VACCINES IN THE PREVENTION OF VIRAL PNEUMONIA

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DISCLOSURE STATEMENT

PO holds a Wellcome Trust grant supporting vaccine testing with Mucosis BV, and collaborates with GlaxoSmithKline on RSV disease.

KEY WORDS

Influenza; vaccination; respiratory syncytial virus (RSV); parainfluenza; adenovirus; lower respiratory tract infection (LRTI), viral pneumonia.

Published online as article in press on 18th December 2016 in Clinics in Chest Medicine

<http://dx.doi.org/10.1016/j.ccm.2016.11.009>

KEY POINTS

- Viral pneumonias are a major cause of disease and death across the globe.
- Vaccination is a most effective way of preventing infection, but is only available for a limited (but expanding) number of respiratory pathogens.
- Current seasonal Influenza vaccines confer insufficient protection, especially in some high-risk populations (e.g. older adults).
- Research into correlates of protection has identified new ways to develop universal influenza vaccines that induce broad and long –lasting humoral and cell-mediated responses.
- Respiratory Syncytial Virus is a common cause of viral pneumonia and a largely unrecognized killer of frail elderly persons. Many promising vaccines are under development and there are high hopes of effective vaccines in the near future.

SYNOPSIS

Pneumonia is of great global public health importance. Viral infections play both a direct and indirect part in its etiology across the globe. Influenza is a leading cause of viral pneumonia in both children and adults, and respiratory syncytial virus (RSV) is increasingly recognized as causing disease at both the extremes of age. Vaccination offers the best prospect for prevention but current influenza vaccines do not provide universal and durable protection, especially in high-risk populations and require yearly reformulation. In the future, it is to be hoped that influenza vaccines will give better and universal protection, and that new vaccines can be found for other causes of viral pneumonia.

INTRODUCTION

Pneumonia is of huge global public health concern. Viral and bacterial pneumonias are major and leading causes of global mortality, the impact being greatest in children, the elderly and the immunodeficient and those with co-morbidities¹⁻³. In 2015, pneumonia was estimated to cause 41.7 deaths per 100 000 population⁴. In 2010, it is thought that there were approximately 15 million hospital admissions for severe acute lower respiratory infections (ALRI) in children under 5 years and that 265 000 of these resulted in death. However only 62% of children with ALRI are admitted to hospital with the vast majority of deaths happening in the community (81%)⁵.

The introduction of molecular (PCR-based) diagnostics enable pathogens to be identified in many patients with community-acquired pneumonia, but in many cases the initiating infection remains unidentified. Respiratory viruses are implicated in about 45% of pneumonia cases requiring hospitalization in children⁶ but some viruses (rhinovirus and adenovirus in particular) are found both in symptomatic and asymptomatic individuals⁷.

The relative importance of viral infections as a cause of pneumonia has increased not only because of improved diagnostics, but also because of the introduction of bacterial vaccines such as the Hib conjugate (HibCV) and pneumococcal conjugate vaccines (PCVs)⁸. Vaccination is also available for influenza and vaccination against varicella zoster, rubella and measles helps to prevent additional cases of viral pneumonia and its complications.

The burden of ALRI caused by viral pathogens indicates clearly that additional effective, durable and affordable vaccines are urgently needed.

VIRAL VACCINES

Current licensed vaccines include inactivated, subunit, vectored and live attenuated preparations. Inactivated vaccines may be made up of whole virus, split virus, subunit or virus-like particles. Whole virus is grown in culture and then inactivated using a variety of methods including chemical or heat treatments to render them non-pathogenic. Vaccines containing whole killed organisms are generally

cheaper to produce but may have a disadvantageous safety (reactogenicity) or immunogenicity profile. Split virus vaccines are a type of inactivated vaccine, split using organic solvents or detergents. Subunit vaccines comprise isolated or biosynthetic viral proteins that are selected to stimulate appropriate protective immune response while avoiding adverse host reactions.

Some vaccines, especially those that are highly purified and refined, may need to be combined with adjuvants and/or require the inoculation of multiple doses to be immunogenic. Adjuvants augment the host's immune response to vaccination, normally by providing a collateral 'danger' signal via the innate immune system and thus boosting the protective acquired immune response. They enhance immunological memory, allowing greater optimised antigen presentation⁹. Examples of adjuvants include alum (aluminum salts), virosomes, MP59 and AS03.

