop are funded by the HRPU in respiratory infections.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited; externally peer reviewed.

Submitted for peer review: Jan 9, 2024; **Revised manuscript received:** Mar 1, 2024; **Publication date:** Mar 13, 2024.

RESPIRATORY DISEASES

SPOTLIGHT

INTERVIEW

Charting a course for better pneumococcal vaccines

A new platform for developing bacterial vaccines—the multiple antigen presenting system—could enable an affordable alternative to current pneumococcal conjugate vaccines. Here, the multiple antigen presenting system co-inventor **Rick Malley,** Senior Physician in Pediatrics, Boston Children's Hospital, and Professor of Pediatrics, Harvard Medical School, speaks with **Charlotte Barker,** Editor, *Vaccine Insights*, about the fast-evolving pneumococcal vaccine field.

Vaccine Insights 2024; 3(1), 7–12

DOI: 10.18609/vac.2024.003

Q How did you first get involved in vaccine development?

RM: Starting my career in pediatrics at Boston Children's Hospital in the early 1990s, I was very impressed by the impact of the Haemophilus influenzae type B (Hib) conjugate vaccine.

Conjugate vaccines were a new development at the time. By conjugating proteins to polysaccharides found in the bacterial surface we can essentially trick the body into responding to the polysaccharide as if it were a protein, and therefore elicit a more robust immune response. Importantly, it allows babies under the age of 2 years to make antibodies to bacterial polysaccharides, which they could not otherwise do.

During my internship, I cared for many children with meningitis and other forms of invasive disease caused by Hib, but a year later, the disease was virtually eliminated in the US after

the conjugate vaccine was introduced. A few years later, I was a pediatric infectious diseases fellow working in a laboratory when I was lucky enough to meet one of the scientists behind the Hib vaccine: Dr Porter Anderson.

He told me he was working on one last research effort before his planned retirement: the development of an affordable vaccine against pneumococcus (*Streptococcus pneumoniae*), the pathogen most often associated with bacterial pneumonia. I was inspired by this mission, and eventually turned it into a full-blown research project, with Porter as my mentor. Porter has still not retired and the collaboration we started in the mid-1990s persists to this day!

Inspired by Porter, I realized that I wanted to dedicate myself to vaccine development; specifically, vaccines for countries that cannot afford the very expensive vaccines that are currently on the market. That has been one of the guiding principles of my research for the last 20-plus years at Boston Children's Hospital.

Q What impact did you see in your practice—and in the wider population—after the introduction of pneumococcal conjugate vaccine 7?

RM: When pneumococcal conjugate vaccine 7/Prevnar® (which Porter also worked on) was introduced in 2000, some doctors hoped that we would see the eradication of pneumococcus as a cause of pneumonia, bacteremia, meningitis, ear infections, and so on. While there was certainly a very remarkable decline, it was not anywhere near as dramatic as the Hib vaccine experience.

Many bacteria cover their surface with polysaccharides, which define their serotype. Whereas Haemophilus influenzae consists of only a few serotypes that cause disease in humans (of which type b was predominant), pneumococcus has more than 90 serotypes. The polysaccharide included in the vaccine confers protection against only some strains of that serotype; as we vaccinate against some serotypes, others emerge to take their place. It's like a game of whack-a-mole! My friend and colleague Marc Lipsitch predicted this serotype replacement effect as early as 1997 [1] and that is exactly what happened with pneumococcal vaccines [2].

Q How have PCVs evolved?

RM: While serotype replacement did not eliminate the benefit of the conjugate vaccine strategy, it mitigated it significantly. In fact, we saw the rise of several serotypes not included in the original vaccine; serotype 19A was particularly problematic, as it was highly virulent and antibiotic-resistant, causing severe cases of meningitis and sepsis in children.

Inspired by Marc's work, Porter and I realized that a strategy based on polysaccharide capsule immunity would require adding more and more serotypes, such that the cost of the vaccine, instead of reducing over time, would remain high or even rise. Sadly, that is what we have observed with the advent of PCV13, and now PCV15 and PCV20.

It is important to say that these vaccines are phenomenal; they have a very impressive effect on most of the serotypes included. But they are hard to produce and very expensive. Quality control issues make it difficult for other vaccine companies, especially those

in lower- and middle-income countries, to manufacture. And due to serotype replacement, they have not eradicated pneumococcal disease altogether.

Interestingly, if you examine the immune response to the 13 serotypes also covered by PCV13, they are lower in PCV20 [3]. We don't yet know why this happens, nor the clinical consequences (if any) for children or adults. It is possible that simply adding more serotypes means the body is unable to produce as strong a response to each. A phenomenon in conjugate vaccines called carrier-induced epitopic suppression has been described, a situation in which using the same carrier over and over in different vaccines may exhaust the T cells' ability to respond [4].

Q How did you set about trying to overcome these limitations?

RM: Initially, I was working on a whole-cell pneumococcal vaccine. Essentially, we stripped the bacteria of its polysaccharide capsule, made some mutations to enhance immunogenicity, and used the whole cell (minus the capsule) as a vaccine. This is a much cheaper approach than a conjugate vaccine and could be produced for pennies per dose. My group and I worked on the whole-cell vaccine for many years, in collaboration with Instituto Butantan in Brazil and with support from the Bill & Melinda Gates Foundation (BMGF) and PATH [5], and it ultimately reached Phase 2 trials in toddlers in Kenya.

However, while the vaccine itself is cheap to produce, the clinical development plan proved complicated. Since the antigens being targeted are entirely different from the current conjugate vaccines, it is not possible to use existing correlates of protection. While PCV13 and PCV20 could be compared directly with their predecessors, one approach to obtain licensure of a new whole-cell vaccine could potentially require large-scale efficacy clinical trials enrolling thousands of patients. Efforts in this area are being pursued nevertheless, evaluating different clinical endpoints that may require fewer subjects.

Meanwhile, my close colleagues at Children's, Fan Zhang and Yingjie Lu, and I developed a new technology: the multiple antigen presenting system (MAPS).

Q How does MAPS differ from the chemistry used in conjugate vaccines?

RM: In traditional conjugation technology, you chemically couple polysaccharides to a protein. Multiple proteins are entangled with multiple strands of polysaccharide in what we often refer to as a 'spaghetti and meatball' configuration.

It is a very good technology, but it is inefficient and requires a huge amount of expertise to ensure that the chemistry is right. In addition, in existing vaccines, the protein is not an immunogen in its own right and confers no protection.

With MAPS, a biotin molecule is bound to a polysaccharide, and a rhizavidin molecule is fused to a protein (which can be derived from the targeted organism). Biotin and rhizavidin have an extremely high affinity for each other, similar to the affinity between biotin and egg avidin, so when the molecules are combined, the polysaccharide and protein become tightly affinity-linked. These affinity links create a more consistent and ordered structure than traditional conjugation and leave both polysaccharide and protein molecules chemically intact.

"Many have tried and failed to date, but a vaccine targeting pneumococcal proteins is a very attractive idea—it would be inexpensive, and you could even imagine a mucosal administration through the nose or skin."

The manufacturing process for MAPS is less complex and more efficient than traditional conjugation, and therefore cheaper and more suitable for technology transfer to lower- and middle-income countries. Plus, in animal and human studies, we have observed that the immunogenicity of MAPS-based vaccines seems to be superior to conjugate vaccines. Even though this is not a covalent bond, the immune system sees molecules linked by MAPS as if they were conjugated together.

Q What is the status of the MAPS pneumococcal vaccine, now being developed by GSK?

RM: With funding from the BMGF and others, we spun out Affinivax in 2014, to develop vaccines based on the MAPS technology. Affinivax was acquired by GSK in 2022, and I remain a consultant to the MAPS program.

I'm interested in every aspect of vaccine development, but I recognize that a small company like Affinivax successfully bringing vaccines to the market on a global scale would be very unusual, so putting the technology in the hands of a highly experienced pharmaceutical company like GSK is the best way to optimize the chances of success.

At Affinivax, we started by targeting 24 polysaccharides and two pneumococcal proteins [6], then expanded to more than 30 polysaccharides and four proteins. The first version successfully completed Phase 2 in adults, will soon enter a Phase 3 clinical trial in older adults, and is now in Phase 2 trial in infants. We are hoping that the combination of so many polysaccharides, plus multiple proteins, might be the best way to control this organism and avoid the whack-a-mole problem that has plagued previous vaccines.

Q Is there hope for a fully serotype-independent pneumococcal vaccine in the future?

RM: This is an area where theory and practice are still quite far apart, but progress is being made. Our efforts on a whole-cell vaccine are ongoing and an Australian company, GPN Vaccines, is also developing a whole-cell vaccine. Many have tried and failed to date, but a vaccine targeting pneumococcal proteins is a very attractive idea—it would be inexpensive, and you could even imagine a mucosal administration through the nose or skin.

Scientifically, there are lots of exciting approaches that I'm still interested in working on, but practically, you have to compete with the remarkable efficacy of polysaccharide-based vaccines. Therefore, the approach we took with the MAPS pneumococcal vaccine—including proteins primarily as a means of enhancing the immune response to the polysaccharide—offers massive advantages. Time will tell if this vaccine also results in universal protection

through protein-mediated immunity, to protect against serotypes that evade or are not covered by conjugate vaccines, and/or whether a protein-only-based approach can be successful in eradicating pneumococcal disease. That is the holy grail.

Q What does the future hold for pneumococcal vaccines?

RM: All in all, these are very exciting times for pneumococcal vaccine research. There is a lot of energy in this space because it is an important and global public health issue. Sanofi is making a 21-valent vaccine, Vaxcyte is evaluating a 24-valent and a 31-valent vaccine, and GSK is working on a 24-valent as well as a 30-plus-valent vaccine.

It's very exciting to me, not just as a vaccine researcher, but as a clinician who wants to see a vaccine that can tackle the remaining pneumococcal disease in the US and across the world. The COVID-19 pandemic reminded us, if there ever was any need, that we live in a global community. I think people are increasingly recognizing that the massive inequity in vaccine deployment across the world is both morally unacceptable and a huge risk from a pandemic-preparedness standpoint. The idea that we're making vaccines that are not just for the wealthy but for all, regardless of the geographical accident of their birth, is something that has motivated me throughout my career and is still fueling my research today.

