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The Respiratory Syncytial Virus Vaccine Landscape

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older adults. Advances in knowledge of the structural biology of the RSV
surface fusion (F) glycoprotein have revolutionized RSV vaccine
development by providing a new target for preventive interventions. The
RSV vaccine landscape has rapidly expanded to include 19 vaccine
candidates and monoclonal antibodies (mAbs) in clinical trials,
reflecting the urgency of reducing this global health problem and hence
the prioritization of RSV vaccine development. The candidates include
mAbs and vaccines using four approaches: (1) particle-based, (2) live-
attenuated/chimeric, (3) subunit, (4) vector-based. Late phase RSV
vaccine trial failures highlight gaps in knowledge regarding immunologic
protection and provide lessons for future development. In this review we
highlight promising new approaches to RSV vaccine design and provide a
comprehensive overview of RSV vaccine candidates and mAbs currently in
clinical development to prevent one of the most common and severe
infectious diseases in young children and older adults worldwide.

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The global burden of disease caused by respiratory syncytial virus (RSV) is increasingly recognized, not only in infants, but also in older adults. Advances in knowledge of the structural biology of the RSV surface fusion (F) glycoprotein have revolutionized RSV vaccine development by providing a new target for preventive interventions. The RSV vaccine landscape has rapidly expanded to include 19 vaccine candidates and monoclonal antibodies (mAbs) in clinical trials, reflecting the urgency of reducing this global health problem and hence the prioritization of RSV vaccine development. The candidates include mAbs and vaccines using four approaches: (1) particle-based, (2) live-attenuated/chimeric, (3) subunit, (4) vector-based. Late phase RSV vaccine trial failures highlight gaps in knowledge regarding immunologic protection and provide lessons for future development. In this review we highlight promising new approaches to RSV vaccine design and provide a comprehensive overview of RSV vaccine candidates and mAbs currently in clinical development to prevent one of the most common and severe infectious diseases in young children and older adults worldwide.

Search strategy and selection criteria
References for this review were identified through a search of PubMed for clinical trials with "syncytial" in the title published after January 1, 2013 with no language restrictions, through April 3, 2018. We did not intend to do a systematic review of the literature. No inclusion or exclusion criteria were used. Instead, we selected articles that were most relevant to the subheadings used in this review. The PATH RSV vaccine and mAb Snapshot was used as a reference to identify all vaccine and mAb candidates in clinical trials. ClinicalTrials.gov as well as the WHO vaccine pipeline tracker for RSV were used to identify all relevant trials for these vaccine candidates and mAbs. Additional data was collected during the RSV Vaccines for the World Conference on November 29- December 1, 2017 and through pharmaceutical websites for the respective vaccine and mAb candidates.

13 Introduction

14 Respiratory syncytial virus (RSV) acute lower respiratory infection (ALRI) has
15 gained recognition as a global health problem with a high burden of disease and no
16 vaccine licensed for prevention. In children under 5 years, it is estimated that 33.1
17 million episodes of ALRI, 3.2 million hospital admissions and as many as 118,200
18 deaths were attributable to RSV worldwide in 2015(1) [Figure 1]. Although often
19 characterized as a pediatric disease, RSV in adults represents a significant health
20 burden. Mortality attributable to RSV in adults ≥65 years of age is estimated to be
21 7.2 per 100,000 person years(2) and 8% of RSV ARLI among older hospitalized
22 adults was reported to result in death (3) in the United States(US). The. RSV vaccine
23 candidates aim to protect at least three target populations that are at risk for severe
24 RSV disease: (1) young infants through passive immunization, (2) older infants and
25 young children through active immunization, and (3) older adults.

26   Development of effective RSV vaccines and monoclonal antibodies (mAbs)
27 presents both opportunities and challenges. First, concerns of enhanced respiratory
28 disease (ERD) following vaccination with the formalin-inactivated RSV (FI-RSV)
29 vaccine in the 1960s have complicated the design and testing of RSV vaccines(4).
30 Current vaccine candidates, especially those designed for RSV naïve infants and
31 children, must demonstrate safety by avoiding these immunologic hallmarks of ERD.
32 Second, an absolute correlate of protection against a clinically relevant RSV infection
33 remains elusive, although cell-mediated immunity(5), mucosal IgA(6) and potent
34 neutralizing antibodies(7) have been associated with decreased disease severity.

35   Recently, three phase IIb/III trials (two vaccine trials in older adults(8,9) and
36 one mAb trial in infants(10)) failed to meet clinical endpoints. In addition to
37 possible inadequacies in trial design and implementation, the failure of these
38 candidates demonstrates the continued gaps in knowledge regarding immunologic
39 mechanisms of protection in the different target populations. Another challenge to
40 RSV vaccine design is the lack of consensus regarding clinical endpoints though
41 attempts have been made to define these for RSV prevention trials(11–13).
42 Furthermore, these endpoints may differ according to the target population. Finally,
43 a consideration in RSV vaccine development is the limited protection conferred by
44 immune responses elicited by natural RSV infection. Natural immunity provides
45 only transient protection against subsequent infection and re-infection occurs
46 frequently(14) though the most severe RSV disease is usually observed during the
47 primary infection. Disease in older children and healthy younger adults is typically
48 mild. Monoclonal antibodies circumvent the problem of transient immunity to RSV
49 and an immature immune response to vaccination in young infants at risk of severe
50 disease. An ideal RSV vaccine candidate should prevent severe disease in at risk
51 populations. Certain vaccines might also lessen person-to-person transmission and
52 thereby provide secondary benefits in those who cannot benefit directly from
53 vaccination(15).

54   Despite these obstacles, there are several opportunities for RSV vaccine and
55 mAb development. First, RSV disease burden has received increasing attention from
56 international stakeholders such as the World Health Organization (WHO)(16) and
57 the Bill & Melinda Gates Foundation based on better estimates of RSV-associated
58 mortality worldwide(17). Second, the discovery and stabilization of the prefusion

59　(pre-F) conformation of the RSV F protein provided a new target for vaccines and
60　mAbs(18,19) as pre-F specific antibodies may be more potent than postfusion (post-
61　F) antibodies in protecting against RSV ALRI. Third, pharmaceutical companies have
62　recognized the urgent unmet need of RSV prevention and prioritized the
63　development of RSV vaccines and mAbs.
64　　　In 2015, a review of RSV prevention and therapeutic strategies was
65　conducted which demonstrated that 10 vaccines were in clinical development(20).
66　An update of that review is necessary in light of the recent failures and new
67　candidates in the last few years. In this review, we show that only 50% (5/10) of
68　candidates from 2015 are currently continuing in clinical trials and 14 additional
69　new candidates have entered clinical trials [Figure 2]. In the context of RSV as an
70　increasingly recognized global health problem, these rapid changes and expansion
71　show the prioritization of RSV vaccine and mAb development.
72

