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

Simon B Drysdale1,2\*, Rachael S Barr4, Christine S Rollier1, Christopher A Green1,, Andrew J Pollard1,3, Charles J Sande1,5

1 Oxford Vaccine Group, Department of Paediatrics and the NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, United Kingdom

2 Institute of Infection and Immunity, St George’s, University of London, London, United Kingdom

3 Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom

4 Taunton and Somerset NHS Foundation Trust, Taunton, United Kingdom

5 KEMRI-Wellcome Trust Research Programme, Kilifi Kenya

\*Corresponding author

Word count: 9102

*One sentence summary*

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 20151. 99% of deaths occur in low and middle income countries1. 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 rate2. Recently it has been shown that more general practitioner (GP) episodes, hospitalisations and deaths are attributable to RSV in older adults than to influenza3. 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 development4 with new vaccine designs continually being developed5. Severe RSV disease occurs very early in life, typically between the second and third months of life6, 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 7 and the presence of high titres of maternally derived antibodies8 are likely to blunt the infant response to vaccination, making induction of protective responses more challenging at this age9. The risk of severe disease is also elevated in immunocompromised/immunosuppressed10 individuals and older adults11, 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 natural12 nor experimental human infection13 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 vaccines14. 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 1950s15,16 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 vaccination17. 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-fold18. 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 vaccine18. 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% controls18. 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%)18. Enhanced respiratory disease (ERD) was, however, only detected in FI-RSV vaccinees younger than 2 years of age and not older children18. 60% of the FI-RSV vaccinees infected with natural RSV were hospitalised compared with 22% of controls18.

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 group14. 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 group14. 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 eosinophilia14. 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 ERD (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 exposure19. 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-up20.

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 lambs21. 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 virus22. 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 infection23. 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 maturation24. This view, however, has been disputed25. Later studies showed that treatment of RSV antigens with formalin promotes the development of Th2 responses in children 26 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 neutralisation27 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 observation28. Although many animal-based studies have been used to postulate the mechanisms by which FI-RSV vaccines potentiated natural infection22–24,26,29,30, 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 host31 and reduces the risk of immune suppression mediated by passively acquired maternal antibodies9. 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 32. 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 immunity33. 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 models34. 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, nasal congestion - which is a significant concern in the youngest infants who are obligate nasal breathers35. Previous trials of live attenuated RSV vaccines have demonstrated a considerable transmission risk, with one study reporting a transmission rate of 20%-25% of the vaccine virus to placebo recipients. The same study also reported a case of post-vaccination wheezing in a child who had received the vaccine36.

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 replication37, 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 TB38, malaria39, RSV40 and influenza41. They have been tested in different target populations - including 10 week-old infants42 – 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 might reduce the immune response to the antigenic target. This can potentially be surmounted by using higher doses and heterologous prime-boost vaccine regimens 43. 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 neonates44. 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 countries45. Motavizumab, a similar, but more potent RSV monoclonal antibody, was found to be non-inferior to palivizumab in a large multi-centre trial46. 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 47. 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 48. The results of this trial are yet to be formally published but were presented at the 11th International RSV symposium (2018) (https://rsvsymposium.com/). It was highlighted that the reason for the failure of the NURSERY study was the development of a dominant mutation of RSV-B isolates at the binding site.

There are two RSV monoclonal antibody currently undergoing clinical evaluation, MEDI889749 and MK-165450. 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 palivizumab51. 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 49. 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 strains52. 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 202050. 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 age6,53. 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 circulation54. 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 infants44. 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 vaccination55. 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 controls49. 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 women58.

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 countries59. 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 backgrounds59. Overall, about 55% of pregnant women across the globe attend at least four antenatal clinic visits over the course of their pregnancy59. 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 baculovirus60. 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 reinfection56. 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 hypoxemia61. 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 transfer62,63 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 infant53, 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 location64,65. 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 climates66,67.

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 vaccines67. 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 setting68. 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 vaccination69. 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 trials4 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 influenza68,70–74. 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 season11. 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 disease2,75–77. 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 both78. 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 adults79, while their RSV specific T-cell responses appear to become significantly attenuated with age80.

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 disease81. 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-F82. 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 stage40. Although many immunological responses to vaccination in preclinical animal models are reasonably well correlated with human responses57, 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 vaccine83,84 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 vaccination85. Early murine RSV studies showed that there was up to a 100-fold difference in the infectivity of mice with different genetic backgrounds86, 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 children18 can be induced in the BALB/c mouse by pre-sensitization with the RSV attachment (G) protein87 but can be effectively annulled when alternative strains of mice are used88. 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 mediators83 or adjusted by changing experimental parameters such as the route and type of sensitizing antigen89. For example, poor quality antibodies passively administered or transferred from rodents vaccinated with a FI-RSV vaccine have not caused immunopathology or ERD in the rodent models28. In addition, there is concern about using the rodent model to screen for ERD when non-viral components of the FI-RSV vaccine have been shown to cause enhance immunopathology consistent with ERD85. The formalin inactivation procedure has been shown to result in an abundance of carbonyl groups that appeared to mediate a Th2-mediated ERD response in mice, an effect that could be almost completely reversed by chemical-reduction of these carbonyl groups90. 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 studies28.