EXPERIMENTAL VACCINES

There are many different innovative vaccine approaches that are currently in clinical development and for the most part these focus on directing pathogen genomic material to the target host immune cell. DNA vaccines involve the injection of DNA encoding specific antigens into muscle leading to *de novo* antigen expression and the stimulation of both B and T-cells. A major advantage of DNA vaccines is the stability, the absence of any infectious material and the possibility of rapid scale-up. Drawbacks include the difficulty of translating apparent benefits from animal models into man and the need for repeated and relatively large volume injections. Most ongoing trials are in the treatment of HIV and certain cancers, but there are some studies of DNA vaccines for influenza^{10,11}.

Recombinant vector vaccines aim to introduce microbial DNA from an attenuated virus or bacteria using another pathogenic virus or bacteria to deliver genetic material to the appropriate host immune cell. Therefore, this closely mimics natural infection and triggers a corresponding immune response. Dendritic cell vaccines work in a similar way but exploit the immune systems' own antigen presenting cells to present pathogenic DNA. These are mostly being investigated in the context of HIV infection and cancer¹².

CHALLENGE MODELS AND VACCINE DEVELOPMENT

Animal models make for useful preliminary models for analyses of immune biology and identification of potential vaccine candidates. However animals and humans can differ in their immune responses and correlates of protection¹³, confounded by the fact that those who experience the most severe viral disease are not typically those studied in challenge models (infants, pregnant women and the elderly)¹⁴.

Below we will discuss some of the common causes of viral pneumonia, current vaccination programs, their efficacy and potential vaccine strategies for the future, including influenza, respiratory syncytial virus, parainfluenza and adenovirus.

INFLUENZA

Influenza virus is the commonest cause of viral pneumonia in adults and the only virus that has an established global vaccination program. Epidemics have been estimated to cause 2–5 million cases of severe illness and 250,000–500,000 deaths per year across the globe. Annual infection rates are estimated to be 5–10% in adults and 20–30% in children. Those at increased risk of severe disease are infants, the elderly, pregnant women and those with major co-morbidities¹⁵. Vaccination against influenza virus has been available since the 1940s and remains the most effective way of preventing disease.

There are 3 types of influenza: A, B and C (A and B causing most human disease). Influenza B is more genetically stable than influenza A, with less antigenic drift and consequent immunologic variation¹⁶. Influenza vaccines have to be re-formulated due to the constantly evolving nature of influenza viruses due to antigenic shift and drift in response to immunological pressure and reassortment events. Mutations in surface proteins hemagglutinin (HA) and neuraminidase (NA) accumulate under a variety of influences: an error-prone RNA polymerase, host immune pressures and co-infection of a host with multiple strains can lead to gene re-assortment.

Seasonal influenza vaccines vary depending on geographical region and are tailored to the circulating strains. Each year in February and September the World Health Organization (WHO) recommend

viruses for inclusion in seasonal influenza vaccines in the southern and northern hemispheres, using information from classical re-assortment and reverse genetics techniques ¹⁷.

In 2010, the United States Advisory Committee on Immunization Practices (ACIP) expanded the recommendation for influenza vaccination to include all individuals six months of age and older ¹⁸. Globally however due to limited resources this is not always possible and therefore high-risk groups are prioritized ([see Panel 1](#)). Live attenuated vaccination is currently being introduced to protect children in the UK.

Panel 1- Populations to prioritize for Influenza vaccination

Those at high risk for influenza-related complications:

- 6 months through 4 years (59 months) of age
- ≥50 years of age
- Have chronic co-morbidities: pulmonary (e.g. COPD, asthma), cardiovascular (except hypertension), renal, hepatic, haematological (including sickle cell disease), metabolic (including diabetes mellitus), neurological (including neuromuscular/ neurodevelopmental) disorders
- Immunosuppressed (including immunosuppression caused by medications or by HIV)
- Pregnancy
- 6 months through 18 years of age and receiving long-term aspirin therapy (and therefore may be at risk for Reye syndrome after influenza virus infection).
- Morbid obesity (body mass index [BMI] ≥40 for adults or BMI >2.33 standard deviations above the mean for children)

Environmental:

- Nursing home residents and other long term care facilities
- Healthcare personnel
- Household contacts or caregivers of children <5 years and adults ≥50 years of age
- Household contacts or caregivers of persons with medical conditions that put them at increased risk for severe complications of influenza

Adapted from following sources: Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices — United States, 2013–2014. World Health Organization (WHO) Weekly epidemiological record vaccines against influenza WHO position paper, November 2012.