73 Methods
74 A data collection template was designed for all vaccines in clinical development
75 according to the PATH RSV vaccine and mAb Snapshot (updated November 2017
76 (21)) [Supplementary Table 1]. Gaps in knowledge were identified by searching
77 PubMed, clinical trial registries, WHO, European Medicines Agency (EMA) and
78 pharmaceutical websites for each vaccine candidate, with no language restrictions,
79 through April 3, 2018 (NM, ACL, NH, IR, EP, JS). We did not intend to do a systematic
80 review of the literature. No inclusion or exclusion criteria were used. Instead,
81 articles were selected based on relevance to the subheadings used in this review as
82 well as each vaccine candidate or mAb in clinical development. Furthermore, data
83 for this review were systematically collected using a data collection template
84 [Supplemental Table 1] at the RSV Vaccines for the World conference organized by
85 the Respiratory Syncytial Virus Network (ReSViNET) from November 29 -
86 December 1, 2017 in Malaga, Spain. The goal of this meeting was to share scientific
87 data and expertise on RSV vaccine development, and to connect stakeholders
88 involved in RSV research. During the meeting information was collected (NM, ACL,
89 NH, IR, EP, JS) from scientific presentations, posters and personal communications.
90   We included all vaccine candidates and mAbs in clinical development
91 according to the PATH RSV vaccine and mAb Snapshot. Vaccines were divided into
92 four major groups: particle-based, vector-based, live-attenuated/chimeric and
93 subunit vaccines. Immunoprophylaxis with mAbs was included as a fifth category.
94
95 RSV Vaccine History
96 RSV vaccine development started shortly after the first identification of the virus in
97 humans in 1957(22). However, ERD upon natural RSV infection after vaccination
98 with a formalin-inactivated RSV (FI-RSV) candidate in a series of trials in the 1960s
99 severely hindered inactivated virus and subunit vaccine development for many
100 years. In the youngest age group, 20 of 31 of RSV naïve infants were infected with
101 community-acquired wild-type RSV during the next RSV season and 16 (80%)
102 required hospitalization including two deaths(4) in whom ERD was documented.
103 Decades of research have revealed that priming with FI-RSV vaccine triggered a
104 strong but non-neutralizing antibody response(23), followed by a T-helper 2 (Th2)
105 skewed immunologic response(24) which led to ERD upon natural RSV infection.
106 Other aspects of the immune response implicated in ERD include distinct subsets of
107 CD4 T-cells(25) and memory CD8 T-cells(26). The failure to mount a protective
108 cytotoxic T-lymphocyte (CTL) response was coupled with excess lung eosinophilia
109 and neutrophilia, monocytic infiltration, and immune complex deposition in the
110 lungs(27).
111   Nevertheless, work continued on development and human testing of live-
112 attenuated RSV vaccine candidates. In the following 60 years, only two products
113 were licensed for prevention of RSV. The first product was RSV intravenous
114 immunoglobulin (RSV-IVIG), a polyclonal immunoglobulin preparation with high
115 titers of anti-RSV neutralizing activity that was approved in the US and Canada and
116 discontinued after 2003. RSV-IVIG was replaced by the second approved product
117 palivizumab, a humanized mAb directed against the RSV F glycoprotein(28,29).
118 Since its initial approval in 1998, palivizumab remains the only licensed preventive

119   intervention against RSV after demonstrating a reduction of 39% to almost 80%
120   reduction of RSV hospitalizations in preterm infants < 35 weeks gestational age with
121   and without chronic lung disease respectively(29). Palivizumab has an excellent
122   safety profile and is indicated for the prevention of severe RSV ALRI in children
123   born prematurely, with congenital heart disease, for with chronic lung disease(30).
124   Motavizumab, a higher affinity variant of palivizumab, was developed in
125   early 2000 but was discontinued in 2010(31). In a non-inferiority head-to-head
126   comparative trial motavizumab recipients had a slightly higher frequency of mild
127   skin reactions following administration when compared to palivizumab(32).
128   However, in a placebo-controlled trial, motavizumab was highly efficacious against
129   inpatient and outpatient RSV LRI in healthy term American Indian infants (33).
130   Nevertheless, without evidence of superiority compared to palivizumab, for
131   protection from RSV-related hospitalization, evidence of slightly higher side effects,
132   and no plan for dose reduction or cost-saving, the product did not attain regulatory
133   approval(34,35).
134   With respect to vaccines for active immunization, many approaches targeted
135   for RSV naïve children were evaluated preclinically over the years. Live-attenuated
136   vaccine candidates were considered safe for clinical evaluation in these children
137   because these vaccines are not expected to cause ERD(36). Over the past 40 years,
138   several biologically derived live-attenuated vaccine candidates with attenuating
139   temperature sensitivity or cold-passage mutations were evaluated clinically,
140   including in the pediatric population, but the appropriate balance of attenuation and
141   immunogenicity, suitable for RSV-naive children and infants, remained elusive. After
142   reverse genetics techniques became available in the 1990s, it became possible to
143   design vaccines with the appropriate level of attenuation, but with increased
144   immunogenicity(37). While pediatric live-attenuated RSV vaccine candidates were
145   under continued evaluation since the 1970s there were relatively few trials of RSV
146   subunit vaccines conducted before 2000, with the exception of the purified F protein
147   (PFP) vaccines(38,39), and an RSV fusion (F) attachment (G), and matrix (M)
148   subunit vaccine(40).
149   Over the past 10 years development of preventive interventions for RSV has
150   rapidly expanded. Currently, 19 vaccine candidates and mAbs for different target
151   populations are in clinical trials, and many more are in preclinical development(21).
152
153   Lessons from the vaccine and mAb graveyard
154   While vaccine development has accelerated, there have been three recent late-phase
155   vaccine and mAb trial failures. It is important to distil lessons learned from these
156   results to inform future vaccine development.
157   1.   A phase III double-blind, placebo-controlled trial (NURSERY) evaluating
158   REGN2222 (suptavumab), a mAb against antigenic site V on the RSV pre-F
159   protein(41) was conducted at 250 sites in 19 countries. REGN2222 was
160   administered once or twice during the respiratory season to 1,149 healthy
161   preterm infants < 6 months of age with a gestational age ≤35 weeks who were
162   not eligible to receive palivizumab prophylaxis. The trial did not meet its
163   primary efficacy endpoint to prevent medically-attended RSV infections
164   through day 150 of life(42). REGN2222 was accelerated from phase I to phase

165   III due to promising results and the US Food and Drug Administration (FDA)
166   granted Fast Track designation in October 2015. A speculation for this failure
167   may be inadequate dosing schedule in regard to the antibody half-life.
168   Ultimately, the basis for failing to meet the primary clinical endpoint is not
169   known, as analyses of this late-stage failure have not yet been made public.