***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.

**Acknowledgements**

All authors conceived the manuscript, SBD and CJS drafted the manuscript and display item. CSR, CAG, RSB and AJP provided additional information, comments, and critical corrections of the manuscript. This study was supported by the NIHR Oxford Biomedical Research Centre. SBD, AJP and CJS are members of the REspiratory Syncytial virus Consortium in Europe (RESCEU). RESCEU has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement Nº 116019. This Joint Undertaking receives support from the European Union’s Horizon 2020 research and innovation programme and EFPIA. No funding was required for the writing of this review. CJS, AJP and CSR conceived the idea for the article. CJS and SBD wrote the initial draft of the article. All authors reviewed, revised and approved the final manuscript.

**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. SBD has been an investigator on studies funded by Janssen and MedImmune. All funds have been paid to his institution and he has received no personal payments. The other authors have no conflicts of interest to declare.

**References**

1 Shi T, McAllister DA, O’Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet 2017; 390: 946–58.

2 Lee N, Lui GCY, Wong KT, et al. High Morbidity and Mortality in Adults Hospitalized for Respiratory Syncytial Virus Infections. Clin Infect Dis 2013; 57: 1069–77.

3 Ackerson B, Tseng HF, Sy LS, et al. Severe morbidity and mortality associated with Respiratory Syncytial Virus versus Influenza infection in hospitalized older adults. DOI:10.1093/cid/ciy991/5193205.

4 PATH RSV vaccine snapshot. April 2019. Found at: <https://vaccineresources.org/files/RSV-snapshot-2019_04_05_April_High%20Resolution.pdf> (accessed 15th May 2019).

5 Crank MC, Ruckwardt TJ, Chen M, et al. A proof of concept for structure-based vaccine design targeting RSV in humans. Science (80- ) 2019; 365: 505–9.

6 Murray J, Bottle A, Sharland M, et al. Risk factors for hospital admission with RSV bronchiolitis in England: a population-based birth cohort study. PLoS One 2014; 9: e89186.

7 Thornton CA, Upham JW, Wikström ME, et al. Functional maturation of CD4+CD25+CTLA4+CD45RA+ T regulatory cells in human neonatal T cell responses to environmental antigens/allergens. J Immunol 2004; 173: 3084–92.

8 Ochola R, Sande C, Fegan G, et al. The level and duration of RSV-specific maternal IgG in infants in Kilifi Kenya. PLoS One 2009; 4: e8088.

9 Crowe Jr. JE, Modlin JF, Crowe JE. Influence of maternal antibodies on neonatal immunization against respiratory viruses. Clin Infect Dis 2001; 33: 1720–7.

10 Moyes J, Cohen C, Pretorius M, et al. Epidemiology of Respiratory Syncytial Virus-associated acute lower respiratory tract infection hospitalizations among HIV-infected and HIV-uninfected South African children, 2010-2011. J Infect Dis 2013; 208: S217–26.

11 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: 1749–59.

12 Henderson FW, Collier AM, Clyde WA, Denny FW. Respiratory-Syncytial-Virus infections, reinfections and immunity. N Engl J Med 1979; 300: 530–4.

13 Hall CB, Walsh EE, Long CE, Schnabel KC. Immunity to and frequency of reinfection with Respiratory Syncytial Virus. J Infect Dis 1991; 163: 693–8.

14 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.

15 Salk JE. Studies in human subjects on active immunization against poliomyelitis. I. A preliminary report of experiments in progress. J Am Med Assoc 1953; 151: 1081–98.

16 Strebel PM, Sutter RW, Cochi SL, et al. Epidemiology of poliomyelitis in the United States one decade after the last reported case of indigenous wild virus-associated disease. Clin Infect Dis 1992; 14: 568–79.

17 L Potash, AA Tytell, BH Sweet, et al. Respiratory Syncytial and Parainfluenza virus vaccines. Am Rev Respir Dis 1966; 93(4):536-48.

18 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.

19 Weibel RE, Stokes Jr J, Mascoli CC, et al. Respiratory virus vaccines. VII. Field evaluation of Respiratory Syncytial, Parainfluenza 1, 2, 3, and Mycoplasma Pneumoniae vaccines, 1965 to 1966. Am Rev Respir Dis 1967; 96(4):724-39.