Types of Influenza Vaccines

Licensed Influenza vaccines are inactivated (whole virus, split virus, or subunit) or live attenuated. Inactivated Influenza Vaccines (IIVs) are generally produced from highly purified, egg-grown influenza viruses and delivered intramuscularly or intradermally. A trivalent IIV has been available since 1978. In 2003 in the USA and in 2011 in Europe a live attenuated influenza vaccine (LAIV) for intranasal use was approved for use in healthy adults and children.

Trivalent or Quadrivalent?

Trivalent vaccines contain antigens from two A strains and one B strain from a single lineage. Historically, there are several examples of mismatch between the vaccine and the circulating B strain have occurred. In the years 2001 through to 2011, the predominant circulating influenza B lineage was different from that contained in the trivalent vaccine in five out of ten seasons ¹⁹. It is now generally recommended that quadrivalent influenza vaccines should be used that contain two influenza A strains (H1N1 and H3N2 subtypes) and two influenza B strains (Victoria and Yamagata lineages).

Vaccine Efficacy

The effectiveness of influenza vaccines is related to the age and immune competence of the recipient, as well as the antigenic matching of vaccine to circulating strains. A recent Cochrane review of inactivated seasonal flu vaccination in healthy adults showed an overall efficacy in preventing confirmed influenza of 60% (a number needed to vaccinate (NNV) of 719)²⁰. In this review, it was concluded that vaccination did not have a demonstrable effect on hospital admissions or working days lost ²⁰. In years with poor vaccine matching, benefit is much lower, although the vaccine can still provide protection against more severe outcomes ²¹. A case-control study showed previous vaccinees achieved mortality reduction rates of up to 75% (95% CI 31 to 91%), but only a 9% (95% CI 0 to 59%) reduction in mortality was seen in those that had never received a vaccination ²².

Efficacy for the LAIV in healthy adults is similar to that of inactivated vaccine ²⁰, but depends on the 'take' of the vaccine on the mucosal surface. LAIV given intra-nasally as a large particle spray appears

superior to inactivated trivalent influenza vaccine with respect to protection against influenza strains that diverge from the vaccine strain ²³, indicating a degree of heterosubtypic protection.

INFLUENZA VACCINATION IN AT RISK POPULATIONS

For a list of vaccination recommendations for at risk populations, see [Panel 1](#) ^{24–26}.

Pregnant Women

It is recommended that if vaccination is in short supply, the first population group targeted should be pregnant mothers²⁵. This is because influenza may be more severe in pregnancy, and that it increases perinatal infant mortality, prematurity, smaller birth size and weight ²⁷. Maternal vaccination may also benefit the newborn child ²⁸.

The use of the IIV is recommended in pregnancy. It has been shown that vaccination reduces risk to both mother and infant (via passive immunity). An Indian study of 340 women demonstrated a 63% reduction of proven influenza illness in infants up to 6 months of age and prevention of approximately a third of febrile respiratory illnesses in mothers and young infants ²⁹. In addition, maternal immunization results in the presence of antibody titers against Influenza-A vaccine subtypes in a significant proportion of mothers and their infants. Hemagglutination-inhibition antibodies for influenza A subtypes are greater in infants of vaccinated mothers up to 20 weeks of age however the immunogenicity varies dependent on strain^{30, 31}.

Children

LAIV is now the vaccine of choice for healthy children aged 2-18 years, due to the significant weight of evidence suggesting vaccine effectiveness³². A meta-analysis of nine randomized controlled trials compared LAIV to placebo demonstrated a relative efficacy of 77% against antigenically similar strains and 72% efficacy regardless of antigenic similarity, whilst comparison with TIV showed that 46% fewer children experienced influenza illness ³³. Other studies have also showed LAIV to consistently provide a higher level of protection in children when compared to inactivated vaccines or placebo ^{34–36}. Regarding

other vaccine types, a Cochrane review in children aged 6 months to 2 years of age showed inactivated trivalent influenza vaccine is not significantly more efficacious than placebo ³⁴. However a 2011 randomised controlled trial involving 4707 children aged 6 to 72 months (1941 vaccinated) showed that the adjuvant influenza vaccine (MF59 adjuvant trivalent vaccine) was significantly more effective than control or lone trivalent vaccine at preventing influenza like illness ³⁷ Interestingly when given to children (age 6-15 years) the TIV vaccine has been associated with an increase in the rates of non-influenza respiratory virus infection³⁸. This study was carried out in the pandemic season 2008-2009, however this data has not been replicated in different age groups (under 5 years and greater than 50 years)³⁹.