170 2. The second candidate that failed to meet the predefined study endpoint in
171   phase III clinical trials was the RSV F nanoparticle vaccine candidate for older
172   adults, a candidate based on aggregates of full-length post-F. The results of the
173   preceding phase II showed modest efficacy(43) and promising
174   immunogenicity measures, as determined by rise in geometric mean titer for
175   IgG antibodies against the F protein and palivizumab competing antibodies
176   (PCA), in the phase II trial(44). In the phase III trial, 11,850 subjects ≥60 years
177   of age were enrolled in 60 US sites in a double-blind placebo-controlled trial
178   (RESOLVE) over a single season starting November 2015 with 182 days
179   follow-up for the efficacy outcome. The trial was granted fast track designation
180   by the FDA in 2016. (45). However, the vaccine candidate failed to show
181   efficacy against RSV moderate–severe lower respiratory tract disease (ms-
182   LRTD) in phase III results(9). Compared to the previous season, RSV acute
183   respiratory disease (RSV-ARD) and ms-LRTD attack rates were lower than
184   expected in the 2015 – 2016 season (RSV-ARD: 2.0% versus 4.9% and RSV-
185   msLRTD 0.4% versus 1.8% during the vaccine and previous season respectively).
186   The vaccine manufacturer speculates that the difference in vaccine efficacy
187   observed may in part be due to this lower attack rate as well as high pre-
188   existing immunity in the study population(43). Another proposed explanation
189   for failure of this vaccine candidate is that the quantity of the immune
190   response to vaccination may not represent effective immunity. For example,
191   PCA titers may not correspond to effective immunity as non-neutralizing
192   antibodies can also bind the palivizumab binding site and can interfere with
193   the binding of neutralizing antibodies(46). In a post-hoc subgroup analysis, the
194   vaccine candidate showed efficacy against hospitalizations for all-cause
195   chronic obstructive pulmonary disease (COPD) exacerbations(43). Upon
196   further analysis of the phase III results, there was a non-statistically significant
197   trend towards higher RSV microneutralization titers in adults without RSV-
198   ARD when compared to adults with RSV-ARD, but this difference was not
199   statistically significant. One conclusion that can be drawn from this trial is that
200   late-phase clinical research for RSV vaccine candidates should include
201   evaluation across more than one RSV season.

202 3. Development of the MEDI-7510 vaccine candidate, a subunit vaccine candidate
203   for older adults, was discontinued after a phase IIb trial in North America,
204   Europe, South Africa, and Chile. The vaccine candidate was evaluated in 1900
205   adults ≥60 years and the study failed to meet its primary objective, efficacy
206   against RSV-associated respiratory illness between 14 days post vaccination
207   throughout the end of the surveillance period, approximately 7 months. MEDI-
208   7510 was a subunit vaccine using soluble (unaggregated) postfusion (post-F)
209   conformation of the F protein with a TLR4 agonist adjuvant. The vaccine
210   candidate showed safety and immunogenicity with increased B and T cell

211   responses in the vaccine compared to the placebo group in a phase I clinical
212   trial(47) after safety and improved immunogenicity with an adjuvant was
213   demonstrated in a first-in-human trial(48). The incidence of RSV-associated
214   respiratory illness as diagnosed by PCR was 1.7% and 1.6% in the vaccine and
215   placebo groups respectively, for a vaccine efficacy (VE) of -7.1(47). No efficacy
216   was found in secondary subset analyses. On day 29, 93% of vaccinees had an
217   anti-F IgG antibody seroresponse and there was a 4.6 geometric mean fold rise
218   in anti-F IgG titer at the end of the RSV season in vaccine recipients compared
219   to the placebo group(47). One proposed explanation for the negative results
220   may be that the choice of a post-F antigen induced antibodies without
221   appropriate epitope specificity(49). Upon further analysis, other proposed
222   explanations include a low incidence of laboratory-confirmed RSV in the study
223   population, or selection of the study population, which included high-risk and
224   low-risk older adults. Considerations for the future include selection of an
225   older study population at higher risk of RSV infection.
226
227 Vaccine antigens
228 Vaccine antigens included in RSV vaccine candidates are diverse. The majority of
229 vaccines in clinical trials (11/18) use the F protein, a class I viral fusion protein, as
230 an antigenic target. The RSV F protein is highly conserved and facilitates viral fusion
231 with host cells. Understanding the structural differences between pre-F and post-F
232 conformations, as well as stabilization of the pre-F soluble forms, has resulted in
233 advances in vaccine antigen design(19,50). Current vaccine candidates use pre-F
234 and post-F as vaccine antigens [Table 1]. Of note, the predominant conformation
235 displayed on the FI-RSV vaccine candidate was the post-F conformation(51). It
236 remains unclear as to whether there is a trigger for the pre-F to post-F
237 conformational change, but it does occur spontaneously, making it difficult to ensure
238 that a wild-type F vaccine antigen maintains a pre-F conformation. However,
239 stabilizing mutations have been identified that can preserve the pre-F-specific
240 epitopes(50,52). The antigenicity of some stabilized pre-F constructs has not been
241 rigorously investigated, and it remains an open question as to whether certain
242 stabilizing mutations affect the conformation of antibody binding sites Assays to
243 assess antigen conformation are needed. Likewise there is no consensus on cellular
244 receptors that determine viral tropism(53).
245   Other less frequent vaccine antigens, used alone or in combination with other
246 antigens, include the RSV envelope associated glycoproteins G (1/18) and small
247 hydrophobic (SH) protein (1/18) as well as internal proteins: nucleocapsid (N)
248 (3/18), M (1/18), and M2-1 (1/18). Besides the F protein, the G protein is the only
249 other target for neutralizing antibodies on the viral surface. The G protein is most
250 important for viral attachment and is less frequently utilized as a vaccine antigen
251 due to high variability across RSV strains(54), and limited knowledge of its surface
252 structure(55). The G protein exists as an oligomer on the surface of RSV particles
253 and as a monomer when secreted from infected cells in soluble form(56). There is
254 evidence that the soluble form of the G protein can act as a decoy that helps the
255 virus evade the antibody response(57). Another possible vaccine target, the SH
256 protein, is not well understood, but data suggest that it plays a role in viral