20 Weibel RE, Stokes Jr J, Leagus MB, et al. Respiratory virus vaccines. V. Field evaluation for efficacy of heptavalent vaccine. Am Rev Respir Dis 1966; 94(3):362-79.

21 Taylor G. Animal models of respiratory syncytial virus infection. Vaccine 2017; 35: 469–80.

22 Murphy BR, Prince GA, Walsh EE, et al. Dissociation between serum neutralizing and glycoprotein antibody responses of infants and children who received inactivated respiratory syncytial virus vaccine. J Clin Microbiol 1986; 24: 197–202.

23 Murphy BR, Walsh EE. Formalin-inactivated respiratory syncytial virus vaccine induces antibodies to the fusion glycoprotein that are deficient in fusion-inhibiting activity. J Clin Microbiol 1988; 26: 1595–7.

24 Delgado MF, Coviello S, Monsalvo AC, et al. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. Nat Med 2009; 15: 34–41.

25 Shaw CA, Otten G, Wack A, et al. Antibody affinity maturation and respiratory syncytial virus disease. Nat Med 2009; 15: 725–725.

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

27 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.

28 European Medicines Agency. Guideline on the clinical evaluation of medicinal products indicated for the prophylaxis or treatment of respiratory syncytial virus (RSV) disease. Available at: <https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-clinical-evaluation-medicinal-products-indicated-prophylaxis-treatment-respiratory_en.pdf> (Accessed 15 May 2019).

29 Connors M, Collins PL, Firestone C-Y, et al. Cotton rats previously immunized with a chimeric RSV FG glycoprotein develop enhanced pulmonary pathology when infected with RSV, a phenomenon not encountered following immunization with vaccinia—RSV recombinants or RSV. Vaccine 1992; 10: 475–84.

30 Waris ME, Tsou C, Erdman DD, et al. Respiratory syncytial virus infection in BALB/c mice previously immunized with formalin-inactivated virus induces enhanced pulmonary inflammatory response with a predominant Th2-like cytokine pattern. J Virol 1996; 70: 2852–60.

31 Ambrose CS, Wu X, Belshe RB. The efficacy of live attenuated and inactivated influenza vaccines in children as a function of time post-vaccination. Pediatr Infect Dis J 2010; 29: 806–11.

32 Walsh EE, Falsey AR. Humoral and mucosal immunity in protection from natural Respiratory Syncytial Virus infection in adults. J Infect Dis 2004; 190: 373–8.

33 Karron RA, Buchholz UJ, Collins PL. Live-attenuated Respiratory Syncytial Virus vaccines. Curr Top Microbiol Immunol. 2013;372:259-84.

34 Rostad CA, Stobart CC, Gilbert BE, et al. A recombinant Respiratory Syncytial Virus vaccine candidate attenuated by a low-fusion F protein Is immunogenic and protective against challenge in cotton rats. J Virol 2016; 90: 7508–18.

35 Karron RA, Buchholz UJ, Collins PL. Live-attenuated respiratory syncytial virus vaccines. Curr Top Microbiol Immunol 2013; 372: 259–84.

36 Karron RA, Wright PF, Crowe, Jr. JE, et al. Evaluation of Two Live, Cold‐Passaged, Temperature‐Sensitive Respiratory Syncytial Virus Vaccines in Chimpanzees and in Human Adults, Infants, and Children. J Infect Dis 1997; 176: 1428–36.

37 Dudek T, Knipe DM. Replication-defective viruses as vaccines and vaccine vectors. Virology 2006; 344: 230–9.

38 Xing Z, Lichty BD. Use of recombinant virus-vectored tuberculosis vaccines for respiratory mucosal immunization. Tuberculosis 2006; 86: 211–7.

39 Ewer KJ, Sierra-Davidson K, Salman AM, et al. Progress with viral vectored malaria vaccines: A multi-stage approach involving “unnatural immunity”. Vaccine 2015; 33: 7444–51.

40 Green CA, Scarselli E, Sande CJ, et al. Chimpanzee adenovirus- and MVA-vectored respiratory syncytial virus vaccine is safe and immunogenic in adults. Sci Transl Med 2015; 7: 300ra126.

41 Tripp R, Tompkins S, Tripp RA, Tompkins SM. Virus-vectored Influenza virus vaccines. Viruses 2014; 6: 3055–79.

42 Afolabi MO, Tiono AB, Adetifa UJ, et al. Safety and immunogenicity of ChAd63 and MVA ME-TRAP in West African children and infants. Mol Ther 2016; 24: 1470–7.