Older Adults

The majority of deaths associated with influenza occur in frail elderly persons ^{18,40} therefore influenza vaccination is generally recommended for all those over the age of 65 years ¹⁸. This is particularly important for those suffering from co-morbidities, such as chronic lung disease, heart failure or diabetes. Effectiveness of vaccination at preventing influenza in the elderly is of ongoing debate, with a great degree of heterogeneity in published studies⁴¹. A robust randomized control trial in this age group showing significant benefit of vaccine in older adults is lacking⁴².

Comprehensive reviews come to differing conclusions dependent on study design, methodology, inclusion criteria and varying approaches to the correction of season-to-season variation of vaccine matching ^{35,43,44}. Two large meta-analyses assessing the same body of evidence have come to differing conclusions, whilst one group concluded that there was insufficient evidence with respect to older adults ⁴², the other concluded when virus was circulating, vaccination reduced influenza-related and non-fatal complications by 28% and confirmed cases of influenza by 5% ⁴⁵. These differences highlight the importance of taking into account the large number of factors that affect the estimation of influenza vaccine effectiveness and the importance of careful and thorough evaluation. Failure to find an effect may be as much due to methodological shortcomings as to lack of efficacy.

To improve vaccine immunogenicity in older age groups, higher dose vaccines have been trialed, resulting in significantly greater antibody responses ⁴⁶. Promising data has also been seen with the use of adjuvanted vaccines ⁴⁷. LAIV is mostly ineffective in older adults due to previous immunity hindering local mucosal infection and therefore vaccine induced immune response ⁴⁸.

Asthmatics

Globally, asthma is considered a priority group for vaccination ^{24,49}, and clear guidelines can result in greatly increased uptake in vaccination³². However, there are inconsistent reports of vaccine effectiveness in persons with asthma largely due to differences in patient subgroups, research methodology and variability in the definition of asthma.

LAIV has been demonstrated to cause a 35% relative risk reduction in culture-confirmed influenza when compared to TIV in a large trial (n=2229) of asthmatic children aged 6-17 with no increase in exacerbations of their underlying asthma ⁵⁰. A study of younger children aged 6-59 months concluded that LAIV reduced proven influenza cases by 55% when compared to TIV but the youngest children (aged between 6-11 months) experienced a higher hospitalized rate after LAIV compared to TIV (6.1% vs 2.6%)³⁶. Because of the small but significant potential for LAIV to cause wheeze and increased hospital admissions, the Centres for Disease Control (CDC) recommend avoiding its use in children aged 2-4 years who have had an episode of wheeze in the preceding 12 months²⁴.

A Cochrane systematic review concluded that vaccination with inactivated influenza vaccination did not result in a significant reduction in the number, duration or severity of influenza-related asthma exacerbations but did improve symptoms and also noted that there was no evidence that LAIV vaccine itself is actually causative of asthma exacerbations ⁵¹.

Reports of low effectiveness can adversely affect uptake of vaccination amongst patients whilst a healthcare worker recommendation to vaccinate is highly predictive of adherence⁵².

Pandemic Influenza Vaccines

Rapid deployment of vaccines as part of the response to pandemic threats is often problematic, largely due to the delay in production of new vaccines and the speed of spread of novel influenza strains. Three influenza pandemics occurred last century: 1918, 1957 and 1968 with a total mortality of 50–100 million people⁵³. In 2009 an Influenza pandemic was declared by the WHO (caused by the influenza A H1N1 virus). The emergence of a new influenza strain genetically very different caused a pandemic with significant mortality even in persons with no co-morbidities⁵⁴. This is due to lack of herd immunity and vaccinations not being matched to the novel influenza strain.

Seasonal vaccines generally offer little or no protection against novel pandemic strains; although there are hints that weak heterosubtypic protection may occur with LAIV. Developing a novel influenza vaccine requires safety and immunogenicity testing, but generally not field trials of efficacy. Even with expedited testing, production inevitably lags behind the timeline required for novel pandemic strains. A new vaccine typically takes 4-6 months to make, by which time the pandemic peak may be passed. Second, antigens from some novel influenza strains (e.g. avian H5N1) are poorly immunogenic, making effective induction of immunity with vaccination difficult even with the use of adjuvants and multiple doses⁵⁵. Ideally, a universal influenza vaccine that provides broadly cross-reactive protection through the induction of antibodies and/ or T cells to the conserved regions of the virus need to be developed to counter future epidemic and pandemic threats⁵³.