257 replication *in vivo*(53) and inflammasome activation(58). The SH protein contains a
258 transmembrane and extracellular domains(59); the latter has been used as a
259 vaccine antigen(60). Internal proteins are particularly relevant to induce T cell-
260 mediated immunity(55). As such, three non-membrane RSV proteins have been
261 included in RSV vaccine design. The N protein is the major nucleocapsid protein that
262 encapsidates the RNA genome of the virus(61). The M2-1 and M2-2 proteins are
263 specific to RSV and other *Pneumoviridae*. M2-1 is essential for viral transcription
264 (62), and M2-2 deletion is utilized in live vaccine candidates for viral attenuation.
265 Finally, the M protein is a membrane-associated protein that gives virions their
266 filamentous shape(63,64). In summary, different viral proteins are being employed
267 as antigens in RSV vaccine design. Viral surface glycoproteins such as F and G are
268 known to induce antibodies with differing neutralization capacity. The SH protein
269 may be important for induction of antibody dependent cell-mediated cytotoxicity
270 (ADCC), whereas non-membrane proteins are especially important to induce a
271 robust T-cell response(55).
272
273 Target populations
274 RSV prophylactic interventions are designed to protect at least two populations
275 most vulnerable to severe RSV disease: RSV-naïve young infants and children, and
276 older adults, although other high-risk populations are important to consider. It is
277 estimated that 45% of hospital admissions and in-hospital deaths due to RSV-ALRI
278 occur in infants younger than 6 months of age(1), an age at which vaccines are
279 generally less immunogenic. Older adults and adults with chronic cardiopulmonary
280 conditions have emerged as an important target for RSV prevention due to an
281 increased understanding of RSV burden in this population. An overview of all RSV
282 vaccine candidates per target population is shown in Table 2.
283   Maternal vaccination is utilized to provide passive immunity to young infants
284 by boosting maternal vaccine-specific antibody titers that are actively transferred
285 through the placenta, thereby extending the period of protection conferred by
286 maternal antibodies. Historically, epidemiologic studies have demonstrated an
287 association between higher maternal RSV antibody concentrations and protection
288 from ALRI in infants(65). Passive transfer of antibodies to infants has been shown to
289 be protective against severe RSV infection through the administration of high-titer
290 polyclonal and monoclonal antibodies (RSV-IVIG and palivizumab) (28,29). The
291 duration of protection of maternal vaccination is defined by the antibody half-life.
292 Administration of mAbs is an alternative form of passive vaccination that can
293 circumvent this hurdle due to extended antibody half-life through Fc alterations(66).
294 The proof-of-principle of maternal vaccination as a tool to prevent infant disease
295 has been demonstrated by the effective near-elimination of maternal and neonatal
296 tetanus worldwide through tetanus toxoid vaccination in pregnancy(67). Maternal
297 vaccination may also play a role in preventing RSV infection in pregnant women and
298 adverse birth outcomes, however data on the burden of RSV disease in pregnant
299 women and the effect of RSV infection during pregnancy on the fetus is limited(68–
300 71)
301   Premature infants, a population at high risk for severe RSV disease, may be
302 insufficiently protected by maternal vaccination given that the majority of IgG

303 transport occurs after 32 weeks gestational age(72). Globally 10% of children are
304 born preterm(73). The burden is especially relevant in low and middle-income
305 countries (LMICs) as more than 60% of preterm birth occurs in Sub-Saharan Africa
306 and South Asia(74). Thus, a maternal vaccination strategy may not be sufficient to
307 protect the high-risk preterm population if administered during the third trimester
308 of pregnancy. Tetanus-diphtheria-acellular pertussis (Tdap) immunization in the
309 second trimester is associated with higher cord-blood antibody titers as compared
310 to third trimester immunization(75). A strategy of earlier vaccination could be
311 considered for maternal RSV immunization to maximize protection for preterm
312 infants. Other populations in which impaired transplacental antibody transfer may
313 limit protection by maternal vaccination include infants of mothers with chronic
314 infection, hypergammaglobulinaemia, malaria, and HIV infection(76). The ratio of
315 transplacental antibody transfer and antibody decay kinetics are currently
316 considered the main parameters to assess protection conferred via maternal
317 vaccination. However, protection may also be mediated by breast milk antibodies
318 transferred postnatally.
319   A combined strategy that utilizes passive immunization to protect young
320 infants, via maternal vaccination or mAbs, followed by pediatric active
321 immunization may be effective to prevent severe RSV infection in young
322 children(77). The combined strategy is estimated to avert at least twice as many
323 admissions per 100 births and four times as many in-hospital deaths per 1000
324 births than maternal vaccination alone(77). This strategy will be particularly
325 relevant to prevent morbidity and mortality in children with comorbidities who are
326 at risk of severe RSV disease at older ages(78,79). A similar maternal and pediatric
327 combined passive and active immunization strategy is currently employed for
328 pertussis and influenza vaccination(76).
329   Although RSV is frequently considered a pediatric pathogen, it is important
330 to consider the older adult population with regard to prevention of severe RSV
331 disease. RSV has been identified as an important disease in older and high-risk
332 adults, with a disease burden similar to that of influenza(3). It is estimated that RSV
333 accounts for 10,000 – 14,000 deaths annually in adults over the age of 65 years in
334 the US(2,3). In addition, older adults with comorbidities such as underlying heart or
335 lung disease are at elevated risk of severe RSV disease; 4-10% of high-risk adults
336 will develop acute RSV infection annually(3).
337
338 <u>Immunologic endpoints</u>
339 Antibodies are thought to be the key players in limiting RSV ALRI as evidenced by
340 proven protection in immunoprophylaxis trials in children (28,29,33). Recent
341 evidence from experimental human infection in adults shows a protective role for
342 nasal RSV-specific IgA against RSV infection(6), underscoring the importance of
343 mucosal immunity. A limited ability to generate memory IgA responses after RSV
344 infection may be in part responsible for incomplete immunity and subsequent RSV
345 re-infection. Antibodies directed against different antigenic sites of the F protein
346 display different neutralization capacities with the most neutralization-sensitive
347 epitopes exclusive to the pre-F conformation. Antibodies with specificity for
348 antigenic sites Ø and V show high neutralizing activity and are exclusive to the pre-F

14

conformation(41,80). Antigenic site Ø is located at the apex of the pre-F conformation, the most variable region of the highly conserved F protein(19). Antibodies against antigenic site III prefer the pre-F conformation and exhibit high neutralizing activity(81). Antibodies directed against site II and IV, present on both pre-F and post-F, exhibit medium to high neutralization potency(80,82). Finally, antibodies against antigenic site I, present primarily on post-F, show weak or no neutralization. Escape mutants of these antigenic sites have been identified, but global RSV genetic data are needed to assess the molecular heterogeneity of RSV and the subsequent susceptibility or resistance to mAbs targeting RSV among circulating viruses.