43 Ewer KJ, Lambe T, Rollier CS, et al. Viral vectors as vaccine platforms: from immunogenicity to impact. Curr Opin Immunol 2016; 41: 47–54.

44 The IMpact-RSV Study Group. Palivizumab, a humanized Respiratory Syncytial Virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. Pediatrics 1998; 102(3):531-7.

45 Public Health England. Immunisation against infectious disease (2015): Green Book. Chapter 27a Respiratory syncytial virus. Available at:

<https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/458469/Green_Book_Chapter_27a_v2_0W.PDF> (Accessed 15 May 2019).

46 Carbonell-Estrany X, Simões EAF, Dagan R, et al. Motavizumab for prophylaxis of Respiratory Syncytial Virus in high-risk children: a noninferiority trial. Pediatrics 2010; 125: 35-51.

47 AstraZeneca withdraws BLA for motavizumab for serious respiratory syncytial virus (RSV) - MPR. https://www.empr.com/home/news/drugs-in-the-pipeline/astrazeneca-withdraws-bla-for-motavizumab-for-serious-respiratory-syncytial-virus-rsv/ (accessed March 5, 2019).

48 Regeneron Pharmaceuticals I. Regeneron to discontinue development of Suptavumab for Respiratory Syncytial Virus- Regeneron Pharmaceuticals Inc. 2017.

https://investor.regeneron.com/news-releases/news-release-details/regeneron-discontinue-development-suptavumab-respiratory?releaseid=1037184 (accessed Jan 4, 2019).

49 Domachowske JB, Khan AA, Esser MT, et al. Safety, tolerability and pharmacokinetics of MEDI8897, an extended half-life single-dose respiratory syncytial virus prefusion F-targeting monoclonal antibody administered as a single dose to healthy preterm infants. Pediatr Infect Dis J 2018; 37: 886–92.

50 ClinicalTrials.gov. Safety, tolerability, and pharmacokinetics of MK-1654 in infants (MK-1654-002). https://clinicaltrials.gov/ct2/show/NCT03524118?term=merck&cond=RSV+Infection&rank=1 (accessed March 5, 2019).

51 Zhu Q, McLellan JS, Kallewaard NL, et al. A highly potent extended half-life antibody as a potential RSV vaccine surrogate for all infants. Sci Transl Med 2017; 9(388):eaaj1928.

52 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: 572–80.

53 Sande CJ, Cane PA, Nokes DJ. The association between age and the development of respiratory syncytial virus neutralising antibody responses following natural infection in infants. Vaccine 2014; 32: 4726–9.

54 Hashira S, Okitsu-Negishi S, Yoshino K. Placental transfer of IgG subclasses in a Japanese population. Pediatr Int 2000; 42: 337–42.

55 Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet 2015; 385: 430–40.

56 Glenn GM, Fries LF, Thomas DN, et al. A randomized, blinded, controlled, dose-ranging study of a respiratory syncytial virus recombinant fusion (F) nanoparticle vaccine in healthy women of childbearing age. J Infect Dis 2016; 213: 411–22.

57 Glenn GM, Smith G, Fries L, et al. Safety and immunogenicity of a Sf9 insect cell-derived respiratory syncytial virus fusion protein nanoparticle vaccine. Vaccine 2013; 31: 524–32.

58 MacDorman MF, Gregory ECW. Fetal and Perinatal Mortality: United States, 2013. Natl Vital Stat Rep 2015; 64: 1–24.

59 World Health Organization. World Health Statistics: 2013. Available at: <https://www.who.int/gho/publications/world_health_statistics/EN_WHS2013_Full.pdf>

60 Raghunandan R, Lu H, Zhou B, et al. An insect cell derived respiratory syncytial virus (RSV) F nanoparticle vaccine induces antigenic site II antibodies and protects against RSV challenge in cotton rats by active and passive immunization. Vaccine 2014; 32: 6485–92.

61 Novavax announces topline results from Phase 3 PrepareTM trial of ResVaxTM for prevention of RSV disease in infants via maternal immunization- Novavax Inc. - IR Site. http://ir.novavax.com/news-releases/news-release-details/novavax-announces-topline-results-phase-3-preparetm-trial (accessed March 5, 2019).

62 Palmeira P, Quinello C, Silveira-Lessa AL, et al. IgG placental transfer in healthy and pathological pregnancies. Clin Dev Immunol 2012; 2012;2012:985646.

63 Atwell JE, Thumar B, Robinson LJ, et al. Impact of placental malaria and hypergammaglobulinemia on transplacental transfer of respiratory syncytial virus antibody in Papua New Guinea. J Infect Dis 2016; 213: 423–31.