REFERENCES

1. Lipsitch M. Vaccination against colonizing bacteria with multiple serotypes. *Proc. Natl. Acad. Sci. USA* 1997; 94(12), 6571–6576.
2. Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. *Lancet* 2011; 378(9807), 1962–1973.
3. Watson W. PCV20 Phase 2/3 study results among children. Presented at: Advisory Committee on Immunization Practices (ACIP) Meeting. Feb 22–24, 2023.
4. Dagan R, Eskola J, Leclerc C, Leroy O. Reduced response to multiple vaccines sharing common protein epitopes that are administered simultaneously to infants. *Infect. Immun.* 1998; 66(5), 2093–2098.
5. Keech CA, Morrison R, Anderson P *et al.* A Phase 1 randomized, placebo-controlled, observer-blinded trial to evaluate the safety and immunogenicity of inactivated Streptococcus pneumoniae whole-cell vaccine in adults. *Pediatr. Infect. Dis. J.* 2020; 39(4), 345–351.
6. Chichili GR, Smulders R, Santos V, *et al.* Phase 1/2 study of a novel 24-valent pneumococcal vaccine in healthy adults aged 18 to 64 years and in older adults aged 65 to 85 years. *Vaccine* 2022; 40(31), 4190–4198.

BIOGRAPHY

RICK MALLEY received his early education at L'École Active Bilingue in Paris, France, then a BA from Yale University, MD from Tufts University, and pediatrics, pediatric infectious diseases and emergency medicine training at Boston Children's Hospital (BCH). A chance meeting with Dr Porter Anderson led to his interest in the development of a universal pneumococcal vaccine and vaccinology in general. Under Anderson's mentorship, he shifted his research to the development of novel vaccines. His current clinical activities include attending on the inpatient Infectious Diseases consult service, and directing the Travel and Geographic Medicine clinic at BCH.

Malley runs a research laboratory with funding from the National Institutes of Health, PATH, and the Bill & Melinda Gates Foundation (BMGF). In collaboration with PATH and the BMGF, Malley led an international effort for the development of a pneumococcal vaccine for developing countries. In 2014, Malley and collaborators started Affinivax, a biotechnology company seed-funded by BMGF, and based on a novel technology called MAPS (multiple antigen presenting system) to develop vaccines for both developed and developing countries. From December 2021 to August 2022, he served as Chief Scientific Officer at Affinivax (part-time, split with BCH activities); with Affinivax's acquisition by GSK in August 2022, he became Chief MAPS scientist and the clinical representative of the pneumococcal vaccine program at GSK-Affinivax, a role he held until March 31, 2023, after which he returned full time to Boston Children's Hospital.

AFFILIATION

Rick Malley PhD
Senior Physician in Pediatrics,
Boston Children's Hospital,
and
Professor of Pediatrics,
Harvard Medical School

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Malley R has potential future royalties on MAPS vaccines (via being an employer at Boston Children's Hospital). Malley R was a Member of the Board of Directors for Affinivax from 2014 to 2023. Malley R has several patents issues/planned/pending on MAPS technology. Malley R has stock options in Corner Therapeutics and Amplitude.

Funding declaration: Malley R received consulting fee payments from GSK and Merck Vaccines. Malley R has received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Merck Vaccines. Malley R received travel grants for travel to ISPPD12 (Toronto), Merck Vaccines (Les Pensieres), ACPID meeting, and Asian Pneumococcal Symposium (South Korea).

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited; interview held on Nov 19, 2023.

Revised manuscript received: Jan 24, 2024; **Publication date:** Jan 26, 2024.

VACCINE INSIGHTS

RESPIRATORY DISEASES

SPOTLIGHT

What's next for COVID vaccines?

Jeffrey B Ulmer and Lbachir BenMohamed
TechImmune LLC

"...several exciting [COVID-19 vaccines] are being pursued, which have the potential to confer broad and durable protection across the spectrum of coronavirus strains."

VIEWPOINT

Vaccine Insights 2024; 3(1), 23–27

DOI: 10.18609/vac.2024.006

While first-generation COVID-19 vaccines were highly successful, waning immunity and the rapid evolution of the virus mean that new approaches are needed. In this Viewpoint, we describe several promising strategies with the potential to overcome the limitations of current vaccines.

COVID-19 REMAINS A MAJOR PUBLIC HEALTH CONCERN

The COVID-19 pandemic has created one of the largest global health crises in nearly a century. As of today, the number of confirmed cases has reached over 770 million and the disease has caused nearly 7 million deaths. As grim as these numbers seem, it would have been far worse without the rapid development and implementation of the first generation of COVID-19 vaccines, based primarily on viral vector and modified mRNA technologies. It has been estimated that tens of millions of lives were saved by these vaccines. However, waning immunity in the population has fueled the emergence of heavily spike-mutated and highly contagious SARS-CoV-2 variants and sub-variants that escaped immunity induced by the current clinically proven spike-alone-based vaccines, disrupted the effectiveness of the COVID-19 vaccine booster paradigm, and outpaced the development of variant-adapted spike-alone vaccines. Since early 2020, over 20 variants of concern have emerged and contributed to repetitive surges in morbidity and mortality.

Consequently, COVID-19 remains a major threat to human health, with rates of hospitalizations and deaths rising markedly in the past few months. COVID-19 now accounts for over 3% of all deaths in the US and recently exceeded 6,000 deaths every month [1]. While it is difficult to assess true infection rates, since proactive diagnostics and testing have declined and positive home test cases are not reported to authorities, the amount of the virus in wastewater is the only accurate reflection of the amount of virus being circulated in the human population. Recently, this number reached the second-highest level ever recorded [2]. The rate of emergence of new heavily spike-mutated virus variants, such as the recent JN-1, has accelerated. Of particular concern is the dramatically higher rate of change in the virus that facilitates escape from immunity conferred by the current spike-alone-based vaccines [3]. In addition, COVID fatigue and complacency in the general population, due in part to decreasing confidence in the effectiveness of the currently available vaccines, has led to low rates of uptake of the updated vaccines and is compounding the problem. It may be only a matter of time before we return to a much more widespread and severe COVID-19.

This bleak outlook of a prolonged COVID-19 pandemic emphasizes the urgent need for developing a next-generation broad-spectrum pan-coronavirus vaccine capable of conferring strong cross-strain protective immunity that would prevent immune evasion and breakthrough infection. Importantly, an effective vaccine that obviates the need for frequent updates and boosting could restore confidence and increase uptake, thereby providing greater individual protection and a population benefit that could break the transmission cycle.

TOWARD BROAD-SPECTRUM COVID-19 VACCINES

Current COVID-19 vaccines, except for whole inactivated virus vaccines, focus immune responses solely on the surface spike glycoprotein and confer protection mainly via neutralizing antibodies. This approach has been shown to work well when there is a good match between the spike protein in the vaccine and the circulating virus strain, as was the case early during the pandemic. But, unfortunately, this breaks down when there is a mismatch, such as has been the case since the appearance of viral variants. Furthermore, because the spike protein gene is not well conserved across the coronavirus family, the current spike-based vaccines are strain-specific.

Fortunately, several promising strategies have the potential to overcome these limitations.

First, broadly cross-reactive antibodies that can neutralize diverse coronavirus variants and strains have been identified from human samples, similar to those observed in people with HIV. Thus, in principle, it may be possible to elicit such antibodies with a vaccine. Approaches being undertaken to achieve this are utilizing novel antigen design strategies including:

- Mosaic antigen delivery where multiple spike antigens or receptor-binding domains derived from them are presented to the immune system in the context of nanoparticles or virus-like particles [4];
- Identification of naturally occurring consensus sequences presented as a combination of conserved epitopes [5]; and
- Computationally derived cross-reactive sequences identified from large amounts of sequence data using bioinformatics and machine learning approaches [6].

While more cross-reactive antibodies will be an improvement over the relatively narrow immunity induced by the current spike-based vaccines, by themselves such antibodies are unlikely to confer broad protection across the coronavirus family due to the diversity of spike gene sequences and the susceptibility to immune evasion.

Second, the function of T cell responses in protection against COVID-19 is becoming clear. Animal models have demonstrated the protective effect of antigen-specific CD4 and CD8 T cells against live virus challenge and evidence for the important role T cells play in humans is growing [7]. For example, we have demonstrated that preexisting cross-reactive $CD4^+$ and $CD8^+$ T cells directed against conserved coronavirus antigens correlated with good outcomes in COVID-19 patients, suggesting that vaccines capable of inducing such T cell responses could confer cross-protective immunity in humans [8]. Unlike antibodies, which can prevent virus infection, T cells can result in abortive infections by facilitating clearance of virally infected cells and preventing or minimizing disease. Given that these antigens have undergone far fewer mutations than the spike protein throughout coronavirus evolution, vaccines targeting these conserved antigens have the potential to provide a superior breadth of protection than the current spike-only vaccines. A rational strategy is to build on the demonstrated success of antibody-inducing vaccines by the inclusion of non-spike antigens to provide the added benefit of T cell responses targeting conserved epitopes.

Finally, we have not yet taken advantage of an important part of the immune system that is particularly relevant for protection against respiratory pathogens, namely mucosal immunity. Since most viruses, including coronaviruses, enter through mucosal surfaces, the presence of antigen-specific tissue-resident effector and memory lymphocytes could provide a key first line of defense. In addition, because most viruses are also shed via the mucosal route, active local immunity could reduce the levels of virus transmission. The main challenge for the development of successful mucosal vaccines has been inefficient delivery, usually requiring a live organism or viral vector. However, progress is being made in overcoming this limitation with improved non-viral delivery systems for nucleic acid vaccines and recombinant subunit proteins with adjuvant [9]. If successful, this approach would be complementary or synergistic with those targeting broadly neutralizing antibodies against spike and T cell responses against conserved antigens of the virus.

PROSPECTS

In summary, several exciting approaches to designing next-generation COVID-19 vaccines are being pursued, which have the potential to confer broad and durable protection across the spectrum of coronavirus strains. From a technical perspective, based

on our increasing insights into the virology and immunology of coronaviruses, this seems achievable. From a practical perspective, however, for these innovations to make a meaningful difference in the ongoing endemic and prevention of future outbreaks, it will be critical to ensure acceptance of these new vaccines by the general population and sustainable local manufacturing to enable global equitable access. If not, the virus will continue to circulate, evolve, and cause unnecessary morbidity and mortality.

