Priorities for developing respiratory syncytial virus (RSV) vaccines in different target populations

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There is now a real prospect of respiratory syncytial virus (RSV) disease control with up to 44 candidate vaccines and monoclonal antibodies in clinical development, for a variety of the target populations including pregnant women, infants, children and older adults.

Abstract

Respiratory syncytial virus (RSV) is a major cause of severe respiratory tract infection worldwide. There is a monoclonal antibody, palivizumab, which can be used for prophylaxis, but no licensed vaccine or clinically effective antiviral therapy. The development of an effective vaccine has been hampered over the last 50 years by significant difficulties in the 1960s in which a formalin-inactivated vaccine led to increased severity of RSV disease following acquisition of the virus in the RSV season after vaccination. However, renewed efforts to develop a vaccine have resulted in up to 44 candidate vaccines and monoclonal antibodies now being in pre-clinical or clinical development (as of April 2019). The target populations for effective vaccination are varied and include neonates, young children, pregnant women and older adults. The reasons for susceptibility to infection in each of these groups may be different and could therefore require different vaccine types for induction of protective responses, adding a further challenge for vaccine development. Here we review the current knowledge of vaccine development for these target populations, and we propose a view of the priorities for RSV vaccines and their rationale.

Introduction: the challenges of developing an effective RSV vaccine

Respiratory syncytial virus (RSV) is a major cause of severe respiratory tract infection worldwide and a major pathogen for which there is no vaccine or clinically effective treatment. RSV infection results in the hospitalisation of vast numbers of children under five years of age: a large systematic review estimated that RSV caused 33·1 million episodes of RSV-acute lower respiratory tract infection (ALRI), 3·2 million hospital admissions, and 59,600 in-hospital deaths worldwide in 2015[1]. 99% of deaths occur in low and middle income countries[1]. RSV infection in infancy is also associated with the subsequent development of chronic respiratory morbidity (e.g. asthma). Epidemiological data on RSV are more sparse in adults but it is estimated to cause up to 5% of community acquired pneumonia, mainly in older adults and those with co-morbidities in whom there is a 9-12% case fatality rate[2]. Recently it has been shown that more general practitioner (GP) episodes, hospitalisations and deaths are attributable to RSV in older adults than to influenza[3]. Due to major advances in novel biological platforms for antigen delivery and advances in structural biology for improved epitope presentation, there is now the real prospect of disease control through vaccination.

There are currently (as of April 2019) 44 vaccine and monoclonal antibody candidates in development[4]. Severe RSV disease occurs very early in life, typically between the second and third months of life[5], providing limited opportunity for programmatic intervention. This means that a single-dose vaccine would have to be given, or several doses given at very short intervals, which provided protection within the first month of life. Antibody responses are typically of lower magnitude in early infancy [6] and the presence of high titres of maternally derived antibodies[7] are likely to blunt the infant response to vaccination, making induction of protective responses more challenging at this age[8]. The risk of severe

disease is also elevated in immunocompromised/immunosuppressed[9] individuals and older adults[10], in whom immunosenescence and underlying comorbidities compromise vaccine responses.

The demographic and immunological risk factors for developing severe RSV disease are different in infants and adults, although any significant cardiac, respiratory or immunological comorbidity increases the risk at any age. It is, therefore, likely that vaccine-induced immune responses required to provide protection against RSV will be different in each population and an RSV vaccine may not result in sterilising immunity but prevention of severe disease. The argument that future RSV-vaccines are unlikely to achieve sterilising immunity is supported by the fact that neither natural[11] nor experimental human infection[12] induce robust immunity against re-infection. In addition, regulators will probably require large safety databases to ensure there is not an increased risk of severe disease or death upon subsequent natural infection as happened with historical vaccines[13]. In this review we explore the past and present RSV vaccine landscape and examine the different vaccines and monoclonal antibodies currently in development.