64 Hogan AB, Campbell PT, Blyth CC, et al. Potential impact of a maternal vaccine for RSV: A mathematical modelling study. Vaccine 2017; 35: 6172–9.

65 Nyiro JU, Kombe IK, Sande CJ, et al. Defining the vaccination window for respiratory syncytial virus (RSV) using age-seroprevalence data for children in Kilifi, Kenya. PLoS One 2017; 12: e0177803.

66 Cromer D, van Hoek AJ, Newall AT, et al. Burden of paediatric respiratory syncytial virus disease and potential effect of different immunisation strategies: a modelling and cost-effectiveness analysis for England. Lancet Public Heal 2017; 2: e367–74.

67 Janet S, Broad J, Snape MD. Respiratory syncytial virus seasonality and its implications on prevention strategies. Hum Vaccin Immunother 2018; 14: 234–44.

68 Taylor S, Taylor RJ, Lustig RL, et al. Modelling estimates of the burden of respiratory syncytial virus infection in children in the UK. BMJ Open 2016; 6: e009337.

69 Jefferson T, Rivetti A, Di Pietrantonj C, Demicheli V. Vaccines for preventing influenza in healthy children. Cochrane Database Syst Rev 2018; published online Feb 1. DOI:10.1002/14651858.CD004879.pub5.

70 Mullooly JP, Bridges CB, Thompson WW, et al. Influenza- and RSV-associated hospitalizations among adults. Vaccine 2007; 25: 846–55.

71 Jansen AGSC, Sanders EAM, Hoes AW, van Loon AM, Hak E. Influenza- and respiratory syncytial virus-associated mortality and hospitalisations. Eur Respir J 2007; 30: 1158–66.

72 Zhou H, Thompson WW, Viboud CG, et al. Hospitalizations Associated With Influenza and Respiratory Syncytial Virus in the United States, 1993–2008. Clin Infect Dis 2012; 54: 1427–36.

73 van Asten L, van den Wijngaard C, van Pelt W, et al. Mortality attributable to 9 common infections: significant effect of influenza A, respiratory syncytial virus, influenza B, norovirus, and parainfluenza in elderly persons. J Infect Dis 2012; 206: 628–39.

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

75 Volling C, Hassan K, Mazzulli T, et al. Respiratory syncytial virus infection-associated hospitalization in adults: a retrospective cohort study. BMC Infect Dis 2014; 14: 665.

76 Widmer K, Zhu Y, Williams JV, et al. Rates of hospitalizations for respiratory syncytial virus, human metapneumovirus, and influenza virus in older adults. J Infect Dis 2012; 206: 56–62.

77 Dowell SF, Anderson LJ, Gary HE, et al. Respiratory syncytial virus is an important cause of community-acquired lower respiratory infection among hospitalized adults. J Infect Dis 1996; 174: 456–62.

78 Openshaw PJM, Chiu C, Culley FJ, Johansson C. Protective and harmful immunity to RSV infection. Annu Rev Immunol 2017; 35: 501–32.

79 Walsh EE, Falsey AR. Age related differences in humoral immune response to respiratory syncytial virus infection in adults. J Med Virol 2004; 73: 295–9.

80 Cherukuri A, Patton K, Gasser RA, et al. Adults 65 years old and older have reduced numbers of functional memory T cells to respiratory syncytial virus fusion protein. Clin Vaccine Immunol 2013; 20: 239–47.

81 Novavax. Novavax announces topline RSV F vaccine data from two clinical trials in older adults- Novavax Inc. - IR Site. 2016. http://ir.novavax.com/news-releases/news-release-details/novavax-announces-topline-rsv-f-vaccine-data-two-clinical-trials (accessed Jan 5, 2019).

82 Marcandalli J, Fiala B, Ols S, et al. Induction of potent neutralizing antibody responses by a designed protein nanoparticle vaccine for respiratory syncytial virus. Cell 2019; 176: 1420-1431.e17.

83 Connors M, Giese NA, Kulkarni AB, et al. Enhanced pulmonary histopathology induced by respiratory syncytial virus (RSV) challenge of formalin-inactivated RSV-immunized BALB/c mice is abrogated by depletion of interleukin-4 (IL-4) and IL-10. J Virol 1994; 68: 5321–5.

84 Murphy BR, Sotnikov A V., Lawrence LA, et al. Enhanced pulmonary histopathology is observed in cotton rats immunized with formalin-inactivated respiratory syncytial virus (RSV) or purified F glycoprotein and challenged with RSV 3–6 months after immunization. Vaccine 1990; 8: 497–502.

85 Bem RA, Domachowske JB, Rosenberg HF. Animal models of human respiratory syncytial virus disease. Am J Physiol Cell Mol Physiol 2011; 301: L148–56.