Correlates of Protection – Influenza Vaccination

Influenza vaccination induces antibodies, primarily against the major surface glycoproteins HA and NA. Antibodies directed against HA are the most widely accepted correlate of protection⁵⁶. HA is the viral receptor-binding protein and antibodies that are directed to epitopes located in or near to this can prevent binding of the virus to its receptor and thus its infectivity. Titres of 1:40 HA inhibition antibody or greater have been shown to provide 50% level of protection in healthy adults and this concentration of vaccine induced antibody is used as a benchmark during vaccine development⁵⁷. This is not necessarily the case for LAIV, where secretory immunoglobulin A is a better correlate of mucosal protection⁵⁸.

T cells are abundant in the lung mucosa and have the ability to recognise conserved internal viral proteins. Although not preventing infection higher peripheral CD4+ T cell are associated with lower virus shedding and less severe influenza illness, even in the absence of specific antibodies ⁵⁹. Presence of cross-reactive CD8+ T cells have also been associated with a reduced symptom burden as well as higher levels of interferon- γ (IFN- γ) and interleukin-2 (IL-2) in influenza infection ⁶⁰. As T lymphocytes (CD4+ or CD8+) preferentially recognize the more conserved internal proteins, this creates great potential for broad responses ⁶¹.

As already mentioned, frail elderly persons do not mount as good an immune response as younger people. T cell senescence is well described, particularly in studies of cytotoxic T cells. In a study of non-institutionalized elderly persons, pre-vaccination interferon- γ was ten-times lower, IL-10 three-times greater and the Th1:Th2 ratio was lower in those that developed influenza in the weeks following vaccination ⁶².

The Future of Influenza Vaccines

Given the increasing emergence of influenza strains that are resistant to varying antiviral therapies: (adamantanes⁶³, rimantadine, zanamivir and oseltamivir⁶⁴) and the fact that classical influenza vaccines must be updated regularly to match the new strains, prevention of disease using novel vaccine platforms should be given some urgent priority.

Cell culture technology offers the potential to produce large quantities of vaccine antigen, and adjuvants allowing for less viral antigen to be used. MF59 and AS03 have both been shown to be safe and effective and are now licensed adjuvant vaccines⁶⁵. Recently two synthetic Toll-like receptor (TLR) adjuvants (IZ105 and IV270) have shown promise in murine challenge studies ⁶⁶.

The most promising universal influenza vaccines are those that induce cross-reactive humoral and cell mediated responses. Universally conserved antigens with some promise include the extracellular

domain of M2 ⁶⁷, but this locus is poorly immunogenic ⁶⁸. The stalk portion of HA (HA2) is an attractive target as it contains several conserved epitopes that are highly conserved ⁶⁹.

The non-structural NS1 protein deletion blocks viral replication and stimulates an antibody and cellular immune response. Intranasal delivery of a live NS1 vaccine has been shown to be safe and immunogenic⁷⁰. Vaccines that induce CD8 T cell responses targeting viral nucleoprotein (NP), matrix protein 1 (M1) and polymerase basic 1 (PB1) offer the prospect of universal influenza vaccines that might additionally accelerate viral clearance⁷¹ ([Panel 2](#)).

Panel 2: Current Issues in Influenza Vaccinology

Improving Traditional Approaches

How can durability of protection be enhanced?

How might adjuvants be best used?

How can vaccines be targeted to appropriate risk groups?

Better influenza strain prediction

Future Vaccine Development

How to develop universal vaccines against all existing and emerging strains

Evaluation of cross reactive stalk/stem antibodies

Overcoming weak immunogenicity of some influenza antigens

Optimization of vaccine delivery methods

Improving Manufacturing

Acceleration of production techniques

Manufacture of new vaccines to meet emerging/zoonotic strains

Improved stability and convenient formulation

RESPIRATORY SYNCYTIAL VIRUS

Burden of Disease and Early Attempts at Vaccination

As recently reviewed⁷² respiratory syncytial virus (RSV) is responsible for more than 30 million episodes of acute respiratory tract infection (ARI) globally. With up to 20% of infants under the age of 1 requiring medical attention because of RSV disease, the need for an effective vaccination that can elicit durable protection in at-risk groups is clear^{73,74}.

Indeed, it is surprising that whilst other common respiratory pathogens such as influenza virus and *H. influenza* have established immunization programs, a vaccine against RSV remains elusive. This is in part due to studies of in the 1960s, which showed that alum-precipitated formalin-inactivated RSV vaccine (FI-RSV) can augment subsequent disease^{75,76}. Such vaccines not only fail to prevent infection, but dramatically increase hospitalization rates in young children during natural infection. This appears to be associated with an induction of poorly-neutralizing antibody, skewing towards the production of Th2 cytokines^{77,78} and a local deficit in regulatory T cells⁷⁹. These events hampered efforts in vaccine discovery, but in recent years academics, vaccine manufacturers and international organizations such as WHO have made development of safe and effective vaccines a realistic prospect⁸⁰.