REFERENCES

1. COVID Data Tracker *CDC*; https://covid.cdc.gov/covid-data-tracker/#datatracker-home (accessed Feb 2024).
2. Wastewater Surveillance *CDC*; https://covid.cdc.gov/covid-data-tracker/#wastewater-surveillance (accessed Feb 2024).
3. Yang S, Yu Y, Xu Y, *et al.* Fast evolution of SARS-CoV-2 BA.2.86 to JN.1 under heavy immune pressure. *Lancet Infect. Dis.* 2024; 24(2), e70–e72.
4. Cohen AA, Gnanapragasam P, Lee YE, *et al.* Mosaic nanoparticles elicit cross-reactive immune responses to zoonotic coronaviruses in mice. *Science.* 2021; 371, 735–741.
5. Prakash S, Srivastava R, Coulon PG, *et al.* Genome-wide B Cell, CD4(+), and CD8(+) T cell epitopes that are highly conserved between human and animal coronaviruses, identified from SARS-CoV-2 as targets for preemptive pan-coronavirus vaccines. *J. Immunol.* 2021; 206, 2566–2582.
6. Vishwanath S, Carnell GW, Ferrari M, *et al.* A computationally designed antigen eliciting broad humoral responses against SARS-CoV-2 and related sarbecoviruses. *Nat. Biomed. Eng.* 2023; Epub ahead of print. doi: 10.1038/s41551-023-01094-2.
7. Wherry EJ, Barouch DH. T cell immunity to COVID-19 vaccines. *Science* 2022; 377, 821–822.
8. Prakash S, Dhanushkodi NR, Zayou L, *et al.* Cross-protection induced by highly conserved human B, $CD4^+$, and $CD8^+$ T cell epitopes-based coronavirus vaccine against severe infection, disease, and death caused by multiple SARS-CoV-2 variants of concern. *bioRxiv* 2023; Epub ahead of print. doi: 10.1101/2023.05.24.541850.
9. Dotiwala F, Upadhyay AK. Next generation mucosal vaccine strategy for respiratory pathogens. *Vaccines (Basel)* 2023; 11, 1585–1609.

BIOGRAPHIES

JEFFREY B ULMER spent more than 30 years in vaccines R&D at Merck Research Laboratories, Chiron Corporation, Novartis, and GlaxoSmithKline. His most recent leadership positions included Global Head, External R&D; Head, Preclinical R&D; and Program Head, Technical R&D. His scientific focus has been vaccine technology platforms, including DNA and mRNA vaccines, viral vectors, and adjuvants. He received his PhD in Biochemistry from McGill University, and completed his postdoctoral training in the laboratory of Nobel laureate Dr George Palade in the Department of Cell Biology at Yale University School of Medicine. He has published over 210 scientific articles, is an inventor on 11 patents, and is a Fellow of the International Society for Vaccines where he serves as Treasurer. He is currently President, TechImmune LLC (Newport Beach, California).

LBACHIR BENMOHAMED spent over 20 years studying infection and immunity to viruses, including recent SARS-CoVs at Gavin Herbert Eye Institute, School of Medicine, UC Irvine,

CA, USA. His scientific research focus has been studying viral infections, immunity, and immune evasion, with a focus on vaccine development, including mRNA-based vaccines, adenovirus, and adenovirus-associated vector-based vaccines. BenMohamed received his PhD. in Immunology jointly from Université Paris VII—Denis Diderot, Paris, France, and Pasteur Institute. He then completed his postdoctoral training at Pasteur Institute, Paris, France, and Beckman Research Institute, City of Hope Medical Center, Duarte, CA, USA. He later joined the University of California Irvine (UC Irvine) as a faculty back in 2001, where he founded and served as the Director of the Laboratory of Cellular and Molecular Immunology. He has published over 120 scientific articles as a leading and corresponding author, and is an inventor on 6 patents. BenMohamed was awarded many NIH grants to study infection and immunity, and to develop sub-unit vaccines for herpes simplex viruses and Coronaviruses. In 2020–2021, he discovered the universal coronavirus vaccine and is currently awarded multi-million NIH grants to develop a such vaccine. His lab remains one of the most funded research labs at the School of Medicine, UC Irvine. His most recent leadership positions included a professor of Immunology, at Gavin Herbert Eye Institute, School of Medicine, UC Irvine, Vice-President for Research, TechImmune LLC, Newport Beach, CA, USA.

AFFILIATIONS

Jeffrey B Ulmer PhD
President,
TechImmune LLC,
Newport Beach,
CA, USA

Lbachir BenMohamed PhD
Professor of Immunology,
Gavin Herbert Eye Institute,
School of Medicine,
UC Irvine, and
Vice-President for Research,
TechImmune LLC,
Newport Beach,
CA, USA

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: LBM has an equity interest in TechImmune, LLC, a company that may potentially benefit from the re-search results and serves on the company's scientific advisory board. BenMohamed L's relationship with TechImmune, LLC has been reviewed and approved by the University of California, Irvine by its conflict-of-interest policies.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Revised manuscript received: Feb 5, 2024; **Publication date:** Feb 9, 2024.

INTERVIEW

Avian influenza and the risk of pandemic

With the potential for avian influenza to transmit and spread amongst humans, an effective vaccine to increase pandemic preparedness is a global priority. **Charlotte Barker**, Editor, *Vaccine Insights*, speaks with **Mathilde Richard**, Principal Investigator and Associate Professor at Erasmus MC, about investigating antigenic evolution of avian influenza viruses, developing better vaccines, and the need for proactive measures to ensure global preparedness in the face of evolving influenza threats.

Vaccine Insights 2024; 3(1), 41–46

DOI: 10.18609/vac.2024.009

Q How did you get involved in the field of virology and, specifically, avian influenzas?

MR: My academic background lies in biochemistry engineering, culminating in a Master's degree from a French engineering school. However, very early on in my career, I realized that I did not want to work in the private sector, or even in engineering. So, during the final year of my Master's, I pursued a parallel research Master's degree in microbiology. During my Master's internship on influenza viruses, I found the topic of virology, and more precisely RNA viruses in general, very interesting.

My subsequent PhD further delved into influenza, with a focus on the resistance of seasonal influenza viruses to antivirals. After my PhD, I took on a postdoctoral research position in the Netherlands, keen to explore how research was done in countries outside of France. In this position, I continued working on influenza viruses but moved away from seasonal viruses. I was fascinated by the highly pathogenic avian influenza (HPAI) viruses because of the threat

that they pose to animal health and the potential for these viruses to infect humans. I did a lot of work on trying to understand the determinants of adaptation of avian influenza viruses to infect a human host and transmit among humans.

In 2018, I established my own research team, securing funding for investigations into the emergence of HPAI viruses and their antigenic evolution.

Q What HPAI subtypes are most concerning for human health?

MR: At the moment, the subtypes that are of most concern are the H5N1 viruses. The hemagglutinin protein of this virus strain traces its origin to the goose/Guangdong lineage, the viruses of which are characterized by high pathogenicity in poultry. The initial emergence of these viruses dates back to 1997 in Hong Kong, marking the first instance of direct human infection with avian influenza virus.

Following the outbreak in Hong Kong, a poultry cull was carried out in an attempt to eradicate the virus. However, the virus persisted in wild birds within reservoirs in China, reemerging in 2002. After that, the virus spread across Asia and entered Europe in 2005. At this point, the virus was still an H5N1 virus, but after about 2014, the virus started to reassort, exchanging gene segments with different low-pathogenicity avian influenza viruses that circulate in wild birds.

This period marked a significant juncture, characterized by a specific subclade of the hemagglutinin 2.3.4.4b reemerging, and leading to outbreaks in 2016, 2017, 2020, and 2021. Since 2021, these viruses have become endemic in wild bird populations. Before that, outbreaks were predominantly observed in poultry, with sporadic occurrences in wild birds. However, the virus has now spread extensively to Europe, the Americas, and even to Antarctica.

H5N1 is now a panzootic virus, affecting many different species. For instance, there have been outbreaks in wild carnivores, like foxes and stone martens, and also in marine mammals, like sea lions. The severity of the situation has escalated, reaching unprecedented levels.

However, at the same time, we should not forget about the H7N9 viruses that have mostly remained in China. These viruses emerged in 2013 and have caused five waves of human infections. Subsequent vaccination efforts in poultry populations in China led to a decline in virus prevalence. However, reports from China suggest continued circulation, and due to ongoing viral evolution accelerated by vaccination, we must continue to keep an eye on the situation. The transition from low to high pathogenicity occurred around 2019, adding another layer of concern that warrants sustained attention.

Q What projects are you and your team working on right now?

MR: Currently, my team is working on two lines of research. The first is foundational research aimed at unraveling the emergence of HPAI viruses. We are particularly interested in understanding the transition from low to high pathogenicity in avian species, focusing on subtle changes in the hemagglutinin protein. For a long time, the actual molecular mechanism behind that genetic change was unknown. Our recent publication in bioRxiv sheds light on one of the two putative mechanisms underlying this transition [1].

"Understanding the genetic underpinnings of antigenic changes and phenotypes crucial for mammalian adaptation is imperative. Enhanced knowledge, potentially assisted by artificial intelligence, could facilitate high-throughput screening of circulating viruses."

We are also trying to understand why these viruses originally only emerged in poultry and not in wild birds. Additionally, we are investigating subtype specificity, particularly the factors behind the exclusive evolution of H5 and H7 viruses towards high pathogenicity. This fundamental understanding of the virus's biology can hopefully help us to mitigate further outbreaks and emergencies.

Our second line of research revolves around trying to understand the antigenic evolution of HPAI viruses. We are specifically focusing on the H5 virus from the goose/Guangdong lineage. Because these viruses have been circulating extensively in birds for more than 20 years, the main antigens of the virus have evolved at both the genetic level and the antigenic level. At the moment, different antigenic variants are circulating in different parts of the world, making it very difficult for us to prepare for a potential pandemic. There is no universal vaccine to protect against all of the variants.

What is missing in this field of research is a powerful tool to monitor antigenic evolution of these zoonotic viruses. We have been using antigenic cartography, which is a tool developed by Ron Fouchier at Erasmus University Medical Center and Derek Smith at Cambridge University to visualize the antigenic evolution of viruses. This approach, applied to H7 and H5 viruses, offers valuable insights into mutations in the hemagglutinin that may contribute to antigenic differences, facilitating the identification of potential new viral variants. Furthermore, we employ these antigenic maps to design broadly reactive antigens and to visualize antibody responses.

While a lot of our work is done in animals, we aim to test some of our antigens in humans in a clinical trial. The goal of these trials would be to visualize individual antibody responses and gain insights into the breadth of immune responses.

Q How can your work be applied to develop more effective vaccines?

MR: It is very hard to design good vaccines against influenza viruses because they are so variable. Efforts to create a universal flu vaccine, capable of protecting against viruses from all subtypes—be they seasonal or zoonotic—have been underway. The focus has been on targeting regions of the virus that undergo fewer changes compared to the primary immunodominant components. Some of that work is quite promising, but challenges persist because those parts of the virus are conserved and are not very immunogenic.

While the aspiration for a fully universal flu vaccine should remain, a more practical approach involves developing subtype-specific universal vaccines. Our ongoing efforts, especially on the H5 subtype, revolve around understanding the mediators of antigenic phenotypes in hemagglutinins and include strategies to enhance both the height and breadth of the immune response.