The history of paediatric RSV vaccines: formalin-inactivated RSV vaccines

Following the successful development of formalin-inactivated vaccines for poliovirus, measles and parainfluenza in the 1950s[14,15] studies of formalin-inactivated RSV (FI-RSV) vaccines were conducted in the United States in the mid to late 1960s, within 10-years of the first description of RSV. A preliminary study of an FI-RSV vaccine showed that children and adults inoculated intramuscularly developed modest serum neutralising antibodies and did not exhibit any severe vaccine-related adverse effects for up to 10 days after vaccination[16]. This vaccine was made from a crude extract from RSV infected Vervet

monkey kidney cells, clarified by centrifugation, formalin-inactivated and alum precipitated, and concentrated 100-fold[17]. A series of large-scale clinical trials of that FI-RSV vaccine were subsequently carried out in infants and young children in the 1960s. In one study, infants and children between four months and ten years old (n=191) were given two intramuscular doses of the FI-RSV vaccine while children in an active control arm (n=194) received a trivalent parainfluenza vaccine[17]. In concordance with previous results, 68% of the FI-RSV vaccinees had a 4-fold or greater rise in RSV antibodies in their post-vaccination sera, compared with only 0.9% controls[17]. However, in the subsequent RSV season, the incidence of severe disease in the FI-RSV vaccine group (7.9%) was almost double that in the control group (4.7%)[17]. 60% of the FI-RSV vaccinees infected with natural RSV were hospitalised compared with 22% of controls[17].

In another study, infants between two and seven months of age were vaccinated with a FI-RSV vaccine and post-vaccination serum RSV neutralising antibody titres were found to be six-fold greater in the FI-RSV vaccine group compared with the parainfluenza control group[13]. However, despite serological evidence of comparable exposure between the two groups in the subsequent RSV season, 80% of FI-RSV vaccinees in this study required hospitalisation following natural infection compared with only 5% of the control group[13]. Tragically, two toddlers who had received the FI-RSV vaccine died upon natural exposure to RSV. Post-mortem examinations found evidence of extensive bronchopneumonia, pneumothorax and eosinophilia[13]. The outcome from these studies was that while the FI-RSV vaccine appeared safe, immunogenic and well tolerated by conventional measures in the post-vaccination period, it had induced an aberrant immune response to natural virus. This resulted in a more severe, potentially life-threatening, pulmonary immunopathology. These disastrous trials mandated extensive investigation,

which persist to this day, into understanding the mechanisms behind the FI-RSV vaccine associated enhanced respiratory disease (FI-RSV ERD).

Interestingly, an entirely different formulation of FI-RSV was tested in children in the mid-1960s. In one trial conducted in Pennsylvania, an alum-adjuvanted, formalin-inactivated RSV formulation was concentrated 22-fold and administered intramuscularly to children between the ages of 3 and 5 years, in parallel with similarly formalin-inactivated parainfluenza and *Mycoplasma pneumoniae* vaccines. A priming dose of each vaccine was given between late October and early November 1965 and booster doses of each formulation administered 3-4 weeks later. About 45% of children who had initially been classified as RSV-seronegative developed a greater than four-fold increase in antibody following the boosting dose while only about 11% of previously seropositive children seroconverted. In the post vaccination surveillance period that ran until May 1966, active clinical assessment visits were undertaken and it was determined that the vaccines were generally safe, with only a few children reporting respiratory symptoms that were classified as severe. Unlike the trials described above, there did not appear to be ERD attributable to vaccination. Despite this, compared with an unvaccinated control group, the vaccinated group was not protected against RSV disease following natural exposure[18]. In a separate trial carried out in the same location between October and December 1966, these vaccines (FI-RSV, FI-Parainfluenza [1, 2 and 3] and FI-*M.pneumoniae*) were combined into a single vaccine formulation and administered to toddlers between the ages of 3 and 5 years. In the five-month post vaccination follow-up period, there appeared to be a protective effect against severe respiratory disease, although this effect was only apparent in the first two months of follow-up[19]. From these two trials, it can be surmised that the protective effect observed in the second trial of the heptavalent vaccine did

not reflect protection against natural RSV infection, but most likely against either *M. pneumoniae* or Parainfluenza.