Antigenic Targets for Prophylaxis and Vaccination

RSV is a negative-sense, single stranded RNA virus consisting of 10 genes that encode 11 proteins; the surface glycoproteins F and G are responsible for fusion and attachment to the host cell membrane respectively. F and G are both targets for neutralizing antibody and are the key vaccine antigens⁸¹.

F protein is crucial in facilitating the fusion of the virus and cell membrane, thereby permitting cellular infection (and incidentally, of cell fusion into syncytia). Prior to fusion, (pre-F) protein has a metastable conformation and forms trimeric structures extending 11 nm from the surface. Upon fusion with the host cell membrane the structure extends to 16 nm and assumes a new post-fusion conformation that is highly stable. Both pre-F and post-F forms can be found on the viral surface and share two major sites for antibody binding; however, neutralizing antibody is predominantly against the less stable pre-fusion form of F, which is now the generally preferred component of RSV vaccine candidates⁸².

The G protein can be either membrane bound or secretory. The soluble form may act as a decoy to assist in viral evasion⁸³, but G (in either form) also engages the CX3CR1 fractalkine (CX3CL1) receptor, demonstrated to impair interferon (IFN) production by epithelial and dendritic cells *in vitro*⁸⁴. The CX3C motif is located within the central conserved domain of G, flanked by mucin-like serine-threonine rich domains that are heavily *O*-glycosylated.

Vaccination with layer-by-layer nanoparticles (LbL-NP) carrying the G protein CX3C motif has recently been shown to induce blocking antibodies and attenuate RSV pathogenesis in mice⁸⁵. However, given the degree of glycosylation and variability of G protein coupled with recent advancements in structural biology techniques, F protein has been identified as being the most promising target for vaccination⁸⁶.

Although antibody induction is the major goal of vaccination against RSV disease, the recent demonstration that local mucosal CD8 T cells play a part in controlling viral load offers the prospect that T cell induction by vaccines might also be beneficial^{87–89}.

RSV Prophylaxis

Palivizumab is a humanized monoclonal antibody against F protein. It targets antigenic site II on the viral protein and works prophylactically (but not therapeutically) to reduce hospitalization rates due to RSV bronchiolitis in infants⁸². It is licensed for use in selected high-risk infants less than 1 year of age, and is given as five monthly doses by intramuscular injection. In some cases, it can be given in the second year of life⁹⁰, but finds little use outside these indications. Disadvantages include prohibitive cost and the lack of efficacy in those with established disease. Most failures of prophylaxis reflect delay in onset of prophylaxis or in the administration or scheduling of monthly injections. The seasonality is variable depending on geographical location^{72,91}.

Recent evidence suggests that palivizumab also reduces the prevalence of wheeze in the first year of life, presumably by delaying RSV infection beyond the critical first few months of life. This effect occurs

after the end of treatment, lending strength to the argument that wheeze is a delayed effect of bronchiolitis in premature infants ⁸³.

Another monoclonal antibody, motavizumab, was developed with greater affinity to site II; this showed an 87% relative reduction (relative risk [RR] 0.13, 95% CI 0.08–0.21) in the proportion of healthy-term infants admitted to hospital with RSV when compared to placebo but has subsequently been discontinued due to non-superiority to palivizumab and side effects related to skin reactions. In native American (Navajo) infants born at 36 weeks' gestational age, motavizumab is surprisingly ineffective at preventing post-RSV wheeze ⁹².

RSV Vaccine Development

Due to the requirement of vaccines for diverse target populations (young infants, older infants, pregnant women and the elderly), multiple strategies may be required. These include live attenuated, particle-based, subunit and vector-based vaccine approaches. Young RSV-naïve infants, for example, might benefit from live-attenuated vaccines while adults (who have experienced multiple infections) or immunosenescent older individuals may require different approaches.

An RSV F nanoparticle vaccine (Novavax Inc., 9920 Belward Campus Drive, Rockville, MD 20850, USA) is currently the most advanced vaccine in clinical trials for the protection of older adults (60 years of age and older), having been shown to be well-tolerated and immunogenic in healthy adults. However, a phase 3 clinical trial in 11,856 older adults failed to meet its primary objective of preventing RSV-associated lower respiratory tract disease or its secondary objective of reducing all symptomatic respiratory disease due to RSV [NCT02608502]. The reasons for this failure of efficacy are yet to be established but a milder RSV season was noted during the study and the vaccine was tolerated safely. A related study in women in their third trimester of pregnancy is being carried out with the aim of reducing RSV lower respiratory infection in their infants up to 3 months of age [NCT02624947].