86 Prince GA, Horswood RL, Berndt J, et al. Respiratory syncytial virus infection in inbred mice. Infect Immun 1979; 26: 764–6.

87 Alwan WH, Kozlowska WJ, Openshaw PJ. Distinct types of lung disease caused by functional subsets of antiviral T cells. J Exp Med 1994; 179: 81–9.

88 Hussell T, Georgiou A, Sparer T, et al. Host genetic determinants of vaccine-induced eosinophilia during respiratory syncytial virus infection. J Immunol 1998; 161: 6215–22.

89 Johnson TR, Teng MN, Collins PL, Graham BS. Respiratory syncytial virus (RSV) G glycoprotein is not necessary for vaccine-enhanced disease induced by immunization with formalin-inactivated RSV. J Virol 2004; 78: 6024–32.

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

91 Falsey AR, Walsh EE, Capellan J, et al. Comparison of the Safety and Immunogenicity of 2 Respiratory Syncytial Virus (RSV) Vaccines— Nonadjuvanted Vaccine or Vaccine Adjuvanted with Alum—Given Concomitantly with Influenza Vaccine to High‐Risk Elderly Individuals. J Infect Dis 2008; 198: 1317–26.

92 Langley JM, Sales V, McGeer A, et al. A dose-ranging study of a subunit Respiratory Syncytial Virus subtype A vaccine with and without aluminum phosphate adjuvantation in adults ≥65 years of age. Vaccine 2009; 27: 5913–9.

93 Power UF, Nguyen TN, Rietveld E, et al. Safety and immunogenicity of a novel recombinant subunit Respiratory Syncytial Virus vaccine (BBG2Na) in healthy young adults. J Infect Dis 2001; 184: 1456–60.

94 Murata Y. Respiratory syncytial virus vaccine development. Clin Lab Med 2009; 29: 725–39.

95 Karron RA, Luongo C, Thumar B, et al. A gene deletion that up-regulates viral gene expression yields an attenuated RSV vaccine with improved antibody responses in children. Sci Transl Med 2015; 7: 312ra175-312ra175.

96 Karron RA, Wright PF, Belshe RB, et al. Identification of a recombinant live attenuated Respiratory Syncytial Virus vaccine candidate that is highly attenuated in infants. J Infect Dis 2005; 191: 1093–104.

97 Karron RA, Wright PF, Crowe, Jr. JE, et al. Evaluation of two live, cold-passaged, temperature-sensitive Respiratory Syncytial Virus vaccines in chimpanzees and in human adults, infants, and children. J Infect Dis 1997; 176: 1428–36.

98 Wright PF, Belshe RB, Kim HW, et al. Administration of a highly attenuated, live respiratory syncytial virus vaccine to adults and children. Infect Immun 1982; 37: 397–400.

99 Malkin E, Yogev R, Abughali N, et al. Safety and immunogenicity of a live attenuated RSV vaccine in healthy RSV-seronegative children 5 to 24 months of age. PLoS One 2013; 8: e77104.

100 Bernstein DI, Malkin E, Abughali N, et al. Phase 1 study of the safety and immunogenicity of a live, attenuated respiratory syncytial virus and parainfluenza virus type 3 vaccine in seronegative children. Pediatr Infect Dis J 2012; 31: 109–14.

101 Gomez M, Mufson MA, Dubovsky F, et al. Phase-I study medi-534, of a live, attenuated intranasal vaccine against respiratory syncytial virus and parainfluenza-3 virus in seropositive children. Pediatr Infect Dis J 2009; 28: 655–8.

102 Tang RS, Spaete RR, Thompson MW, et al. Development of a PIV-vectored RSV vaccine: Preclinical evaluation of safety, toxicity, and enhanced disease and initial clinical testing in healthy adults. Vaccine 2008; 26: 6373–82.

103 Watt PJ, Robinson BS, Pringle CR, Tyrrel DAJ. Determinants of susceptibility to challenge and the antibody response of adult volunteers given experimental respiratory syncytial virus vaccines. Vaccine 1990; 8: 231–6.

104 Pringle CR, Filipiuk AH, Robinson BS, et al. Immunogenicity and pathogenicity of a triple temperature-sensitive modified respiratory syncytial virus in adult volunteers. Vaccine 1993; 11: 473–8.

105 Karron RA, Buonagurio DA, Georgiu AF, et al. Respiratory syncytial virus (RSV) SH and G proteins are not essential for viral replication in vitro: clinical evaluation and molecular characterization of a cold-passaged, attenuated RSV subgroup B mutant. Proc Natl Acad Sci U S A 1997; 94: 13961–6.