The mechanisms of protection may differ according to vaccine type, and therefore, many different immunologic assays are employed in clinical trials. Neutralizing activity of serum is a frequent immunologic endpoint of vaccine trials. A measure of functional antibody response can be elucidated by the ratio of fold-increase in RSV-binding antibodies to fold-increase in RSV-neutralizing antibodies (ELISA-to-neutralization response ratio). A ratio of <1 may be an important correlate of protection(83). Furthermore, rather than a definitive protective threshold for antibodies, fold-rise in antibody titer may be a relevant correlate of protection for live-attenuated vaccines, since that may be the best indicator of B-cell priming. Recent efforts by PATH, the WHO, and the National Institute for Biological Standards and Control (NIBSC) examined the variability of RSV neutralization assays across laboratories and recommended steps for improved standardization globally(84), resulting in the development of a new WHO International Standard for Antiserum to RSV with 1000 International Units of RSV subtype A neutralizing activity per vial now available through the NIBSC(85). Standardization of other frequently used immunologic assays such as PCA, ELISA and T-cell assays has not yet taken place.

Once infection of the lower airways is established, CD8 T-cells play an important role in viral clearance(86). Th2-biased responses have been associated with animal models of RSV ERD and measurement of Th1 and Th2 responses are considered important to predict safety of vaccine candidates other than live-attenuated vaccines in clinical trials in young children.

Animal models are important for preclinical development of vaccine candidates and assessing the possibility of enhanced disease. Alveolitis in the cotton rat and priming of a Th2 response in mice are considered markers to assess possible ERD; there is no consensus on the ability to reproduce ERD in calves(87).

Although we discuss several potential immunological correlates of protection for vaccine trials, we considered cell-mediated immunity beyond the scope of the manuscript. However, we highlight the different aspects of the expected immune response for all 19 vaccine candidates and mAbs in clinical development in Table 3. A definitive threshold for protection against RSV disease remains elusive. So far no vaccine candidates have been tested in the experimental human infection model, but the model provides a unique opportunity to test vaccine candidates in the natural host despite practical and ethical challenges(88).Ultimately, the outcome of large-scale vaccine trials will inform which immunologic measures correspond to protection from clinical RSV disease.

396 <u>Vaccine strategies</u>
397 We have divided vaccines in clinical development into four categories in accordance
398 with the PATH RSV vaccine and mAb snapshot: particle-based, vector-based, subunit
399 and live-attenuated/chimeric vaccines(21). We have also included mAbs in clinical
400 development for the prevention of RSV ALRI. In the snapshot there are 43 vaccines
401 and 4 mAbs in development of which 19 are in clinical stage development. An
402 important consideration for all vaccines is not only to prevent severe RSV disease,
403 but also to avoid the risk of priming for RSV ERD. Based on our current
404 understanding of the underlying mechanisms leading to RSV ERD, caution should be
405 taken in the use of protein-based vaccines in RSV naïve individuals. Replication
406 deficient vectors, engineered to induce CD8 T cell responses expressing RSV
407 antigens intracellularly, are considered more similar to live-attenuated virus
408 vaccines which have been shown not to cause ERD in this population. In Table 1 we
409 provide a comprehensive overview and more detailed comparison of all
410 characteristics of the 19 vaccine candidates and mAbs in clinical development.
411
412 <u>Particle-based vaccines</u>
413 The RSV F nanoparticle-based vaccine platform is currently being evaluated for
414 protection of three target populations: (1) infants through maternal vaccination, (2)
415 children between 6 months and 5 years, and (3) older adults. These vaccine
416 candidates utilize aggregates of a modified stabilized F protein which exhibits the
417 post-F morphology(89). The maternal RSV F nanoparticle vaccine candidate is
418 farthest along in clinical development and the PREPARE trial has entered the third
419 year of a phase III trial to enroll up to 8,618 pregnant women at 80 sites in 11
420 countries(43). In January 2018 an informational analysis of the phase III trial was
421 announced in which the vaccine candidate successfully targeted an efficacy
422 threshold against the primary endpoint in infants at day 90 of >40%(90). Second in
423 clinical development is the RSV F nanoparticle vaccine for older adults. Despite lack
424 of efficacy in a phase III trial (RESOLVE) with a non-adjuvanted vaccine candidate,
425 development was continued in a phase II roll-over study initiated in January 2017 in
426 Australia in 300 adults. The aim of this rollover trial is to determine whether 2 dose
427 regimens with an adjuvant (Matrix-M, a saponin-based adjuvant, or aluminum-
428 phosphate) may increase the magnitude and quality of the immune response in this
429 population. The results from the RESOLVE trial in older adults suggested vaccine
430 efficacy in adults with COPD, leading to considerations to initiate a future trial in this
431 older adult population at high risk for severe RSV infection(43). Finally, the phase I
432 trial was completed in young children 24-72 months of age in 2016, but no data
433 have been published yet(91).
434 SynGEM is a particle-based needle-free vaccine candidate containing the RSV
435 F protein attached to empty bacterial particles made from *Lactococcus lactis*. In this
436 vaccine platform an antigen is presented by a bacterial particle. An influenza vaccine
437 candidate in clinical trials which uses the same vaccine platform, has shown both
438 local and systemic antibody responses(92) but needs further optimization for RSV
439 vaccination. The preliminary results of immunogenicity testing have been reported.
440 The immunogenicity of this vaccine was evaluated after delivery as a nasal spray to