A promising live attenuated vaccine, MEDI-559 (MedImmune/National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA) induces a substantial immune response in 59% of children

aged 5 to 24 months of age⁹³. A range of other candidate vaccines are moving forwards from the pre-clinical testing stage to clinical trials, including a mucosal vaccine based on bacteria-like particles coated in F (Mucosis BV, NL). The RSV vaccines in different phases of development are shown in [Table 1](#).

Table 1– Snapshot of Current RSV Vaccines					
	Preclinical	Phase I	Phase II	Phase III	Market approved
Live attenuated	7	5	0	0	0
Inactivated	1	0	0	0	0
Particle	12	1	1	2	0
Subunit	10	2	2	0	0
RNA/DNA	4	0	0	0	0
Vector	8	3	0	0	0
Other (incl. antibody)	2	1	0	0	1
Total n=62	44	12	3	2	1

From PATH. Vaccine development – Vaccine Development Global Program. Available at: <http://sites.path.org/vaccinedevelopment/respiratory-syncytial-virus-rsv/vaccine-development/>. Accessed May 19, 2016.

Overcoming Hurdles for RSV Vaccine Development

The ideal RSV vaccine would elicit long-lasting upper and lower respiratory tract protection, be well tolerated in infants and adults alike and be free of risk of subsequent disease augmentation. Natural infection causes only partial protection against re-infection despite the low rates of antigenic variability, which indicates that an effective vaccine needs to surpass natural infection but avoid causing excessive T-cell mediated host immune disease. This is especially important in infants with an immature immune system⁹⁴. These young infants are especially at risk of severe RSV infection, and may benefit from maternal immunization⁹⁵. This aims to induce protective antibody that is transferred to the child via the

placenta and breast milk, but accurate correlates of protection as well as an understanding of the optimal route and timing of maternal vaccination is lacking. Vaccine effectiveness is typically assessed by the level of induced systemic RSV-specific neutralizing antibodies but this may not be an accurate predictor of durable immunity, with a human challenge model of RSV infection suggesting that the presence of mucosal RSV-specific IgA may provide a better correlate of protection⁹⁶. A non-profit organization, PATH, is developing an international RSV antibody reference standard to permit greater comparability between research studies⁹⁷. These wide-ranging and intensive efforts to develop a range of suitable vaccines, raises the hope of successfully combating RSV-mediated disease in the not too distant future.

PARAINFLUENZA VACCINATION

Parainfluenza viruses (PIV) are single-stranded RNA viruses that, like RSV, are members of the *Paramyxoviridae* family and cause an approximately similar spectrum of symptoms. There are four major serotypes of PIV (1-4) with PIV1 and PIV2 being associated with croup, PIV3 with pneumonia and PIV4 with upper respiratory tract infection⁹⁸.

The usual course of infection results in a mild self-limiting illness but susceptible individuals, particularly those who are immunocompromised, can experience a more serious, protracted illness marked by severe pneumonia and prolonged viral shedding^{99,100}. Most children by the age of 5 years and virtually all adults have antibodies to PIV with multiple reinfections common but usually characterized by only mild disease limited to the nose and pharynx^{101,102}. As PIV3 is the most common serotype, which has the ability to cause bronchiolitis in infants, most vaccine efforts have been directed towards it.

The virus contains six proteins that are common across serotypes. Two of them (fusion glycoprotein F and hemagglutinin-neuraminidase glycoprotein HN) are present on the viral surface and are the main targets for neutralizing antibodies. There is as yet no vaccine to prevent PIV infection despite nearly 60 years of research and development. Current strategies include the use of nasally delivered live-attenuated vaccines, the use of reverse genetics, bovine/human chimeras and subunit vaccines that express F and HN proteins¹⁰³.

The live-attenuated vaccine HPIVcp45 is derived from a live strain of HPIV3 that has undergone cold-passage (*cp*) 45 times. The accumulation of 15 attenuating mutations has rendered it non-pathogenic, and it is undergoing phase 1 trials in both adults and infants. It is reported to have a 94% infectivity rate and lacks transmissibility^{104–106}. A larger phase II study with a population size of 380 children aged 6-18 months, consisting of 226 seronegative children demonstrated that it generated adequate antibody response in 84% of seronegative recipients with no significant difference in adverse events when compared to placebo¹⁰⁷.