106 Wright PF, Karron RA, Madhi SA, et al. The interferon antagonist NS2 protein of Respiratory Syncytial Virus is an important virulence determinant for humans. J Infect Dis 2006; 193: 573–81.

107 Friedewald WT, Forsyth BR, Smith CB, et al. Low-temperature-grown RS virus in adult volunteers. JAMA 1968; 204: 690.

108 Kim HW, Arrobio JO, Pyles G, et al. Clinical and immunological response of infants and children to administration of low-temperature adapted RSV. Pediatrics 1971; 48(5); 745-55.

109 Wright PF, Mills V J, Chanock RM. Evaluation of a Temperature-Sensitive Mutant of Respiratory Syncytial Virus in Adults. J Infect Dis 1971; 124: 505–11.

110 Kim HW, Arrobio JO, Brandt CD, et al. Safety and antigenicity of temperature sensitive (TS) mutant RSV in infants and children. Pediatrics 1973; 52;56-63.

111 Wright PF, Shinozaki T, Fleet W, et al. Evaluation of a live, attenuated respiratory syncytial virus vaccine in infants. J Pediatr 1976; 88: 931–6.

112 Wright PF, Karron RA, Belshe RB, et al. Evaluation of a Live, Cold‐Passaged, Temperature‐Sensitive, Respiratory Syncytial Virus Vaccine Candidate in Infancy. J Infect Dis 2000; 182: 1331–42.

113 Gonzalez IM, Karron RA, Eichelberger M, et al. Evaluation of the live attenuated cpts 248/404 RSV vaccine in combination with a subunit RSV vaccine (PFP-2) in healthy young and older adults. Vaccine 2000; 18: 1763–72.

114 Belshe RB, Anderson EL, Walsh EE. Immunogenicity of purified F glycoprotein of respiratory syncytial virus: clinical and immune responses to subsequent natural infection in children. J Infect Dis 1993; 168: 1024–9.

115 Paradiso PR, Hildreth SW, Hogerman DA, et al. Safety and immunogenicity of a subunit respiratory syncytial virus vaccine in children 24 to 48 months old. Pediatr Infect Dis J 1994; 13: 792–8.

116 Piedra PA, Glezen WP, Kasel JA, et al. Safety and immunogenicity of the PFP vaccine against respiratory syncytial virus (RSV): the Western blot assay aids in distinguishing immune responses of the PFP vaccine from RSV infection. Vaccine 1995; 13: 1095–101.

117 Tristram DA, Welliver RC, Hogerman DA, et al. Second-year surveillance of recipients of a respiratory syncytial virus (RSV) F protein subunit vaccine, PFP-1: Evaluation of antibody persistence and possible disease enhancement. Vaccine 1994; 12: 551–6.

118 Tristram DA, Welliver RC, Mohar CK, et al. Immunogenicity and safety of Respiratory Syncytial Virus subunit vaccine in seropositive children 18-36 months old. J Infect Dis 1993; 167: 191–5.

119 Walsh EE, Hall CB, Schlesinger JJ, et al. Comparison of antigenic sites of subtype-specific Respiratory Syncytial Virus attachment proteins. J Gen Virol 1989; 70: 2953–61.

120 Munoz FM, Piedra PA, Glezen WP. Safety and immunogenicity of respiratory syncytial virus purified fusion protein-2 vaccine in pregnant women. Vaccine 2003; 21: 3465–7.

121 Falsey AR, Walsh EE. Safety and immunogenicity of a respiratory syncytial virus subunit vaccine (PFP-2) in ambulatory adults over age 60. Vaccine 1996; 14: 1214–8.

122 Falsey AR, Walsh EE. Safety and immunogenicity of a respiratory syncytial virus subunit vaccine (PFP-2) in the institutionalized elderly. Vaccine 1997; 15: 1130–2.

123 Groothuis JR, King SJ, Hogerman DA, et al. Safety and immunogenicity of a purified F protein Respiratory Syncytial Virus (PFP-2) vaccine in seropositive children with bronchopulmonary dysplasia. J Infect Dis 1998; 177: 467–9.

124 Welliver RC, Tristram DA, Batt K, et al. Respiratory syncytial virus-specific cell-mediated immune responses after vaccination with a purified fusion protein subunit vaccine. J Infect Dis 1994; 170: 425–8.

125 Piedra PA, Cron SG, Jewell A, et al. Immunogenicity of a new purified fusion protein vaccine to respiratory syncytial virus: a multi-center trial in children with cystic fibrosis. Vaccine 2003; 21: 2448–60.

126 Falloon J, Yu J, Esser MT, et al. An adjuvanted, postfusion F protein-based vaccine did not prevent Respiratory Syncytial Virus illness in older adults. J Infect Dis 2017; 216: 1362–70.