The choice of vaccine platform is crucial, and the emergence of mRNA vaccines offers a promising avenue. Ongoing exploration by various companies and researchers is adapting this

platform for influenza viruses. Traditional inactivated vaccines often lack T cell responses and mRNA vaccines might fill that gap. T cell responses represent an area we should invest in. Furthermore, there is a call to move beyond a singular focus on either antibodies or T cells. Collaborative efforts should converge to develop vaccines that effectively elicit both components of the immune response.

In our approach, we design robust antigens with the aim of integrating them into potent platforms, optimizing the potential for a heightened and comprehensive immune response. This holistic strategy represents our ongoing commitment to advancing vaccine development in the context of influenza viruses.

Q Where do you hope to be with your research in 5 years?

MR: Regarding the project on antigenic evolution, I hope that in 5 years we will have done a clinical trial on one of our antigenically central antigens— those positioned in the center of the antigenic space to provoke a broad immune response and hopefully protect against a wide range of antigenically diverse viruses. Further, while we have engineered hemagglutinins in such a way that we get a better immune response, we do not yet understand the mechanism behind it. I hope that in the next few years, we will have more understanding of how the changes that we have introduced in those antigens boost the immune response. I also hope that our knowledge of H5 will be applied to other viruses and that we will be at a point where we can also design better H7 and H9 vaccines.

As far as the emergence aspect of our research, we have made significant strides in understanding the transition from low to high pathogenicity. The mechanism involves a multibasic cleavage site in the hemagglutinin, with nucleotide insertions occurring through polymerase activity. While our recent work has elucidated this mechanism, we must refine the model and discern potential species-specific differences.

Our upcoming endeavors involve understanding the second mechanism involved in this process—non-homologous recombination between the hemagglutinin gene and RNA from other parts of the virus genome or the host. This intricate process is currently poorly understood, and the next 5 years hold the promise of shedding light on its molecular intricacies. We have secured funding for this research, signifying our commitment to understanding this mechanism and potentially revolutionizing our comprehension of influenza virus evolution.

Q Are we doing enough to prevent or prepare for a possible influenza pandemic?

MR: I do not think that we are doing enough. While I love my work, the nature of our research environment, driven by individual career pursuits and the imperative of securing research funding, hampers our collective ability to address global challenges in a coordinated fashion. We need coordinated actions, ideally orchestrated by governmental institutions. Rather than redundant efforts, a more effective strategy would involve assembling individuals with diverse expertise to collaboratively tackle substantial issues through coordinated actions.

After the COVID-19 pandemic, we anticipate that there will be an increased emphasis on preparedness programs. There are some programs, but we need more. One of the big issues

we face when confronted with outbreaks of emerging viruses is that we are always too late. This reactive approach, where we mobilize in response to an outbreak, often results in delayed studies and analyses.

Keeping up with the evolution of viruses is extremely difficult. It is already difficult for seasonal viruses. To address this, we are actively working on predictive models for seasonal viruses, aiming to shift from a reactive to a proactive approach. However, the complex nature of avian viruses, with their propensity for reassortments across diverse species, poses a unique challenge. Understanding the genetic underpinnings of antigenic changes and phenotypes crucial for mammalian adaptation is imperative. Enhanced knowledge, potentially assisted by artificial intelligence, could facilitate high-throughput screening of circulating viruses.

A lot is being done, but to be truly prepared, we need to step up our game.

REFERENCE

1. M Funk, MI Spronken, TM Bestebroer, *et al*. Transient RNA structures underlie highly pathogenic avian influenza virus genesis. *bioRxiv* 2024; Epub ahead of print. doi:10.1101/2024.01.11.574333.

BIOGRAPHY

MATHILDE RICHARD is a biochemist engineer and molecular virologist. She completed her PhD in 2010 under the supervision of Professor Bruno Lina, studying the resistance of influenza A viruses to neuraminidase inhibitors. She then joined the Viroscience Department at Erasmus MC for her post-doctoral studies, which have focused on the pathogenesis, virulence, and transmissibility of influenza A viruses, with special emphasis on genetic and phenotypic viral factors involved in the emergence of new pandemics. Since 2018, she has led a research team at the Viroscience Department, focusing on the understanding of highly pathogenic avian influenza virus emergence, pathogenesis, and the development of new vaccination strategies to cope with antigenic diversity in the context of pandemic preparedness.

AFFILIATION

Mathilde Richard
Principal Investigator, and
Associate Professor,
Erasmus MC

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The authors have no conflicts of interest.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

Article source: Invited. This article is based on a podcast, which can be found here.

Interview held: Jan 16, 2024; **Revised manuscript received:** Feb 19, 2024;

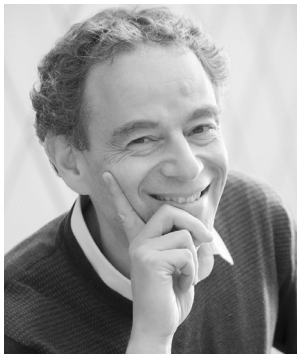
Publication date: Mar 1, 2024.

We hope you enjoyed reading this interview.
You can also listen to the recorded podcast here:

LISTEN NOW

INTERVIEW

Toward global RSV vaccination coverage



Charlotte Barker, Editor, *Vaccine Insights*, speaks with **Louis Bont**, Professor, UMC Utrecht, and Chairman, ReSViNET, about the impact of respiratory syncytial virus vaccines, the future of respiratory syncytial virus prevention, and the critical need for outreach and awareness programs.

Vaccine Insights 2024; 3(1), 29–33

DOI: 10.18609/vac.2023.007

Q How did you get involved in respiratory syncytial virus (RSV) research?

LB: I'm a pediatric infectious disease specialist and I started working on this virus 25 years ago during my PhD studies. I stayed in this line of research because I enjoyed working in an area that has a global perspective and is tackling a major problem for society. I like the mechanistic aspect—trying to understand what others could not—and the opportunity to contribute to something bigger.

Q Which of your current research projects are you most excited about?

LB: I'm excited about working on everything to do with RSV vaccine development. We work across several areas, including quantifying mortality related to RSV infection worldwide, understanding escape from RSV immunization, exploring the health-economic aspects of RSV prevention, and identifying biomarkers of RSV infection. All of these things are very interesting to me.

Q Is RSV underappreciated as a danger to young children?

LB: What we learned from our RSV-GOLD study is that all children are at risk of dying from RSV—it's not just a specific subpopulation [1]. Some children are at a higher risk, but the majority of children dying from RSV were perfectly healthy until getting RSV. We also learned that a large proportion of children in developing countries who die from RSV do so without being diagnosed. It is a disease that often goes unnoticed, and yet about 30% of all children dying at intensive care units during the winter season have RSV. It isn't routinely tested for in many countries, and until you start looking for it, you don't see it.

Q Why have RSV vaccines proved challenging to develop?

LB: Natural immunity against RSV is mostly directed against the post-fusion conformation of the fusion (F) protein (post-F), which is not adequately protective. Indeed, early inactivated virus vaccines were found to cause dangerous immune-mediated enhancement. The vaccines now on the market target the pre-fusion conformation of the F-protein (pre-F), which has been shown to afford protective immunity. The understanding that you need immunity against pre-F was a revolution in RSV vaccine development. That discovery came 10 years ago and now we are seeing the results, with vaccines for pregnant women, infants, and older adults.

Q What impact can we expect the RSV vaccines now on the market to have?

LB: The impact will vary between different populations. The vaccine for older adults will likely have an impact comparable to influenza vaccination. The vaccines for pregnant women and infants both have the objective of preventing RSV infection in babies and while they will not eradicate or eliminate the virus, I believe they will prevent the majority of severe cases during the first winter season.

We're currently carrying out research into the health economics of RSV vaccines. We don't have the results to share yet, but the burden of the disease is so high in every country around the world that the health economic evaluation will almost certainly prove favorable.

"My hope is that decision-makers in low- and middle-income countries decide that this is a vaccine that their country needs—and that mothers know about it."

Q You recently co-authored an article discussing differing recommendations for maternal RSV vaccines in Europe and the US—what is your main concern?

LB: The maternal vaccine has been licensed by the European Medicines Agency from 24 weeks of pregnancy, as intended by the manufacturer and researchers. In the US, the US FDA has decided to limit the use of the maternal vaccine to 32 weeks of pregnancy onwards, because they were concerned about the possibility of preterm birth.

My concern is that the FDA decision substantially limits the use of the vaccine because the window of administration becomes so small. If a child is born before administration, the vaccine can have no impact. If the FDA recommendation is followed by other countries, I believe this could cost a lot of lives. It is an issue that requires further study.

There is a trial planned in developing countries using the maternal vaccine, funded by the Bill & Melinda Gates Foundation, which should offer some further reassurance that the vaccine is safe from 24 weeks.

Q What further advances in RSV prevention do you expect to see in the coming years?

LB: I hope that in 5–8 years every pregnant woman is vaccinated against RSV, or their baby is immunized with a monoclonal antibody. Maternal vaccination has been well implemented in many developing countries so I'm very optimistic. Nearly every expectant mother in the developing world is vaccinated against tetanus and I hope to see the maternal RSV vaccine gain that level of coverage.

I expect we will also see new vaccines emerge. mRNA-based vaccines are in development and may be used for school-aged children. RSV is not usually life-threatening in school-aged children, but it does cause disease and can be transmitted to babies and the elderly. A vaccine for this age group could provide valuable indirect protection for vulnerable groups.

Q How can we ensure global access to RSV vaccines?

LB: There are a number of challenges. For developing countries, we need support from Gavi and country-specific safety and efficacy data, because there is evidence that outcomes may vary between high- and low-income countries. Plus, there is always the issue of establishing cold chain capacity, which should not be underestimated.

One critical aspect is awareness. If decision-makers are not aware of the disease, they will never prioritize it. I am the founding chairman of ReSViNET [2], the only international non-profit foundation focused on RSV specifically. We organize regular conferences, bringing together scientists, politicians, and patients in locations around the world.

We also give lectures and provide educational materials, and we are carrying out health economic evaluations in 11 countries. We have worked very hard to get RSV on the agenda of the WHO over the past 10 years.

My hope is that decision-makers in low- and middle-income countries decide that this is a vaccine that their country needs—and that mothers know about it. We made a video about RSV in Soweto [3] and in it, a mother who lost her child to RSV says, "Every mother should know about RSV." We believe everybody should know about RSV.

REFERENCES

1. Mazur NI, Löwensteyn YN, Willemsen JE, *et al.* Global respiratory syncytial virus-related infant community deaths. *Clin. Infect. Dis.* 2021; 73(S3), S229–S237.