Further clinical trials of new RSV vaccine candidates, except for live-attenuated vaccines, would need to wait until animal models of ERD were sufficiently developed and capable of reproducing FI-RSV vaccine-like associated immunopathology after experimental challenge. Animals models have been developed including using *Sigmodon hispidus* (cotton rat), mice, African green monkeys, colostrum-deprived calves (challenged with bovine RSV as a translational model for seronegative infants) and lambs[20]. Animal challenge studies and the post-mortem findings from the infant fatalities have been used to extensively investigate the FI-RSV vaccine associated ERD. Early investigations found that children vaccinated with the FI-RSV vaccine failed to develop neutralising antibody titres comparable with those of age-matched individuals who had undergone natural infection. These studies postulated that these non-neutralising antibodies could have potentiated disease either through the formation of immune complexes in the lung or through the stimulation of a suboptimal anti-G response in young infants or that severe disease was the result of poorly neutralising antibodies that delayed the development of effective responses to clear the virus[21]. Subsequent studies found that in addition to the poorly neutralising response, F-specific antibodies to the FI-RSV vaccine were deficient in fusion-inhibiting activity, promoting the spread of the virus in the respiratory tract upon natural infection[22]. Later work suggested that the failure to develop an effective neutralising response following FI-RSV vaccination was not due to formalin disruption of neutralising epitopes but rather due to the development of low avidity anti-RSV antibodies due to the lack of affinity maturation[23]. This view, however, has been disputed[24]. Later studies showed that treatment of RSV antigens with formalin promotes the development of Th2 responses in children [25] and that disease exacerbation was the result of an over exuberant inflammatory response to infection.

More recent analyses have demonstrated that formalin and heat-inactivation of RSV promotes a fast and irreversible transition from the pre- to the post-fusion conformation of the F protein, and in its wake, an almost complete loss of epitopes that are sensitive to antibody neutralisation[26] This history continues to cast a long shadow over further RSV vaccine development.

Development of vaccines for active infant immunisation

Current and future RSV vaccine candidates require careful pre-clinical evaluation in animal challenge models and, provided no FI-RSV vaccine immunopathology is observed, can then progress from phase 1 trials in healthy adults through a series of age de-escalation trials towards seronegative infants. Studies should include the response in infants over the subsequent RSV transmission season and a longer period of safety observation[27]. Although many animal-based studies have been used to postulate the mechanisms by which FI-RSV vaccines potentiated natural infection[21–23,25,28,29], there are uncertainties as to which, if any, of these mechanisms can be feasibly extrapolated to human infants. The FI-RSV vaccine also raised concerns regarding the use of non-replicating vaccines in seronegative infants. To date the only vaccine type that has been safely used in seronegative infants is live-attenuated vaccines (Table 1). These vaccines have a number of features that make them particularly attractive as a platform for delivering virus antigens to the seronegative infant. The intranasal delivery of the vaccines provides an opportunity to directly stimulate mucosal immunity, resulting in the development of functional immunity at the point of contact between the virus and the host[30] and reduces the risk of immune suppression mediated by passively acquired maternal antibodies[8]. In adults, the quantity of RSV-specific nasal IgA has been identified as a significant factor in the risk of RSV infection despite the background of

robust immune responses in blood [31]. Live-attenuated RSV vaccines also have the advantage of a strong safety track record in seronegative infants. A consistent feature of these vaccines has been the lack of ERD upon subsequent infection with wild-type virus. Notwithstanding this safety record, these vaccines have historically struggled to strike the right balance between achieving enough attenuation for safety and sufficient virulence to induce and maintain protective immunity[32]. Despite this, encouraging developments have emerged in this field; by leveraging powerful reverse genetics approaches, recent studies have investigated vaccines containing novel attenuating mutations on the virus backbone that yield high levels of attenuation while retaining immunogenicity in animal models[33]. These developments raise the prospect of licensure of a replicating RSV vaccine for the seronegative paediatric population in the years ahead. However, this must be tempered with potential concerns about reversion to wild-type virus, transmission of vaccine virus between household and other contacts and nasal congestion, which is a significant concern in the youngest infants who are obligate nasal breathers[34].