441   healthy adult volunteers. Two intranasal doses of SynGEM were administered 28
442   days apart at low or high dose in 24 subjects per group (6 subjects in each group
443   receiving placebo, double blinded). Assays of serum RSV F-specific antibodies, PCA,
444   and F-specific IgA indicated some immunogenicity, but the results did not reach the
445   threshold set for continuation to viral challenge and the studies were suspended in
446   2017 (Openshaw and Chiu, personal communication).
447
448   <u>Vector-based vaccines</u>
449   There are five vector-based vaccines in clinical development. The first uses a
450   modified vaccinia virus Ankara (MVA), a replication-defective smallpox viral vector,
451   and the remaining four vaccine candidates employ an adenovirus vector to display
452   viral antigens. The MVA vector has been safely used in vaccines for other infectious
453   diseases(93). This vaccine candidate, MVA-BN-RSV, induces both humoral and cell-
454   mediated responses by displaying four vaccine antigens: F, G, N and M2-1. Phase II
455   results in healthy older adults from this candidate will soon be announced.
456   The second vector-based vaccine candidate, VXA-RSV-f, uses an innovative
457   platform with an adenovirus 5 based oral tablet that is stable at room temperature.
458   Using the same oral adenovirus vaccine delivery platform, a phase I trial for
459   influenza has been conducted, which showed neutralizing antibody responses
460   against influenza and no interference of pre-existing vector immunity(94).
461   Preclinical studies for the RSV vaccine candidate in the cotton rat model showed an
462   increase in anti-F antibodies and protection against RSV challenge(95). In the older
463   adult population immunosenescence may be characterized by impaired T-cell
464   responses to RSV(96,97). This vaccine candidate which induces a humoral response
465   may be a promising intervention in this population..
466   Third and fourth, Ad26.RSV.preF, is a vaccine candidate being developed for
467   two populations: the older adult and the pediatric population. In this candidate pre-
468   F antigen is expressed in the human adenovirus strain 26, a vector with a favorable
469   safety profile when used for other infectious diseases(98,99). Previously, the
470   vaccine candidate vector expressed post-F as antigen (FA2) but has now been
471   changed to stabilized pre-F conformation. The stabilized pre-F protein has 5 amino
472   acid changes from wild-type, and is stable at 4C and heat-stable(50). With the
473   expectation that this vaccine candidate will induce highly neutralizing antibodies
474   against pre-F, phase II trials will be conducted in RSV-seropositive children. In
475   December 2017 a phase II trial was initiated comparing concomitant administration
476   of RSV vaccine and seasonal influenza vaccine versus seasonal influenza vaccine
477   alone in healthy older adults(100).
478   Fifth, ChAd155-RSV, a replication-incompetent chimpanzee adenovirus 155
479   has been used as a vector for the F, N and M2.1 proteins. The anticipated use for this
480   pediatric vaccine is to start immunization at two months of age, and to use two
481   doses alongside the normal pediatric vaccination schedule, instead of
482   seasonally(101). This vaccine candidate is currently being evaluated in 12-23 month
483   old RSV seropositive children. In the future, there are plans to conduct clinical trials
484   in seronegative children sequentially from older to younger ages (12-24 months
485   followed by 6-12 months and subsequently 2-6 months of age) to ensure safety in

486   RSV-naïve populations. Results of phase II trials are expected to be announced in
487   2020.
488   In summary, vector-based vaccines are used to display various RSV viral
489   proteins and three of these vaccine candidates are in phase II trials.
490
491   Subunit vaccines
492   Due to concerns of ERD associated with protein-based vaccines, subunit vaccines
493   are only in development for pregnant women and older adult populations. One
494   subunit vaccine in development is the GSK RSV F vaccine candidate, which uses a
495   version of soluble secreted F protein empirically engineered to maintain the Pre-F
496   conformation. Phase I results demonstrated safety and immunogenicity as
497   evidenced by RSV neutralizing antibody response in healthy men(102). However, a
498   phase II trial scheduled for 2017 was halted due to instability of the pre-F antigen
499   during manufacturing.
500   Structure-guided stabilization of the pre-F conformation has yielded a
501   subunit vaccine candidate, DS-Cav1. The stabilization includes a foldon
502   trimerization domain, the introduction of cysteine residues to form a disulfide bond,
503   and cavity-filling hydrophobic residues(52). The vaccine is able to preserve
504   neutralization-sensitive epitopes on a functional pre-F form of the viral surface
505   protein. In preclinical studies the subunit vaccine induced high levels of RSV-
506   neutralizing antibodies in mice and non-human primates(52). Preliminary results
507   from the phase I trial, VRC 317, are promising and are expected to be published
508   soon.
509   DPX-RSV is a vaccine candidate with a unique choice of vaccine antigen; the
510   extracellular domain of the SH protein of RSV(60). The DepoVax technology allows
511   for a prolonged exposure of antigen and adjuvant, and aims to induce ADCC using a
512   liposome and oil-based depot(103). The antigen and adjuvant are encapsulated in a
513   liposome, lyophilized and suspended in oil and the process is expected to produce
514   vaccines with long shelf-life stability(104). Phase I results on safety and
515   immunogenicity in the older adult population have been released and are expected
516   to be published from this investigator-initiated study.
517
518   Live-attenuated and chimeric vaccines
519   In the context of historical concerns for enhanced RSV disease, live-attenuated
520   vaccines can be considered safe for RSV naïve infants, based on consistent clinical
521   study results showing that these candidates do not prime for ERD following
522   subsequent exposure to wild-type RSV after vaccination(105). Another benefit of
523   live-attenuated vaccines against RSV in young infants is their ability to replicate in
524   the respiratory tract despite the presence of maternally-acquired antibodies, and to
525   elicit a broad humoral and cellular response(106). Live-attenuated vaccines are
526   likely limited to the pediatric population under two years of age, as pre-existing
527   immunity in older populations might not permit sufficient replication to generate
528   protective immune responses. Safety could be a concern for intranasal live-
529   attenuated vaccines, in particular if attenuation is insufficient. However, evaluation
530   of current vaccines has not shown evidence of increased rates of vaccine-associated

531 ALRI or fever, though there may be increased rates of rhinorrhea, similar to what
532 has been observed with the live-attenuated influenza vaccines.
533   Five live-attenuated vaccine candidates in phase I clinical trials are being
534 developed in partnership with the National Institutes of Health. Live-attenuated
535 vaccines face the challenge of achieving sufficient attenuation to be safe while
536 remaining immunogenic enough to induce a protective immune response. An
537 improved understanding of the RSV viral genome has informed the development of
538 new vaccine candidates that may overcome this challenge. Two main modifications
539 to the RSV genome have been engineered through reverse genetics: the ΔM2-2
540 deletion which attenuates viral replication and upregulates antigen expression(37)
541 as well as the ΔNS2 deletion which reduces viral suppression of host interferon
542 thereby boosting the innate immune response. RSV MEDI ΔM2-2 reduced viral
543 replication while inducing a strong primary serum neutralizing antibody as well as
544 potent anamnestic response in RSV-seronegative infants and children(37). Further
545 results from phase I clinical trials with the other live-attenuated vaccine candidates
546 are expected.
547   The only chimeric vaccine candidate, rBCG-N-hRSV, currently in clinical
548 development is delivered via a BCG strain. BCG has a safe profile in newborns and
549 infants, induces a Th1 response(107,108), and allows for combined vaccination
550 against two major respiratory pathogens: *Mycobacterium tuberculosis* and RSV. Not
551 only is the Th1 cellular response important in protecting against lung pathology,
552 inflammation and viral replication(109) but the candidate also induces a humoral
553 response. The antigen presented by this vaccine candidate is the RSV N protein(110).
554 Presently, this candidate is the only vaccine candidate intended for administration
555 to newborn infants(110).
556
557 Monoclonal antibodies
558 A promising highly potent monoclonal antibody has emerged as a passive
559 administration strategy to prevent severe RSV infection. MEDI8897, also known as
560 nirsevimab, was optimized from the human antibody D25 that targets antigenic site
561 Ø on the pre-F conformation, which is more neutralization sensitive than the
562 palivizumab epitope, antigenic site II. Using the YTE technology which extends
563 antibody half-life as well as modulates ADCC(111), the three-fold increase in half-
564 life of MEDI8897(112) compared to palivizumab offers the possibility of passive
565 protection for all infants for an entire season through a single intramuscular
566 injection. The intended use is for both term and preterm infants entering their first
567 RSV season. Passive vaccination with an extended half-life antibody offers an
568 approach to protecting infants that is safe and may be reasonably priced.
569 Representatives of the pharmaceutical company have indicated that they expect
570 vaccine-like pricing for MEDI8897. Given the increased potency, the extended half-
571 life, and the required dose, it is expected that the cost to protect an infant during the
572 RSV season can be kept relatively low(66).
573
574 Other approaches not in clinical development
575 Other emerging approaches not yet in clinical development include nucleic acid-
576 based vaccines(113). Importantly these vaccines induce a T-cell response mimicking