In other trials, a recombinant form of the vaccine (rHPIV3cp45), re-derived from cDNA, shows a shorter passage time and reduced the risk of contamination or reversion¹⁰⁸. It has been used in a phase 1 study of 40 seronegative children between 6-35 months and generates virus-specific antibodies with a tolerable safety profile¹⁰⁹. Another trial utilizing rHPIV3cp45 in healthy young seronegative infants between 6-12 months demonstrated strong immunogenicity. These studies involved the delivery of multiple doses of vaccine with appropriate intervals which is necessary to sustain durable immune responses and children in particular are likely to benefit from the nasal route of administration due to the ability to generate protective mucosal and systemic immunity¹⁰⁸.

An antigenically similar bovine PIV3 (BPIV3) vaccine has been developed either alone or as a chimeric recombinant virus with human PIV3 (rB/HPIV3). The latter was highly restricted in replication in adults and seropositive children but caused significant infectivity amongst seronegative children^{110–112}.

HPIV1 and HPIV2 vaccines are in development but are at a less advanced stage with only three trials that have undergone human clinical trial testing at present [ClinicalTrials.gov identifiers: NCT00641017, NCT00186927, NCT01139437]. Results from a recent live-attenuated HPIV1 vaccine trial suggested that it is appropriately restricted in adults and seropositive children but inadequately infectious in seronegative children highlighting the challenges faced in developing novel vaccines that are safely tolerated but also have the ability to cause sufficient immunogenicity in their target populations¹¹³.

Delivery of combined vaccines to young infants that target PIV and RSV to elicit broad protective immunity may also be a feasible prospect in this age group and if successful could reduce the significant burden of disease due to these common respiratory pathogens^{114,115}.

ADENOVIRUS

Adenoviruses were first discovered in 1953¹¹⁶. They display diverse respiratory manifestations from simple upper respiratory tract infections to severe fatal pneumonia¹¹⁷. Those most at risk are children under 5 years old, the immunocompromised (most notably those who have received bone marrow transplants¹¹⁸ and those living in crowded conditions such as the military. The majority of acute respiratory infection among U.S. military trainees is attributable to adenovirus¹¹⁹.

The introduction of an adenovirus vaccine has greatly reduced military respiratory infection morbidity since its introduction in the 1970s. However in 1995 the sole manufacturer ceased production of this vaccine. Over a 2 year period in the nineties when adenovirus vaccines were diminishing 3413 throat cultures were taken from trainees with acute respiratory illnesses with 1814 (53.1%) being positive for adenovirus. Those who were unvaccinated (n=2322) were more likely to yield a positive adenovirus culture (OR, 13.2) especially for serotypes 4 or 7 (OR, 28.1). During this period there were also a number of epidemics of adenovirus in these military bases affecting thousands of trainees¹²⁰.

In 2011 a live vaccine against adenovirus type 4 and 7 (oral) was licensed in the US and rolled out within the military in that year. The phase 3 trial revealed a vaccine efficacy rate of 99.3% (with only one episode of febrile respiratory illness in the vaccinated group) 73% of vaccine recipients seroconverted to ADV-4 while 63% seroconverted to ADV-7 by Day 28¹²¹. After re-introduction of the vaccine episodes of febrile respiratory illness reduced dramatically¹²².

THE FUTURE OF VIRAL VACCINATION

Vaccination is one of the most successful disease prevention strategies of the last century. However, we sadly lack vaccines against some important respiratory viruses; with the exception of influenza and adenovirus, there are no licensed vaccines against common causes of viral pneumonia. One of the

major issues it to better understand the ways in which viruses evade immune protection, and how this information can be used to induce long lasting, robust and non-pathogenic immunity at the mucosal surface. It is possible that vaccines might be designed that would prevent symptomatic disease, while allowing infection (and possibly onward transmission) to occur. Such a vaccine would have the disadvantage of having a lesser impact on community circulation, thereby protecting the vaccine recipient but not others at risk. It is to be hoped that a new generation of vaccines will be developed that induce a balanced protective cell mediated and antibody response and confer durable cross-protection against novel viral strains. With advances in virology, immunology and vaccinology, it is to be hoped that this new dawn will soon be upon us ([Panel 3](#)).

Panel 3: Current Research Gaps

Questions

How can optimal mucosal immunity be induced?
Does T cell immunity reduce severity or enhance recovery?
How can universal vaccination be best achieved?

Risks

Might novel vaccines enhance disease?
Will viral resistance emerge?
What are the drawbacks of adjuvants?

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