In addition to live-attenuated vaccines, one platform that is likely to be appropriate for delivering RSV antigens to seronegative infants is genetically modified viral-vectored vaccines. Viral vectors can be genetically engineered to limit or abolish their replication[35], a safety feature that reduces the risk of unchecked viral replication within the host and potential transmission to others. Viral-vectored vaccines have been shown to induce immune responses against diseases such as TB[36], malaria[37], RSV[38] and influenza[39]. They have been tested in different target populations - including 10 week-old infants[40] – where they have been reported to be safe. Coupled with the relative ease with which transgenes can be inserted into the vector backbone, viral vectors appear to be an ideal platform for the delivery of RSV antigens to seronegative infants. The biggest hurdle to overcome with viral vector vaccines is the immune response to the viral vector reducing the immune response to the antigenic target. This can

potentially be surmounted by using higher doses and heterologous prime-boost vaccine regimens [41]. A further potential disadvantage of this vector-specific immunity is the possibility that the build-up of host immunity against the vector might increasingly preclude its sequential use as a delivery platform for alternative vaccine antigens. Two trials of RSV viral-vectored vaccines (ClinicalTrials.gov Identifier: NCT03303625 using an adenovirus serotype 26 RSV pre-fusion conformation stabilized F protein vaccine and ClinicalTrials.gov Identifier: NCT03636906 using a recombinant chimpanzee adenovirus Type 155-vectored RSV vaccine) are ongoing in this population.

Monoclonal antibodies for protecting the neonatal population

Due to the difficulties in developing a vaccine against RSV for neonates, as outlined above, another approach is passive immunisation with a monoclonal antibody. Palivizumab, a humanised mouse monoclonal antibody which is directed against the RSV fusion (F) protein, was developed in the 1990s and has been shown to be up to 80% effective in preventing severe RSV infection in selected groups of neonates[42]. It has a relatively short half-life (approximately 20 days) and thus monthly intramuscular injections are required during the RSV season to provide protection. It is also expensive, thus limiting its use to very high-risk individuals (e.g. those born extremely prematurely with chronic lung disease of infancy or infants with severe combined immunodeficiency) in high income countries[43]. Motavizumab, a similar, but more potent RSV monoclonal antibody, was found to be non-inferior to palivizumab in a large multi-centre trial[44]. However, after the US Food and Drug Administration (FDA) declined a licensing request, partly due to the lack of evidence of superiority to palivizumab, motavizumab's development was discontinued [45]. The Phase 3 NURSERY study recently investigated suptavumab, an RSV monoclonal antibody requiring only one or two doses over the RSV season. Over 1110 healthy preterm infants were

recruited but unfortunately the study failed to meet its primary endpoint of preventing RSV infection requiring a medical attendance and its development has been discontinued [46]. The results of this trial are yet to be formally published.

There are two RSV monoclonal antibody currently undergoing clinical evaluation, MEDI8897[47] and MK-1654[48]. MEDI8897 is being investigated in a Phase 2 clinical trial (ClinicalTrials.gov Identifier NCT02878330). *In vitro* it has been shown that MEDI8897 targets the prefusion conformation of the RSV fusion (F) protein and neutralizes both RSV A and B strains with more than 50-fold greater activity than palivizumab[49]. A phase 1b/2a dose-escalation study including healthy prematurely born infants (gestational age 32-35 weeks) demonstrated five months after a single intramuscular dose of MEDI8897 90% of infants still had a ≥4-fold rise from baseline in serum RSV-neutralizing antibody levels and 87% had serum concentrations above the 90% effective concentration target level [47]. Those data suggest a single dose of MEDI8897 would provide protection throughout a typical RSV season, except perhaps in regions where RSV circulates throughout the year. One potential concern with any immunisation is mutation in the virus leading to viral escape. An *in vitro* study investigating this for MEDI8897 found natural resistance-associated mutations were rare and that escape variants and their parental virus replicated at similar rates, suggesting the resistance-associated substitutions may not develop a replication advantage over naturally circulating strains[50]. A Phase 1 clinical trial investigating MK-1654 (ClinicalTrials.gov Identifier NCT03524118) in preterm and full term infants commenced in September 2018 and is due to complete in August 2020[48]. The development of a cheap, single dose monoclonal antibody to protect infants over a whole RSV season could substantially reduce the burden of disease in this cohort and thus the results of these studies are eagerly awaited.