127 August A, Glenn GM, Kpamegan E, et al. A Phase 2 randomized, observer-blind, placebo-controlled, dose-ranging trial of aluminum-adjuvanted respiratory syncytial virus F particle vaccine formulations in healthy women of childbearing age. Vaccine 2017; 35: 3749–59.

128 Fries L, Shinde V, Stoddard JJ, et al. Immunogenicity and safety of a respiratory syncytial virus fusion protein (RSV F) nanoparticle vaccine in older adults. Immun Ageing 2017; 14: 8.

129 Langley JM, Aggarwal N, Toma A, et al. A randomized, controlled, observer-blinded phase 1 study of the safety and immunogenicity of a Respiratory Syncytial Virus vaccine with or without alum adjuvant. J Infect Dis 2017; 215: 24–33.

130 Falloon J, Ji F, Curtis C, et al. A phase 1a, first-in-human, randomized study of a respiratory syncytial virus F protein vaccine with and without a toll-like receptor-4 agonist and stable emulsion adjuvant. Vaccine 2016; 34: 2847–54.

131 Langley JM, MacDonald LD, Weir GM, et al. A Respiratory Syncytial Virus vaccine based on the small hydrophobic protein ectodomain presented with a novel lipid-based formulation is highly immunogenic and safe in adults: a first-in-humans study. J Infect Dis 2018; 218(3): 378-387.

132 Buchholz UJ, Cunningham CK, Muresan P, et al. Live Respiratory Syncytial Virus (RSV) vaccine candidate containing stabilized temperature-sensitivity mutations is highly attenuated in RSV-seronegative infants and children. J Infect Dis 2018; 217: 1338–46.

133 McFarland EJ, Karron RA, Muresan P, et al. Live-attenuated Respiratory Syncytial Virus vaccine candidate with deletion of RNA synthesis regulatory protein M2-2 is highly immunogenic in children. J Infect Dis 2018; 217: 1347–55.

134 Beran J, Lickliter JD, Schwarz TF, et al. Safety and immunogenicity of 3 formulations of an investigational Respiratory Syncytial Virus vaccine in nonpregnant women: results from 2 phase 2 trials. J Infect Dis 2018; 217: 1616–25.

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-RSV14,17  | *FIV#* |  |  |  |  |  |
| F, G, M subunit 91,92 | *Subunit* |  |  |  |  |  |
| F-nanoparticle56,57 | *nanoparticle* |  |  |  |  |  |
| Chimpanzee adenovirus RSV vaccine40 | *VVV ±* |  |  |  |  |  |
| BBG2Na93,94 | *Subunit* |  |  |  |  |  |
| ΔM2-295 | *LAV\** |  |  |  |  |  |
| rA2cp248/404/1030ΔSH96 | *LAV* |  |  |  |  |  |
| *cpts*530/100997 | *LAV* |  |  |  |  |  |
| RSV *ts*-298 | *LAV* |  |  |  |  |  |
| MEDI-55999 | *LAV* |  |  |  |  |  |
| MEDI-534100–102 | *LAV* |  |  |  |  |  |
| RSV *ts*-1 A, B C103,104 | *LAV* |  |  |  |  |  |
| *cpts*248/95597 | *LAV* |  |  |  |  |  |
| *cp*-52B105 | *LAV* |  |  |  |  |  |
| rA2cpΔNS2106 | *LAV* |  |  |  |  |  |
| *cp*RSV107,108 | *LAV* |  |  |  |  |  |
| RSV *ts*-1109–111 | *LAV* |  |  |  |  |  |
| *Cpts*248/404112,113 | *LAV* |  |  |  |  |  |
| rA2cp248/404ΔSH96 | *LAV* |  |  |  |  |  |
| rA2cp248/404/1030ΔNS2106 | *LAV* |  |  |  |  |  |
| rA2cp530/1009ΔNS2106 | *LAV* |  |  |  |  |  |
| PFP1114–119 | *Subunit* |  |  |  |  |  |
| PFP2113,120–124 | *Subunit* |  |  |  |  |  |
| PFP3125 | *Subunit* |  |  |  |  |  |
| MEDI7510126 | *Subunit* |  |  |  |  |  |
| F-nanoparticle127,128 | *Nanoparticle* |  |  |  |  |  |
| Pre-F129 | *Subunit* |  |  |  |  |  |
| Soluble post-F130 | *Subunit* |  |  |  |  |  |
| Small hydrophobic protein ectodomain131 | *Subunit* |  |  |  |  |  |
| RSVcps2132 | *LAV* |  |  |  |  |  |
| LIDΔM2-2133 | *LAV* |  |  |  |  |  |
| RSV PreF134 | *Recombinant* |  |  |  |  |  |