2. Respiratory Syncytial Virus Foundation. https://resvinet.org/.

3. RSV GOLD. Why we should all know about RSV (short version). https://www.youtube.com/watch?v=inJQX4uH2zM.

BIOGRAPHY

LOUIS BONT is a Pediatrician Infectiologist-Immunologist, and head of the Pediatric Department of the University Medical Center Utrecht, the Netherlands. He is the Founding Chairman of ReSViNET, an international RSV research consortium. His specific research interest is RSV pathogenesis and burden of disease. His work focuses on unraveling the role of neutrophils, RSV-related mortality and long-term airway disease following RSV infection. Bont is the lead investigator of the INFORM study, a large prospective global clinical virology study to unravel the molecular epidemiology of RSV in about 4000 children. He is one of the co-leads of the Respiratory Syncytial Virus Consortium in Europe (RESCEU), aiming to define the RSV burden of disease in Europe. He is leading the RSV GOLD mortality registry, funded by the Bill & Melinda Gates Foundation. His group collaborates with the World Health Organization on RSV surveillance and vaccine development. Bont's research focuses on clinical and translational mechanisms of disease and identifying targets for intervention of RSV bronchiolitis. He has been the lead author of over 200 publications in peer-reviewed medical journals. He founded the Training of Upcoming Leaders in Pediatric Science (TULIPS), a career training network for clinician scientists in the field of child health.

AFFILIATION

Louis Bont MD PhD
Professor,
UMC Utrecht, and
Chairman,
ReSViNET

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Bont L has regular interaction with pharmaceutical and other industrial partners. He has not received personal fees or other personal benefits. UMCU has received major funding (>€100,000 per industrial partner) for investigator initiated studies from AbbVie, MedImmune, AstraZeneca, Sanofi, Janssen, Pfizer, MSD, and MeMed Diagnostics. UMCU has received major funding for the RSV GOLD study from the Bill & Melinda Gates Foundation.

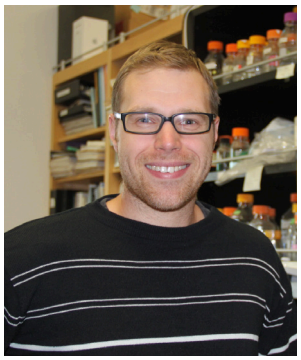
Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

Article source: Invited; interview held Jan 25, 2024.

Revised manuscript received: Feb 12, 2024; **Publication date:** Feb 15, 2024.

INTERVIEW

Exploring hybrid mRNA vaccine technology for lasting immunity to COVID-19



While COVID-19 vaccines have proven effective, the need for lasting immunity has prompted exploration beyond conventional approaches. **Casey Nevins,** Assistant Editor, *Vaccine Insights*, speaks with **Magnus Hoffmann,** Merkin Institute Fellow in the Merkin Institute for Translational Research at Caltech, about his work on developing a hybrid mRNA vaccine technology and its potential to address the limitations of current COVID-19 vaccines.

Vaccine Insights 2024; 3(1), 13–16

DOI: 10.18609/vac.2024.004

Q What influenced you to start working with vaccines?

MH: I was really fascinated by trying to understand how drugs work and how they affect the human body, so I ended up studying pharmacy at Bath University, UK. However, I became frustrated with how limited our treatment options were for a lot of conditions. I wanted to work on developing new and better drugs so I decided to do a PhD in Pamela Bjorkman's lab at Caltech. Pamela's group has a long-standing interest in the characterization of monoclonal antibodies against various infectious diseases such as HIV-1 and SARS-CoV-2. The lab also focuses on the design of immunogens to make more effective vaccines against those diseases.

During my PhD, we developed a new hybrid mRNA vaccine technology—ESCRT-and ALIX-binding region (EABR) technology. Based on that work, I received a National Institute of Health Director's Early Independence Award to launch my own laboratory at Caltech as an independent postdoctoral fellow. My team is focused on continuing to develop this technology.

Q What current gaps or challenges in existing COVID-19 vaccine approaches led you to explore different vaccine technologies?

MH: The current COVID-19 vaccines are effective, but they do have two main problems. The first problem is that the antibody titers that get elicited by COVID-19 vaccines contract relatively quickly over time. You get your shot, and then a few months later, you might not be fully protected anymore.

The second problem is that viruses like SARS-CoV-2 rapidly evolve to escape from immune responses elicited by vaccination or previous infections. For instance, the initial prime/boost regimens of the COVID-19 mRNA vaccines did not elicit effective antibody responses against the omicron-based variants that emerged later in the pandemic. As a result, we need to get frequent and updated booster shots, which is expensive and inconvenient.

There is a great need for innovative vaccine technologies to be able to induce more lasting protection.

Q Does your vaccine prototype offer an enhanced antibody response compared with existing mRNA vaccines?

MH: In mouse studies, our hybrid mRNA vaccine approach elicited about fivefold higher neutralizing antibody titers against the original variant as well as the Delta variant. The binding titers were also higher. Furthermore, in some cases, this technology elicited over tenfold higher titers against some of the omicron-based variants.

That being said, against the BA.5 omicron variant and some of the variants that came after that, like BQ.1.1 and XBB.1, the titers dropped considerably. This was, however, just an initial prime/boost regimen. In the human population, BA.5 only appeared after we had already received three immunizations. Had we given a third immunization in our mouse study, we may have seen high titers against these variants. We are currently testing this theory, evaluating our technology as a booster shot in pre-immunized animals.

Overall, these are promising results, but these responses were elicited in mice. We will have to evaluate this technology in larger animals, and eventually in humans. It is challenging to evaluate durability in mouse studies, so one of the key questions we want to answer is whether the higher peak antibody titers that we are seeing will translate into more durable responses.

"[mRNA and Novavax] vaccines stimulate the immune response by either mimicking an infected cell or mimicking the virus. We are trying to develop a technology that does both in one."

Q How does the hybrid vaccine technology combine features of mRNA and protein nanoparticle-based vaccines?

MH: The conventional mRNA vaccine leads to cellular expression of the SARS-CoV-2 spike protein, which activates the adaptive immune system in two ways: the spike protein is presented on the surface of the cell, which activates B-cells; and the spike protein gets cleaved into peptides that get presented at major histocompatibility complex molecules, which activates T cells. An mRNA vaccine essentially mimics an infected cell to stimulate the immune response.

Other vaccines, such as the Novavax vaccine, present dense arrays of spike protein on the surface of virus-like particles (VLPs). These vaccines are also very effective at activating B-cell responses, and they do so by mimicking the virus.

These two types of vaccines stimulate the immune response by either mimicking an infected cell or mimicking the virus. We are trying to develop a technology that does both in one. In practice, our technology looks very similar to a conventional mRNA vaccine, but we have engineered the spike protein so that when it gets to the cell surface, the cytoplasmic tail of the spike protein recruits host proteins from the ESCRT pathway. That induces the self-assembly and budding of VLPs that pinch off from the plasma membrane and circulate in the body, which could activate immune cells more effectively. In our initial published study, we found that this approach can elicit higher binding and neutralizing antibody titers.

We also saw a slight improvement in T helper 2 responses, but the T helper 1 responses were very similar to the conventional mRNA vaccine. We are in the process of doing more work to characterize potential differences between these vaccine approaches to understand exactly how the immune responses are different.

Q Beyond COVID-19, do you see potential applications of the EABR technology in the development of other vaccines? Are there specific challenges or considerations when applying this technology to different pathogens?

MH: There is great potential for this technology in the development of effective vaccines against various viral as well as non-viral pathogens. Any associated challenges are quite similar to those involved with a conventional mRNA vaccine. One of the key considerations is that the immunogen has to express well. If the immunogen expresses poorly, you are not going to make a lot of VLPs. Therefore, a lot of work needs to be put into immunogen selection, optimization, and design.

Q Looking to the future, what are your key goals or priorities for your research?

MH: In addition to continuing the optimization, evaluation, and application of the hybrid mRNA vaccine technology, we are very interested in characterizing the immune responses that are being elicited by different vaccine approaches. There is a lot of information out there

that can help us to further improve our vaccine designs. In addition, we are very interested in engineering the EABR nanoparticles to package and deliver nucleic acid-based cargoes. That could be very interesting for drug delivery applications.

BIOGRAPHY

MAGNUS HOFFMANN is the Merkin Institute Fellow at the Merkin Institute for Translational Research at the California Institute of Technology. Based on his graduate work in Pamela Bjorkman's laboratory at Caltech, he received the Milton and Francis Clauser Prize for the best PhD thesis across all disciplines, and was awarded an NIH Director's Early Independence Award to launch his own laboratory as an independent postdoctoral scholar at Caltech. Hoffmann's research focuses on the development of innovative vaccine technologies and gaining a deeper understanding of the immunological mechanisms that shape vaccine-induced immune responses. He developed the EABR technology, an innovative approach to genetically encode nanoparticles for vaccine applications. This vaccine platform combines features of mRNA- and protein nanoparticle-based vaccines, resulting in superior neutralizing antibody responses against original and variant SARS-CoV-2 in mice. Ongoing and future research in his group focuses on the continued optimization, evaluation, and application of this technology.

AFFILIATION

Magnus Hoffmann PhD
Merkin Institute Fellow,
Merkin Institute for Translational Research,
California Institute of Technology

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: The work for this manuscript was supported by the NIH, the Bill & Melinda Gates Foundation, Wellcome Leap, George Mason University Fast Grants, and the Rothenberg Innovation Initiative.

Disclosure and potential conflicts of interest: A patent has been filed for the EABR technology.

Funding declaration: Travel to meetings to present this work was supported by the NIH and Bill & Melinda Gates Foundation.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited; externally peer reviewed.

Revised manuscript received: Jan 30, 2024; **Publication date:** Feb 2, 2024.

VACCINE INSIGHTS

RESPIRATORY DISEASES

SPOTLIGHT

Progress and future directions for RSV prevention in older adults

Angela Branche
University of Rochester

"...older adults hospitalized with RSV were 1.5 times more likely to be admitted to an intensive care unit than those hospitalized with either COVID-19 or influenza..."

VIEWPOINT

Vaccine Insights 2024; 3(1), 35–39

DOI: 10.18609/vac.2024.008

Respiratory syncytial virus has long been recognized as a major contributor to acute respiratory illnesses, morbidity, and mortality in young children. Less recognized, but not necessarily less important, is the impact respiratory syncytial virus has on adult populations, for which it remains a major cause of lower respiratory tract disease, resulting in prolonged and often severe illnesses, morbidity from exacerbations of underlying medical conditions, and changes in function, cognition, and quality of life [1]. In a pivotal and landmark 2004 US study involving 608 healthy persons older than 65 and 540 high-risk patients

with cardiopulmonary disease over a 4-year period, respiratory syncytial virus infection was identified in 3–7% of healthy subjects and 4–10% of high-risk patients using a combination of reverse transcription (rt)-PCR and serology [2]. Estimates of the burden of disease range between 600,000–1 million medically attended visits, 140–177,000 hospitalizations, and approximately 11,000–14,000 deaths in US adults 65 years and older, annually [2,3]. Moreover, in a recent report from the US CDC IVY network, older adults hospitalized with respiratory syncytial virus were 2–3 times more likely to require supplemental oxygen and 1.5 times more likely to be admitted to an intensive care unit than those hospitalized with either COVID-19 or influenza, and had two-times higher odds of mechanical ventilation or death than those with influenza [4].