577 the response to live virus infection. Both DNA and messenger RNA (mRNA) vaccines
578 against RSV have shown promising results in preclinical studies(113). Notably,
579 through a collaboration with the Bill & Melinda Gates Foundation, an mRNA
580 technology vaccine platform for HIV and rotavirus has also expanded to include RSV.
581 Another vaccine approach in preclinical development is a whole-inactivated vaccine
582 to be delivered intranasally via a nanoemulsion technology, for which development
583 has been supported by the Bill & Melinda Gates Foundation(114). Furthermore,
584 with the first of the palivizumab patents expiring in October 2015 and the last in
585 2022, there has been active development to produce a biosimilar in order to provide
586 a low-cost RSV preventive intervention..
587
588 <u>Considerations by regulatory agencies and the World Health Organization</u>
589 The FDA has articulated that differences between high income countries (HICs) and
590 LMICs are not particularly relevant to regulatory decisions, though a bridging study
591 in the US must be performed if all clinical trials have been performed outside of the
592 US(115). The EMA does not require that trials intended to support a regulatory
593 decision are conducted in the European Union. Other considerations in population
594 selection for vaccine trials mentioned by EMA include: first testing a vaccine
595 candidate in a seropositive before testing in a seronegative population, testing a
596 maternal vaccine in non-pregnant women of child-bearing age before testing in
597 pregnant women, and including older adults with comorbidities in vaccine trials. No
598 particular considerations were mentioned for population selection in studies for
599 mAbs. In October 2017 the EMA released draft guidelines for the clinical evaluation
600 of RSV prophylactic interventions which included guidance regarding trial design,
601 assessment of efficacy, and safety(116). The draft guidelines will be revised after a
602 period of public consultation based on comments and new publications.
603   The WHO has recognized the importance of RSV as a global health problem
604 and has identified the development of RSV vaccines as a priority for the WHO
605 Initiative for Vaccine Research and for Biological Standardization. WHO recently
606 developed RSV vaccines preferred product characteristics and research and
607 development technical roadmap documents(117,118). Further guidance for
608 development will contribute to adequate policy-making. WHO standardization
609 activities led to the development and establishment of the first international
610 standard for antiserum to RSV. Development of guidelines for evaluation of quality,
611 safety and efficacy of RSV vaccines has been initiated and will be part of consultation
612 with regulators, manufacturers and academia in 2018 with the aim of finalizing it in
613 2019. Further discussion on guiding principles for mAbs is needed before
614 proceeding with the development of the WHO Guidelines. These and other WHO
615 standards serve as a basis for setting national regulatory requirements as well as
616 WHO prequalification.
617   Finally, the WHO is now performing a surveillance pilot study in 14 countries
618 to test the feasibility of using the Global Influenza Surveillance and Response System
619 platform for RSV surveillance and it is expected that this pilot will contribute to our
620 understanding of the RSV disease burden and seasonality in different geographical
621 regions(119).
622

623   Discussion
624 Challenges in RSV vaccine design include concerns of ERD post-vaccination, lack of
625 definitive immunologic correlates of protection, lack of consensus regarding clinical
626 endpoints, and limited natural immunity following RSV infection. Despite these
627 challenges, recent developments such as an understanding of the structural biology
628 of the RSV fusion protein as well as lessons learned from late-phase vaccine trial
629 failures have informed the field as it moves forward.
630   We attempted to collect data regarding expected plans for access to a
631 preventive intervention in LMICs and expected pricing for all vaccine candidates,
632 however this information is not publicly available. The only information obtained
633 regarding expected pricing was for MEDI8897, though a more specific estimate than
634 vaccine-like pricing was not available. Given that the most severe RSV infection
635 occurs in LMICs(17), information regarding LMIC target countries and potential
636 pricing for vaccine candidates will be essential to facilitate access to vaccines
637 worldwide, especially in areas where the mortality burden is highest. In LMICs the
638 most important target for vaccine candidates is young children(120). A mechanism
639 should be introduced to ensure that information regarding expected pricing and
640 access to interventions is transparent and available in the public domain. RSV
641 vaccines and mAbs will be considered in the development of the Vaccine Investment
642 Strategy by GAVI, the Vaccine Alliance in 2018(121).
643   A vaccine trial may be considered a probe study to determine whether a
644 causal relationship exists between RSV infection and asthma, a longstanding
645 question in the field. If long-term follow-up had been undertaken during the pivotal
646 RSV prevention trials using palivizumab, these trials would now have provided 20
647 years of follow-up on respiratory morbidity after RSV prevention in high-risk
648 infants. Lack of long-term surveillance for airway morbidity in vaccine trials are
649 missed opportunities to provide novel scientific insights important not only to
650 understand the pathogenesis but also the long-term vaccine efficacy against airway
651 morbidity following RSV infection. In addition to wheeze, objective outcomes, such
652 as lung function measurements including demonstration of bronchial
653 hyperreactivity and IgE measurements will ideally be incorporated in vaccine trials
654 to fully understand the impact of RSV prevention on asthma development.
655   Viral interference, in which RSV inhibits infection by other viruses, is
656 becoming an increasingly important concept to understand in the context of an
657 approved RSV vaccine. RSV vaccination may conceivably result in an increased
658 prevalence of other respiratory viruses. There is evidence supporting viral
659 interference for influenza vaccination(122,123), for RSV prevention(124,125), and
660 during the RSV season in the absence of RSV(126). It is important for vaccine trials
661 to examine this phenomenon by evaluating the incidence of all-cause ALRI, as well
662 as RSV-specific ALRI, to better understand the implications of viral interference for
663 an RSV vaccine.
664   This review provides an extensive overview of the 19 vaccine candidates and
665 mAbs in clinical trials to prevent RSV infection. RSV vaccine development is moving
666 rapidly and shows promise to address an unmet global health problem. Vaccines for
667 various target populations are in clinical development. One vaccine candidate and
668 one mAb are in late phase trials (IIb/III) and aim to prevent the disease burden in