Vaccination of alternative populations

The unfortunate legacy of the FI-RSV vaccine experience and the narrow epidemiological window available for intervention has caused some reluctance by pharmaceutical companies to develop products for the seronegative infant population. This has raised the question of whether alternative population groups can be vaccinated to provide both direct and indirect protection to the infant. In children, older age, even within the first year of life, is an independent protective factor against the development of severe disease, and therefore even a modest extension to the period of protection afforded by maternal antibody could translate into a significant and disproportionate reduction in the burden of severe disease. We consider the most practical vaccination strategies, the barriers that stand in the way of their successful implementation and assess their potential in alleviating the considerable disease burden caused by RSV.

Maternal Vaccination

In infants, the peak of severe RSV disease risk occurs in the first two months of age[5,51]. Maternal vaccines could protect infants during this window of elevated risk. The last few years have seen an increase in the number of RSV vaccine candidates that are targeted at pregnant women with the aim of boosting RSV-specific antibody that is available for transplacental transfer. Transplacental IgG transfer is an active and efficient physiological process that results in the transport of high titres of protective antibody from maternal to foetal circulation[52]. That passive immunoprophylaxis with palivizumab can reduce hospitalisation in infants with risk factors for severe disease by up to 80% has been a powerful

demonstration that F-specific serum antibodies alone can be protective in infants[42]. Maternal vaccination has the potential to deliver enormous health benefits and substantially reduce infant morbidity and mortality as illustrated by the sharp reduction and near elimination of neonatal tetanus, which is largely attributable to maternal vaccination[53]. In addition, a Phase 2 clinical trial of a maternal RSV vaccine (n=330) showed 11% of vaccinees had serological evidence of new RSV infection compared with 21% of controls[49]. These data suggest that besides the benefit to the infant a maternal RSV vaccine would also give some protection to the mother.

Available data suggest that maternal vaccination is safe and not significantly associated with adverse maternal or neonatal outcomes. Analysis of data from the Vaccine Adverse Events Reporting System (VAERS) in the United States shows there is no excess spontaneous abortion in vaccinated women[56].

The potential global impact of maternal RSV vaccines depends upon access to antenatal care. Recent estimates suggest that approximately 81% of pregnant women across the world attend at least one antenatal care visit although specific estimates vary between countries[57]. Women from low income backgrounds have the poorest coverage with about 72% attending at least one antenatal care visit compared with 99% of women from upper/middle income backgrounds[57]. Overall, about 55% of pregnant women across the globe attend at least four antenatal clinic visits over the course of their pregnancy[57]. Although these relatively high access rates provide some reassurance of the global potential of maternal RSV vaccination programs, the timing of these visits is a critical factor for the success of these programs, as is having trained immunisers in antenatal clinics.

The most advanced maternal vaccine candidate is a nanoparticle vaccine, which is a recombinant near-full length RSV F glycoprotein produced in *Spodoptera frugiperda* (Sf9) insect cells with a recombinant

baculovirus[58]. The vaccine targets the RSV F protein and contains a highly conserved antibody epitope (site II) which is the target of palivizumab. Earlier phase trials have shown that antibodies induced by vaccination appear to provide significant protection to vaccinated women against reinfection[54]. Top line data from the recently completed phase 3 clinical trial (ClinicalTrials.gov Identifier: NCT02624947) showed the vaccine just failed to reach its primary endpoint of prevention of medically significant RSV LRTI. However, the study did show the efficacy of the vaccine was 44% against RSV LRTI hospitalizations and 48% against RSV LRTI with severe hypoxemia[59]. There are now discussions ongoing about possible licensure pathways.