RECENT ADVANCES IN RSV VACCINE DEVELOPMENT FOR ADULTS

In June 2023, the US Advisory Committee on Immunization Practices (ACIP) voted to recommend respiratory syncytial virus (RSV) vaccination for adults aged 60 years and over, using shared clinical decision-making. The recommendation for shared clinical decision-making is intended to allow flexibility for healthcare professionals and patients to consider individual risk for RSV disease. These recommendations came shortly after US FDA approval of two RSV vaccines for this age group: RSVpreF and RSVPreF3. Soon, a third vaccine, mRNA-1345, will also seek and likely be granted FDA approval. In the Phase III trials, investigators showed that a single dose of RSVpreF was able to prevent symptomatic lower respiratory tract (LRTD) in 88.9% of patients, and, similarly, a single dose of RSVpreF3 and mRNA-1345 was able to prevent symptomatic LRTD in 82.6% and 83.7% of patients, respectively [5–7]. However, some unique features of the RSV vaccine trials have complicated how these vaccines may be used in practice.

Firstly, the Phase III efficacy trials for RSVpreF, RSVPreF3, and mRNA-1345 were conducted during the COVID-19 pandemic when many nonpharmaceutical interventions (e.g., masking, social isolation) not only curbed the transmission of COVID-19 but decreased transmission of viral infections in general. This resulted in fewer RSV cases during the 2020–2022 US winter seasons than are typically seen, impeding the ability to assess efficacy against severe disease, hospitalization, and death [8]. While it is logical to presume that if these vaccines can prevent LRTD they can also prevent hospitalization, the low case numbers did not allow for that analysis.

Secondly, vaccine trials are often subject to healthy user bias. In other words, the people willing and able to participate in lengthy clinical trials are generally healthier than the group for whom protection is desired. Accordingly, frail older adults and those 80 years and older, nursing homes residents, and those with cardiopulmonary medical conditions (e.g., congestive heart failure and chronic obstructive pulmonary disease), sub-populations of older adults for whom epidemiological data have shown a strong association with increased risk for severe RSV, were not well represented in the clinical trials.

APPLYING ACIP RECOMMENDATIONS

While the shared clinical decision-making aspect of the ACIP recommendation for RSV vaccination permits some flexibility, more specific recommendations are needed to guide clinical practice and improve vaccine acceptance and uptake. The most clearly defined risk factor for severe outcome with RSV infection is age. Studies have demonstrated that rates of RSV-associated hospitalization increase, and in some reports even double, with each subsequent decade of life over 60 years [9–11]. Therefore, for adults 75 years and older, a strong recommendation for vaccination is supported by the epidemiological data and should be made by regulatory

bodies and medical societies. Similarly, residency in a long-term care facility, which may reasonably be considered a correlate of medical frailty, constitutes a significant risk for RSV-associated hospitalization, resulting in often-catastrophic loss of function and these patients should have unequivocal recommendations for RSV vaccination [9,10,12]. Further work will be needed to ensure RSV vaccines are protective in the oldest and most frail of patients, but this should take the form of immune-bridging studies and post-marketing surveillance of effectiveness and safety, without precluding stronger recommendations being made now.

The shared clinical decision-making model is probably most appropriate for adults in their sixties, for whom risk for severe RSV disease is often correlated with the presence or absence of certain underlying chronic comorbid conditions [13]. However, navigating the complexity of determining who will benefit from RSV vaccination may be the justification to develop a larger policy of risk- rather than age-based recommendations; for example, all adults 75 years and older, residents of long term care facilities, and adults of any age with certain conditions including immunocompromise, chronic obstructive pulmonary disease, congestive heart failure, end-stage renal disease and other serious cardiac and endocrine conditions.

Several studies now also report differences in RSV-associated hospitalizations by race, ethnicity, or socioeconomic status [9,10,14]. In an unpublished analysis from a population-based incidence study we conducted in Rochester, NY, when compared to white race, black or African-American race was associated with a three-times higher rate of hospitalization in adults 45–64 years of age but only 1.4 times higher rate of hospitalization in adults 65 years and older [9]. Similarly, the CDC Respiratory Syncytial Virus–Associated Hospitalization Surveillance Network recently reported that the proportion of hospitalized patients whose race was Hispanic or black decreased with increasing age (p-value <0.01), with black patients accounting for 28.2% of hospitalized patients aged 60–64 years and 8.2% of those aged 80 years or over [10]. These findings may be driven by many factors including access to healthcare, higher rates of important chronic comorbid conditions at a younger age for black and Hispanic Americans and shorter life expectancies. Collectively, however, these disparities further highlight the need for risk-based rather than age-based recommendations for RSV vaccination. Notably, studies in younger and immunocompromised populations are still being conducted and any use of RSV vaccine in these populations would be off-label at this time.

REFERENCES

1. Branche AR, Falsey AR. Respiratory syncytial virus infection in older adults: an under-recognized problem. *Drugs Aging* 2015; 32, 261–269.

2. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. *N. Engl. J. Med.* 2005; 352, 1749–1759.

3. Thompson WW, Shay DK, Weintraub E, *et al.* Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 2003; 289, 179–186.

4. Surie D, Yuengling KA, DeCuir J, *et al.* Disease severity of respiratory syncytial virus compared with COVID-19 and influenza among hospitalized adults aged >/=60 years - IVY Network, 20 U.S. States, February 2022–May 2023. *MMWR Morb. Mortal Wkly. Rep.* 2023; 72(40), 1083–1088.

5. Walsh EE, Perez Marc G, Zareba AM, *et al.* Efficacy and safety of a bivalent RSV prefusion F vaccine in older adults. *N. Engl. J. Med.* 2023; 388, 1465–1477.

6. Wilson E, Goswami J, Baqui AH, *et al.* Efficacy and safety of an mRNA-based RSV PreF vaccine in older adults. *N. Engl. J. Med.* 2023; 389, 2233–2244.

7. Papi A, Ison MG, Langley JM, *et al.* Respiratory syncytial virus prefusion F protein vaccine in older adults. *N. Engl. J. Med.* 2023; 388, 595–608.

8. Falsey AR, Cameron A, Branche AR, Walsh EE. Perturbations in respiratory syncytial virus activity during the SARS-CoV-2 pandemic. *J. Infect. Dis.* 2022; 227, 83–86.

9. Branche AR, Saiman L, Walsh EE, *et al.* Incidence of respiratory syncytial virus infection among hospitalized adults, 2017–2020. *Clin. Infect. Dis.* 2022; 74, 1004–1011.

10. Havers FP, Whitaker M, Melgar M, *et al.* Characteristics and outcomes among adults aged >/=60 years hospitalized with laboratory-confirmed respiratory syncytial virus—RSV-NET, 12 States, July 2022–June 2023. *MMWR Morb. Mortal. Wkly. Rep.* 2023; 72, 1075–1082.

11. Fleming DM, Taylor RJ, Lustig RL, *et al.* Modelling estimates of the burden of respiratory syncytial virus infection in adults and the elderly in the United Kingdom. *BMC Infect. Dis.* 2015; 15, 443.

12. Branche AR, Saiman L, Walsh EE, *et al.* Change in functional status associated with respiratory syncytial virus infection in hospitalized older adults. Influenza Other Respir. *Viruses* 2022; 16, 1151–1160.

13. Wyffels V, Kariburyo F, Gavart S, Fleischhackl R, Yuce H. A real-world analysis of patient characteristics and predictors of hospitalization among US medicare beneficiaries with respiratory syncytial virus infection. *Adv. Ther.* 2020; 37, 1203–1217.

14. Zheng Z, Warren JL, Shapiro ED, Pitzer VE, Weinberger DM. Estimated incidence of respiratory hospitalizations attributable to RSV infections across age and socioeconomic groups. *Pneumonia (Nathan)* 2022; 14, 6.

BIOGRAPHY

ANGELA BRANCHE is an Associate Professor of Medicine at the University of Rochester. Branche currently has a clinical inpatient practice comprised of both general infectious diseases and HIV medicine patients. Her research involves the use of viral molecular and immunological diagnostic assays to explore the pathogenesis and host response to acute viral respiratory illnesses in adults. She is currently Co-Principle Investigator for the UR Vaccine Treatment and Evaluation Unit (UR VTEU) one of ten NIH-funded network sites in the US. Her current research activities explore clinical disease, pathogenesis, development of therapeutics, and vaccine biology related to infection with viral and bacterial respiratory pathogens. Studies include assessment of asymptomatic carriage of *Streptococcus pneumoniae* and the impact of pneumococcal vaccination, surveillance of epidemic influenza infections and immunologic mechanisms of protection following natural infection versus vaccination, the development of pandemic influenza vaccines, population-based studies of RSV infection and the development of vaccine, and anti-viral agents for RSV.

AFFILIATION

Angela Branche
Associate Professor of Medicine,
University of Rochester,
USA

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The authors have no conflicts of interest.

Funding declaration: Branche A has received grants to her institution for research from Pfizer, Moderna, Cyanvac, Vaccitech, and NIH NIAID. Branche A has received consulting fees from GSK and Novavax. Branche A has received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Moderna and GSK.

Article source: Invited.

Revised manuscript received: Feb 8, 2024; **Publication date:** Feb 15, 2024.

VACCINE INSIGHTS

RESPIRATORY DISEASES

SPOTLIGHT

Whole-cell pneumococcal vaccines: a future-proof approach to overcoming pneumococcal serotype replacement

Erin B Brazel, Mohammed Alsharifi, Lauren Giorgio, Timothy R Hirst, and James C Paton
The University of Adelaide and GPN Vaccines Ltd

VIEWPOINT

Vaccine Insights 2024; 3(1), 17–22

DOI: 10.18609/vac.2024.005

"[PPV and PCV] advances are being offset by the steady rise in the incidence of disease caused by non-vaccine-covered serotypes..."

Serotype replacement is an issue associated with currently licensed pneumococcal vaccines, all of which target the serotype-specific capsular polysaccharide. The use of pneumococcal polysaccharide vaccines and pneumococcal conjugate vaccines in particular, have profoundly reduced the burden of invasive pneumococcal disease. However, these advances are being offset by the steady rise in the incidence of disease caused by non-vaccine-covered serotypes, which may be observed soon after the introduction of capsule-based vaccines in a given region [1–3].

To address changes in serotype prevalence, there has been a need to expand the valency of pneumococcal conjugate vaccines (PCVs) in order to maintain coverage against a reasonable proportion of circulating disease-causing strains. However, these vaccines impose serotype-specific selective pressure and are inevitably poised to drive further replacement disease. The steady trend toward ever-higher valency PCVs may also come at the cost of immunogenicity, with evidence of expanded valency PCVs displaying dampened responses. Numerically lower immunoglobulin G (IgG) geometric mean concentrations were observed recently for PCV20 for those serotypes shared with PCV13 after doses three and four, suggesting that PCV serotype expansion may be approaching its limit [4]. The selection of serotypes to include in a PCV also represents a challenge. Even if a vaccine is reformulated to include emerging serotypes, these proportions may differ at the time of their licensure. Geographical regions also display widely varied serotype distributions and selection of capsular antigens is a challenge in the absence of clear dominating serotypes common across regions [5, 6]. These are major shortcomings that will continue to plague further expanded or alternative serotype PCVs.

A potential solution to these challenges may be found by drawing insights from naturally acquired immunity to the pneumococcus, where proteins have been shown to play an important role. In fact, studies of natural exposure to the pneumococcus suggest that capsular polysaccharide is not the dominant target of naturally acquired immunity [7]. Rates of invasive pneumococcal disease decline by age 5 years, at which time antibody responses to pneumococcal proteins have been reported to increase [8]. The increase in disease incidence in older adulthood (over 65 years) also coincides with a decline in anti-protein antibodies, whereas the decline in anti-capsular IgG is far less pronounced [9]. As protein antigens are generally well conserved between serotypes, vaccines that bring forward acquisition in the young, and/or boost immunity to pneumococcal proteins in the elderly, are expected to provide effective broad-spectrum immunity.

Several protein-based vaccines have progressed to clinical evaluation and have been reviewed extensively elsewhere [10–12]. However, there are limitations associated with this approach. Purified protein vaccines rely on the selection of a small number of target antigens relative to the 270 surface proteins expressed on the pneumococcus [13], and these may be susceptible to immune evasion through small changes to their protein structure or by dampening their expression [14]. There is also potential for large-scale manufacture of protein antigens to cause changes in protein conformation, which may impact epitopes important for vaccine efficacy. In addition, adjuvants are required to enhance the immunogenicity of protein-based vaccines [15] and there are few adjuvants (such as alum, AS04, MF59, AS03, CpG, and AS01b)

approved by regulatory authorities for human use. Commonly used alum adjuvants induce T helper cell 2-based responses; however, antibody isotypes associated with T helper cell 1 responses (IgG2a in mice and IgG1 in humans) are known to have high affinity to Fc receptors involved in inducing functional antibody responses with opsonophagocytic activity [17]. Despite protein-based pneumococcal vaccines having undergone significant evaluation in preclinical and clinical trials, to our knowledge, no protein vaccine has outperformed or even matched a licensed PCV using the gold standard correlate of protection, the opsonophagocytic assay (OPA). The capacity to elicit an OPA response that is not deemed inferior to existing PCVs is a key consideration for licensure of new expanded formulations, and candidate vaccines that can meet this bar will be well positioned for further progression and licensure [18].

Another promising approach that targets non-capsular antigens is whole-cell vaccines (WCVs), comprised of inactivated and/or attenuated bacteria [19–26]. Such vaccines have been utilized effectively for many years for the control of pertussis and tuberculosis in children. In addition to presenting antigens in a manner most likely to resemble the natural conformation of proteins, WCVs have the potential to elicit responses to a breadth of antigens surpassing all polysaccharide- or purified protein-based vaccine approaches. Such vaccines could provide a future-proof approach to effectively target emerging and entirely new pneumococcal serotypes that may arise. To date, however, there are few reports of WCVs eliciting robust OPA responses. It is important to note that chemical inactivation using formalin or beta-propiolactone is associated with cross-linking between proteins and reduced immunogenicity, while live attenuated vaccines pose a significant biological and health risk. The use of γ-irradiation has been reported as an effective alternative inactivation method for the development of highly immunogenic and safe WCVs [27, 28], because of reduced protein damage and maintenance of the structural integrity of inactivated pathogens [29]. γ-irradiated vaccines have been found to mimic live pathogens in terms of stimulating both innate and adaptive responses [28].

A recent WCV that has advanced to the clinical stages of evaluation is a γ-irradiated vaccine developed by GPN Vaccines Ltd (Gamma-PN) [21, 23]. Gamma-PN is differentiated from other WCV approaches in both design and formulation, which ultimately are associated with improved immune responses. A modification was introduced to remove a manganese import gene from the Gamma-PN vaccine strain, which was associated with enhanced survival after experimental challenge in immunized mice [23, 30]. Restriction of manganese availability is a key feature of the host innate response to an infection [31] and the improved protective efficacy may be attributable to changes in the antigenic profile of the vaccine strain, which better reflects that of pneumococci during an infection. A subsequent study in rabbits demonstrated that vaccination with Gamma-PN induced antibodies against a broad range of pneumococcal proteins known to be associated with natural immunity; with significant reactivity to 50 antigens reported [32], although the total number of antigens that reacted with the immune sera exceeded this number. Most critically, this study also reported positive functional antibody responses. For most serotypes tested, Gamma-PN administered with an adjuvant elicited higher OPA titers than PCV13. However, without adjuvant, Gamma-PN performed even better, eliciting OPA titers that were either comparable to or far superior to those elicited by PCV13 or pneumococcal polysaccharide vaccine (PPV)23 for various vaccine-included serotypes (6A, 23F, 11A, 22F, and 33F). Further, this vaccine also induced high OPA titers against serotypes not covered by any current licensed vaccines (9N, 15A, 23B, and 35B) [32]. This data indicated that a shift toward T helper cell 2 responses in animals vaccinated with

adjuvanted Gamma-PN was associated with reduced OPA responses, highlighting the important role of T helper cell 1 responses in the ability of Gamma-PN (without adjuvant) to induce high OPA responses.

While Gamma-PN is still undergoing clinical evaluation in a Phase 1/2a trial, this broad-spectrum approach could eliminate the need to reformulate vaccines to address new serotypes. Indeed, any new serotypes that may emerge could be immediately tested for OPA responses using existing clinical trial serum samples. This future-proof approach could offer significant advantages, circumventing the need for further vaccine reformulation and extensive clinical trials. With current vaccines suffering from diminished usefulness over time and serotype replacement a growing concern, Gamma-PN may offer a solution to provide enduring and broad-spectrum protection against pneumococcal disease.

REFERENCES

1. Lewnard JA, Hanage WP. Making sense of differences in pneumococcal serotype replacement. *Lancet Infect. Dis.* 2019; 19(6), e213–e220.
2. Feikin DR, Kagucia EW, Loo JD, *et al.* Serotype-specific changes in invasive pneumococcal disease after pneumococcal conjugate vaccine introduction: a pooled analysis of multiple surveillance sites. *PLoS Med.* 2013; 10(9), e1001517.
3. Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. *Lancet* 2011; 378(9807), 1962–1973.
4. Senders S, Klein NP, Lamberth E, *et al.* Safety and immunogenicity of a 20-valent pneumococcal conjugate vaccine in healthy infants in the United States. *Pediatr. Infect. Dis. J.* 2021; 40(10), 944–951.
5. Cui YA, Patel H, O'Neil WM, Li S, Saddier P. Pneumococcal serotype distribution: A snapshot of recent data in pediatric and adult populations around the world. *Hum. Vaccin. Immunother.* 2017; 13(6), 1–13.
6. Lochen A, Croucher NJ, Anderson RM. Divergent serotype replacement trends and increasing diversity in pneumococcal disease in high income settings reduce the benefit of expanding vaccine valency. *Sci. Rep.* 2020; 10(1), 18977.
7. Turner P, Turner C, Green N, *et al.* Serum antibody responses to pneumococcal colonization in the first 2 years of life: results from an SE Asian longitudinal cohort study. *Clin. Microbiol. Infect.* 2013; 19(12), E551–E558.
8. Wilson R, Cohen JM, Reglinski M, *et al.* Naturally acquired human immunity to pneumococcus is dependent on antibody to protein antigens. *PLoS Pathog.* 2017; 13(1), e1006137.
9. Simell B, Lahdenkari M, Reunanen A, Kayhty H, Vakevainen M. Effects of ageing and gender on naturally acquired antibodies to pneumococcal capsular polysaccharides and virulence-associated proteins. *Clin. Vaccine Immunol.* 2008; 15(9), 1391–1397.
10. Pichichero ME, Khan MN, Xu Q. Next generation protein based Streptococcus pneumoniae vaccines. *Hum. Vaccin. Immunother.* 2016; 12(1), 194–205.
11. Briles DE, Paton JC, Mukerji R, Swiatlo E, Crain MJ. Pneumococcal vaccines. *Microbiol. Spectr.* 2019; 7(6).
12. Li S, Liang H, Zhao SH, Yang XY, Guo Z. Recent progress in pneumococcal protein vaccines. *Front. Immunol.* 2023; 14, 1278346.
13. Morsczeck C, Prokhorova T, Sigh J, *et al.* Streptococcus pneumoniae: proteomics of surface proteins for vaccine development. *Clin. Microbiol. Infect.* 2008; 14(1), 74–81.
14. Sempere J, Llamosi M, Del Rio Menendez I, *et al.* Pneumococcal choline-binding proteins involved in virulence as vaccine candidates. *Vaccines (Basel)* 2021; 9(2), 181.
15. Lagousi T, Basdeki P, Routsias J, Spoulou V. Novel protein-based pneumococcal vaccines: assessing the use of distinct protein fragments instead of full-length proteins as vaccine antigens. *Vaccines (Basel)* 2019; 7(1), 9.
16. Kim H, Yu J, Bai D, Nahm MH, Wang P. Potentiating pneumococcal glycoconjugate vaccine PCV13 with saponin adjuvant VSA-1. *Front. Immunol.* 2022; 13, 1079047.
17. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front. Immunol.* 2014; 5, 520.

18. Jodar L, Butler J, Carlone G, *et al.* Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. *Vaccine* 2003; 21(23), 3265–3272.

19. Malley R, Lipsitch M, Stack A, *et al.* Intranasal immunization with killed unencapsulated whole cells prevents colonization and invasive disease by capsulated pneumococci. *Infect. Immun.* 2001; 69(8), 4870–4873.