Maternal RSV vaccination faces a number of important hurdles. A major concern for global roll out is that maternal diseases such as placental malaria, HIV and hypergammaglobinaemia can potentially reduce the efficiency of transplacental antibody transfer[60,61] and whose prevalence is geographically variable. It is conceivable that in parts of the world where diseases such as malaria are endemic, the effectiveness of maternal vaccination might be substantially reduced relative to regions with a lower disease burden. Another concern relates to the likelihood of achieving adequate levels of protection for newborn infants. Naturally acquired maternal RSV antibodies confer limited protection to the infant[51], suggesting that vaccine-induced antibodies will need to substantially exceed the protective efficacy of maternally derived antibodies. Also, prematurity is a significant risk factor for RSV infection, because of the reduced opportunity for transplacental antibody transfer, which may be entirely absent among those born extremely prematurely. Thus, any vaccine given late in pregnancy will not impact on this vulnerable population.

As RSV seasonality varies considerably across the globe, in temperate regions an annual pattern is usually limited to 3–5 months during the autumn and winter seasons whereas in tropical climates RSV transmission is sustained all year round, the duration of protection needed to impact on RSV hospitalisations from a maternal vaccine will be different by geographic location[62,63]. National vaccine programmes may also need to vary to be cost-effective with analyses needing to take into account seasonal vaccination in temperate climates versus year-round vaccination in tropical climates[64,65].

The best time to vaccinate during pregnancy is also unclear. Most maternal vaccine trials have vaccinated during the third trimester, however, there is emerging evidence that vaccinating earlier in pregnancy, from 16 weeks of gestation, may result in significantly higher vaccine-induced neonatal antibodies for maternal influenza vaccines[65]. The impact of other maternal vaccines (e.g. influenza, pertussis) on transfer of RSV antibody to infants after maternal RSV vaccination is also currently unknown.

For infants, combining approaches, i.e. maternal vaccination and subsequent infant immunisation may also be possible, although this would need to be cost-effective.

Vaccination of toddlers and older children

Although the highest burden of RSV disease is in infants and older adults, there are still significant healthcare costs associated with RSV infection in older children, particularly in the primary care setting[66]. In addition, reducing the circulation of RSV by vaccinating older children may reduce the impact on infants and older adults indirectly, by reducing shedding, as is the case with influenza vaccination[67]. However, herd immunity can only be demonstrated in phase 4, post-licensure studies. Efforts have,

therefore, been made to develop vaccines for older children (Table 1). Although live-attenuated vaccines may not be suitable for the youngest infants, as explained above, this is a viable option for older children. There are currently ten vaccines undergoing early stage clinical trials[4] including an adenovirus-vectored RSV vaccine (replication deficient) in a Phase 2 clinical trial (Clinicaltrials.gov Identifier: NCT03303625) recruiting adults and RSV-seropositive toddlers 12-24 months old, with results expected at the end of 2019.

Development of RSV vaccines for older adults

Although there are few data on the global burden of RSV disease in older adults, a consistent feature of the available information suggests that the morbidity and mortality burden due to RSV in older adults is similar to that caused by seasonal influenza[66,68–72]. One of the few prospective studies that investigated the relative incidence of RSV and influenza infections over four winter seasons showed a mean incidence of 5.5 RSV infections per 100 individuals per season compared with an estimate of about 2.2 influenza infections per 100 individuals per season[10]. The seasonal infection rates for RSV for older adults appear to be the same as those measured in young healthy adults, but greater rates of progression towards the lower respiratory tract and severe disease are significantly notable with increasing age after 65 years. It should be noted that these studies were done in populations with influenza vaccination available for older adults thus potentially impacting the influenza epidemiology in this group.