20. Malley R, Morse SC, Leite LC, *et al.* Multiserotype protection of mice against pneumococcal colonization of the nasopharynx and middle ear by killed nonencapsulated cells given intranasally with a nontoxic adjuvant. *Infect. Immun.* 2004; 72(7), 4290–4292.

21. Babb R, Chen A, Hirst TR, *et al.* Intranasal vaccination with gamma-irradiated Streptococcus pneumoniae whole-cell vaccine provides serotype-independent protection mediated by B-cells and innate IL-17 responses. *Clin. Sci. (Lond.)* 2016; 130(9), 697–710.

22. Jwa MY, Jeong S, Ko EB, *et al.* Gamma-irradiation of Streptococcus pneumoniae for the use as an immunogenic whole cell vaccine. *J. Microbiol.* 2018; 56(8), 579–585.

23. David SC, Laan Z, Minhas V, *et al.* Enhanced safety and immunogenicity of a pneumococcal surface antigen A mutant whole-cell inactivated pneumococcal vaccine. *Immunol. Cell Biol.* 2019; 97(8), 726–739.

24. Chan WY, Entwisle C, Ercoli G, *et al.* A novel, multiple-antigen pneumococcal vaccine protects against lethal Streptococcus pneumoniae challenge. *Infect. Immun.* 2019; 87(3), e00846-18.

25. Jang AY, Ahn KB, Zhi Y, *et al.* Serotype-independent protection against invasive pneumococcal infections conferred by live vaccine with *lgt* deletion. *Front. Immunol.* 2019; 10, 1212.

26. Ramos-Sevillano E, Ercoli G, Felgner P, *et al.* Preclinical development of virulence-attenuated Streptococcus pneumoniae strains able to enhance protective immunity against pneumococcal infection. *Am. J. Respir. Crit. Care Med.* 2021; 203(8), 1037–1041.

27. Alsharifi M, Mullbacher A. The gamma-irradiated influenza vaccine and the prospect of producing safe vaccines in general. *Immunol. Cell Biol.* 2010; 88(2), 103–104.

28. David SC, Lau J, Singleton EV, *et al.* The effect of gamma-irradiation conditions on the immunogenicity of whole-inactivated Influenza A virus vaccine. *Vaccine* 2017; 35(7), 1071–1079.

29. Singleton EV, Gates CJ, David SC, *et al.* Enhanced immunogenicity of a whole-inactivated influenza A virus vaccine using optimised irradiation conditions. *Front. Immunol.* 2021; 12, 761632.

30. McAllister LJ, Tseng HJ, Ogunniyi AD, *et al.* Molecular analysis of the PSA permease complex of Streptococcus pneumoniae. *Mol. Microbiol.* 2004; 53(3), 889–901.

31. Aggarwal S, Kumaraswami M. Managing manganese: the role of manganese homeostasis in streptococcal pathogenesis. *Front. Cell Dev. Biol.* 2022; 10, 921920.

32. David SC, Brazel EB, Singleton EV, *et al.* A nonadjuvanted whole-inactivated pneumococcal vaccine induces multiserotype opsonophagocytic responses mediated by noncapsule-specific antibodies. *mBio* 2022; 13(5), e0236722.

AFFILIATIONS

Erin B Brazel
Research Centre for Infectious Diseases (RCID), and Department of Molecular and Biomedical Sciences, The University of Adelaide, SA, Australia,
and
GPN Vaccines Ltd, Yarralumla, ACT, Australia

Mohammed Alsharifi
Research Centre for Infectious Diseases (RCID), and Department of Molecular and Biomedical Sciences, The University of Adelaide, SA, Australia,
and
GPN Vaccines Ltd, Yarralumla, ACT, Australia

Lauren Giorgio
GPN Vaccines Ltd,
Yarralumla, ACT, Australia

Timothy R Hirst
GPN Vaccines Ltd,
Yarralumla, ACT, Australia

James C Paton
Research Centre for Infectious Diseases (RCID), and Department of Molecular and Biomedical Sciences,
The University of Adelaide,
SA, Australia,
and
GPN Vaccines Ltd,
Yarralumla, ACT, Australia

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Brazel EB, Alsharifi M, Giorgio L, Hirst TR, and Paton JC are affiliated with, or employed by, GPN Vaccines Ltd and all authors hold an equity interest in the company. EBB is a principal investigator on a sponsored research agreement between the University of Adelaide and GPN Vaccines Ltd. EBB is a co-inventor on provisional and pending patents owned by GPN Vaccines Ltd. Pa-ton JC, Alsharifi M, and Hirst TR hold a sponsored research agreement between the University of Adelaide and GPN Vaccines Ltd. The authors own stock and stock options in GPN Vaccines Ltd. Giorigo L has received the South Australian Government Research Commercialisation & Start Up Grant. The authors possess pa-tents related to matters disclosed in the article have been granted to GPN Vaccines Ltd with costs of filing, prosecution and award being paid by GPN Vaccines.

Funding declaration: EBB has received support, including travel and registration costs, to attend scientific conferences from GPN Vaccines Ltd. The authors receive consultancy payments from GPN Vaccines Ltd.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited; externally peer reviewed.

Revised manuscript received: Feb 1, 2024; **Publication date:** Feb 7, 2024.

WEBINAR DIGEST

Affinity chromatography for malaria vaccine purification

Eugene Sun, Field Application Scientist, Thermo Fisher Scientific

The COVID-19 pandemic has emphasized the need for readily available commercial-scale vaccine production tools to support global immunization efforts. This poster presents a case study showing how researchers used C-tag technology to capture a candidate malaria vaccine.

In 2021, there were around 247 million cases of malaria, resulting in approximately 619,000 deaths, mostly in children under 5 years **[1]**. However, new vaccines are bringing hope for controlling the disease. Researchers at the Jenner Institute (Oxford University, UK) recently saw their R21/Matrix-M vaccine achieve an efficacy of 77% in Phase IIb clinical trials **[2]**.

In addition to R21/Matrix-M, scientists at the Jenner Institute are developing a range of malaria vaccines targeting different life stages of the parasite, including a vaccine targeting the PfRh5 protein, which plays an important role in cell entry.

During the development of the PfRh5-based vaccine, the researchers first investigated the functionality of a polyhistidine tag (His-tag) to capture the protein. However, initial results indicated that the immobilized metal affinity chromatography (IMAC) process used to capture His-tag was not scalable due to poor yield.

The team next investigated the C-terminal tag (C-tag), which is comprised of four amino acids (glutamic acid-proline-glutamic acid-alanine, or E-P-E-A) coupled to the C terminus of the target protein. For use in conjunction with the C-tag, Thermo Fisher Scientific has created a scalable, cGMP-compliant affinity resin (CaptureSelect™ C-tagXL), which specifically targets the C-tag's four amino acids.

CaptureSelect™ affinity resins allow for elution using mild pH conditions, which maintains the stability of the target molecule. Scientists working on the vaccine were able to elute the PfRh5 protein using a TRIS, tris(hydroxymethyl)aminomethane, buffer with magnesium chloride at pH 7 **[3]**.

Figure 1 shows a comparison of the two purification processes for the PfRh5 protein—one using the His6-tag and IMAC, and the other using C-tag and CaptureSelect C-tagXL. Both processes involved tangential flow filtration, followed by the affinity step, then size exclusion chromatography (SEC). PfRh5 purity was approximately 72% for the C-tag purification, which was much higher than the 20% purity observed using the His-tag process **(Figure 1A)**. CaptureSelect C-tagXL also offered higher resolution **(Figure 1B)**.

Figure 1C shows the process yield at each step. There was a 43.3% overall process yield with the C-tag, compared with a 25.5% process yield with the His-tag. In addition, the affinity step yield for the C-tag was approximately 85%, compared with the 64% step yield for the His-tag, and the SEC pool purity for the C-tag process was greater than 99%, compared with 80% for the His-tag process.

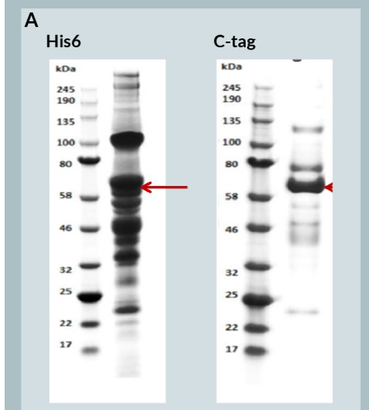
REFERENCES

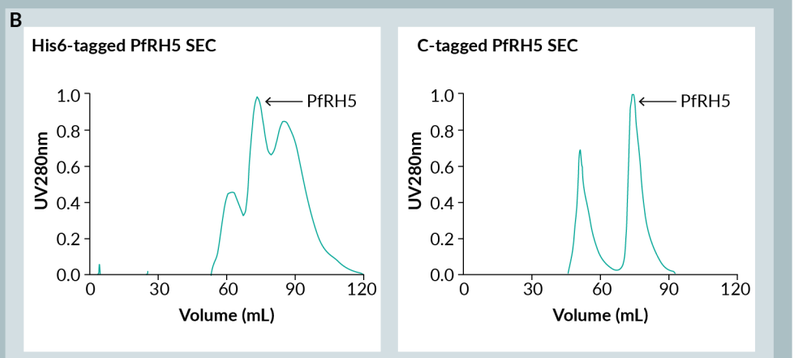
1. *WHO*. World Malaria Report 2022.

2. Datoo MS, Natama MH, Somé A, *et al*. Efficacy of a low-dose candidate malaria vaccine, R21 in adjuvant Matrix-M, with seasonal administration to children in Burkina Faso: a randomised controlled trial. *Lancet* 2021; 397, 1809–1818.

3. Jin J, Hjerrild KA, Silk SE, *et al*. Accelerating the clinical development of protein-based vaccines for malaria by efficient purification using a four amino acid C-terminal 'C-tag'. *Int. J. Parasitol.* 2017; 47, 435–446.

Figure 1. Comparison of C-tag and His-tag used in a protein-based vaccine purification process. **(A)** Purities of the elution pools; **(B)** size exclusion chromatography (SEC) chromatograms of the eluate; **(C)** yields of both processes.





A) Purity comparison on gel

B) Yields comparison of both processes

C) Size exclusion comparison

C

Process yield (after)	His6-tagged construct (%)	C-tagged construct (%)
Culture supernatant	100	100
TFF	82.1	91.0
Affinity chromatography	52.5	77.4
SEC	25.5	43.3

TFF: Tangential flow filtration; SEC: Size-exclusion chromatography

Watch the webinars here | Watch the webinars here | Read the full articles here | Read the full articles here

Vaccine Insights 2024; 3(1), 5 • DOI: 10.18609/vac.2024.002

In partnership with: ThermoFisher SCIENTIFIC