The majority of older adults who are hospitalised with RSV infection have co-morbid conditions; 14%-68% of elderly adults hospitalised with severe RSV infection have underlying lung disease while 14%-63%

have underlying heart disease[2,73–75]. Overall, over 70% of hospitalised older adults will have one or both of these conditions.[2]

Development of RSV vaccines targeted at older adults face several hurdles; the lack of sufficiently sensitive clinical endpoints for detecting disease in older adults, the absence of a population-specific immune correlate of protection, the high prevalence of co-morbid conditions, which are likely to confound the assessment of clinical endpoints of vaccine efficacy, and the low and variable attack rates necessitating very large and expensive studies to demonstrate protective efficacy. There remains uncertainty about whether the increased risk of severe disease in this population is associated with age-related changes in cellular or humoral immunity or both[76]. A widely held view is that the goal of older adult vaccination should be the augmentation of T-cell immunity since there is evidence that serum neutralising antibody levels in older adults appear to be no different from those of younger adults[77], while their RSV specific T-cell responses appear to become significantly attenuated with age[78].

Recent years have seen an expansion of vaccine candidates targeted at older adults. The most advanced of these programs to date is the previously highlighted nanoparticle vaccine, whose Phase 3 clinical trial has recently been concluded. Unfortunately, the results of the trial showed no evidence of protection against lower respiratory tract disease[79]. Although the results of this trial are disappointing, the pipeline of promising antigen delivery platforms that could be suitable for this population continues to expand. Prefusion-stabilized F protein (pre-F) subunit vaccines are undergoing clinical trials, including in older adults (Clinicaltrials.gov Identifier NCT03572062) and trials of viral-vectored vaccines expressing viral targets of both T and B cell immunity are being tested in older adults and carry the potential to overcome age-related immunosenescence by augmenting these critical arms of adaptive immunity against RSV. Recent developments in the structural design of non-replicating vaccines have opened up new prospects

for development of effective vaccines for different adult population target groups, including older adults. A recent study has reported the successful development of self-assembling nanoparticle formulations presenting pre-F in a polymeric array on the nanoparticle scaffold. Preclinical analyses have shown that in this configuration, the pre-F nanoparticle induced neutralising antibodies at levels that were > 10-fold higher than previous trimeric formulations of pre-F[80]. These encouraging developments continue to provide reassurance that a vaccine against RSV in older adults may be achievable in the coming years.

The role of animal models in RSV vaccine research

Well-conducted animal studies can provide powerful data to support the advancement of vaccine candidates to the clinical evaluation stage[38]. Although many immunological responses to vaccination in preclinical animal models are reasonably well correlated with human responses[55], the central role of animal models in RSV vaccine research is as predictors of potential vaccine-induced pathology and as *in vivo* models for the assessment of the complex immune and physiological mechanisms that underlie this outcome.

Animal models, such as the mouse and cotton rat, have been used to replicate the complex immunopathological mechanisms of the FI-RSV vaccine[81,82] and whilst clearly invaluable for such mechanistic research, they suffer significant shortcomings that limit their potential extrapolative value in the forecast of infant responses to vaccination[83]. Early murine RSV studies showed that there was up to a 100-fold difference in the infectivity of mice with different genetic backgrounds[84], suggesting that the genetic background of the animal and not the intrinsic pathogenicity of the virus may be the main determinant of disease severity. The effect of animal genetics on pathological outcome can have profound implications on the interpretation of preclinical data; for example, post vaccination lung eosinophilia which was one of the key features of FI-RSV vaccine pathology in children[17] can be induced

in the BALB/c mouse by pre-sensitization with the RSV attachment (G) protein[85] but can be effectively annulled when alternative strains of mice are used[86]. The modification of pathology by a change in the genetic background of the animal adds an enormous amount of complexity to the interpretation of animal-based safety data, with potential implications for interpreting small human studies, and reduces the value of such data as preclinical safety checkpoints.

The predictive utility of the mouse model in studies of vaccine induced immunopathology is further limited by the fact that pathology can be abrogated by the depletion of certain mediators[81] or adjusted by changing experimental parameters such as the route and type of sensitizing antigen[87]. Taken together, these observations suggest that the patterns of pathology induced by vaccinating small rodents are in part subject to the nuances of experimental design and may deviate substantially from human responses to the same antigens. The mouse, in particular, appears to have a tendency to emphasize the immunopathogenic potential of vaccine candidates, which may not be reflected in humans. In assessing potential vaccine safety issues using animal models, indicators such as lung eosinophilic infiltration should not be rigidly applied as preclinical stop signals that preclude products from further development, but rather as a basis for continued investigation in other animal models in order to demonstrate safety prior to advancing to properly controlled phase 1 safety studies in humans. At present there is no consensus on how this is regulated, i.e. which animal models should be used in preclinical studies[27].

Conclusions

RSV disease is a major burden on paediatric and older adult healthcare services around the world, causing significant morbidity and mortality. Multiple vaccines are in development to try to counter this using a variety of traditional and novel technologies, with one potentially being considered for licensure. The approaches used need to be tailored to each population due to differences in risk factors for severe disease and immunological factors which vary between populations. Although the road has been long, we are now entering an era where an RSV vaccine is likely to become available that could revolutionise paediatric and older adult medicine.

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Declaration of interests

AJP chairs the UK Department of Health and Social Care's (DHSC) Joint Committee on Vaccination and Immunisation (JCVI) and the European Medicine's Agency (EMA) Scientific Advisory Group on Vaccines,

and is a member of the World Health Organization Strategic Group of Experts (SAGE); the views expressed in this manuscript do not necessarily reflect the views of JCVI, DHSC, EMA or SAGE. AJP and CSR are Jenner Institute investigators. CSR is a Jenner investigator at the University of Oxford. The other authors have no conflicts of interest to declare.

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Table 1. Published studies of recent RSV vaccine candidates that have been tested in clinical trials of different population groups. A shaded box signifies the study was carried out in that population.

[#]FIV – Formalin inactivated vaccine, [±]VVV – viral vectored vaccine, [*]LAV – Live-attenuated vaccine

Vaccine	*Class*	*Adults*	*Sero-positive children*	*Sero-negative children*	*Pregnant women*	*Older adults*
FI-RSV[13,16]	*FIV[#]*	■	■	■		
F, G, M subunit [88,89]	*Subunit*					■
F-nanoparticle[54,55]	*nanoparticle*	■			■	■
Chimpanzee adenovirus RSV vaccine[38]	*VVV [±]*	■				■
BBG2Na[90,91]	*Subunit*	■				
ΔM2-2[92]	*LAV[*]*	■	■	■		
rA2cp248/404/1030ΔSH[93]	*LAV*	■	■	■		
*cpts*530/1009[94]	*LAV*	■	■	■		
RSV *ts*-2[95]	*LAV*	■	■	■		
MEDI-559[96]	*LAV*			■		

MEDI-534[97–99]	*LAV*					
RSV *ts*-1 A, B C[100,101]	*LAV*					
*cpts*248/955[94]	*LAV*					
cp-52B[102]	*LAV*					
rA2cpΔNS2[103]	*LAV*					
*cp*RSV[104,105]	*LAV*					
RSV *ts*-1[106–108]	*LAV*					
*Cpts*248/404[109,110]	*LAV*					
rA2cp248/404ΔSH[93]	*LAV*					
rA2cp248/404/1030ΔNS2[103]	*LAV*					
rA2cp530/1009ΔNS2[103]	*LAV*					
PFP1[111–116]	*Subunit*					
PFP2[110,117–121]	*Subunit*					
PFP3[122]	*Subunit*					

MEDI7510[123]	*Subunit*					
F-nanoparticle[124,125]	*Nanoparticle*					
Pre-F[126]	*Subunit*					
Soluble post-F[127]	*Subunit*					
Small hydrophobic protein ectodomain[128]	*Subunit*					
RSVcps2[129]	*LAV*					
LIDΔM2-2[130]	*LAV*					
RSV PreF[131]	*Recombinant*					