669   young infants. Despite some recent failures, RSV vaccine candidates and mAbs in
670   clinical development hold the promise that a preventive intervention for RSV is on
671   the horizon.
672
673

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676   manuscript.
677
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Tables/Figures
Table 1: Overview of RSV vaccines and mAbs in clinical development

Vaccine	Company/ Sponsor	Manufacturing Process	Antigen	Adjuvant	Mechanism of Action	Target Population	Route of Administration	Clinical Phase	Animal Models	Phase I	Phase II	Phase III	Result Summary
PARTICLE-BASED													
RSV F Nanoparticle	Novavax	Sf9/BV recombinant technology	Stabilized F protein exhibiting post-F morphology	Aluminum phosphate	F forms nanoparticle in multimeric micelle format	M	IM	III	Cotton rats (127,128), baboons(129) Guinea pigs(130)	Dec 2010-Dec 2011 NCT01290419 (n=150)	Oct 2012 – May 2013 NCT01704365 (n=330) Oct 2013 – April 2014 NCT01960686 (n=720) Sep 2014 – Jul 2016 NCT02247726 (n=50)	Dec 2015-Jun 2020 NCT02624947 (n=8618)	PhII: all formulations well-tolerated and immunogenic; most robust Ab response with 120ug and 0.4mg aluminum formulation, peak d14 and persistence through d91; RSV infection measured by Western blot was reduced by 52% (p=0.009) in healthy women of childbearing age (n=720)(131,132) Vaccine safe, immunogenic and reduced RSV infection in healthy women of childbearing age (n=330)(133)
RSV F Nanoparticle	Novavax	Sf9/BV recombinant technology	Stabilized F protein exhibiting post-F morphology	Aluminum phosphate & Matrix M	F forms nanoparticle in multimeric micelle format	O	IM	II	Cotton rats (127,128), baboons(129)	Oct 2012-Mar 2014 NCT01709019 (n=220)(134) Oct 2015 – Nov 2016 NCT02593071 (n=1330) Rollover: Jan 2017 – Jul 2018 NCT03026348 (n=1329)	Oct 2014 – Mar 2016 NCT02266628 (n=1599)	Nov 2015 – Dec 2016 NCT02608502 (n=11850)	PhII: safe, VE: 41% against RSV-ARD, 64% VE against RSV-msLRTD(135) PhIII: safe, no efficacy v. RSV-ARD &RSV-msLRTD;; post-hoc efficacy v all-cause hospitalization (n=11850) PhII rollover: no residual protection in 2nd year; second immunization protective against RSV-ARD and msLRTD (n=1329)(131,136)
RSV F Nanoparticle	Novavax	Sf9/BV recombinant technology	Stabilized F protein exhibiting post-F morphology	Aluminum phosphate/ Matrix M-1	F forms nanoparticle in multimeric micelle format	P	IM	I	Cotton rats, (127,128) baboons (129)	Nov 2014 – Apr 2016 NCT02296463 (n=32)	N/A	N/A	PhI: well-tolerates;; Anti-F IgG & PCA increase d14, Peak d28, elevated to d56; 10-fold increase PCA & anti-F IgA adjuvanted 6-fold increase in unadjuvanted(135) (n=32)
SynGEM	Mucosis	Bacterium-like-particle (BLP) mimopath technology carrying F proteins	F protein, unclear which conformation	BLP	BLP allows presentation of F protein and elicits mucosal IgA	O & P	IN	I	Mice	July 2016 – Dec 2017 NCT02958540 (n=48)	N/A	N/A	PhI: some immunogenicity in healthy adults but did not meet threshold; development suspended.
VECTOR-BASED													
MVA-BN RSV	Bavarian Nordic	MVA-BN technology (antigens expressed in attenuated modified vaccinia Ankara)	F, G (subtype A & B), N, M2	none	Virus replication blocked at a late stage	O	IM/IN	II	Cotton rats, BALB/c mice(137)	IM: Aug 2015-May 2016 NCT02419391 (n=63) IN: Sep 2018 – Aug 2019 NCT02864628	Sep 2016 – Aug 2018 NCT02873286 (n=400)	N/A	PhI; safe, 2x increase IgG & IgA; 3-5x increase In T cell responses (n=63)(137) PhII interim results: well-tolerated; broad Ab & T cell response in older adults after single vaccination (n=421) (138)

Name	Developer	Description	Antigen	Adjuvant	Mechanism		Route	Phase	Animal models	Clinical Trial 1	Clinical Trial 2		Results
VXA-RSVf oral	Vaxart	antigen and adjuvant expressed in non-replicating adenovirus vector (Ad5)	F	dsDNA that activates TLR3 receptor	Vector delivers directly to gut (ileum)	O	Oral	I	Cotton rat	Jun 2016-Dec 2017 NCT02830932 (n=66)	2018?	N/A	Preclinical: Systemic Anti-F Ab's and protection against RSV infectio in cotton rat model(95)
Ad26.RSV.preF	Janssen	Antigen expressed in human adenovirus type 26 produced in PER.C6 human cell line	Pre-F (previously FA2)	none	Ad26 vector is replication incompetent but expresses immunogenic F antigen	O	IM	II	Mice, cotton rats(139)	Nov 2016 – Dec 2018 NCT02926430 (n-73)	Dec 2017 – Jul 2018 NCT03339713 (n=180)	N/A	PhI: well-tolerated; durable humoral and cellular immune response for FA2 candidate; comparable or higher for preF candidate in older adults (140)
Ad26.RSV.preF	Janssen	Antigen expressed in human adenovirus type 26 produced in PER.C6 human cell line	Pre-F (previously FA2)	none	Ad26 vector is replication incompetent and expresses immunogenic F antigen	P	IM	I	Mice, cotton rats(139)	Nov 2017-Mar 2019 NCT03303625 (n=60)	Nov 2017-Mar 2019 NCT03303625 (n=60)	N/A	N/A
ChAd155-RSV	GSK	Chimpanzee adenovirus ChAd155-RSV with F, N, M2.1 insert and E1 deletion	F, N, M2.1	none	Intracellular RSV antigen expression; replication incompetent vector	P	IM	II	Mouse, cotton rat, calves (101)	Jul 2015 – Feb 2017 NCT02491463 (n=73)	Jan 2017 – Sep 2020 NCT02927873 (n=96)	Plan to start post 2020 with age de-escalation seronegative infants	PhI: safe, B cell and RSV-neutralizing antibodies in RSV-seropositive adults (n=73)(141)

SUBUNIT

Name	Developer	Description	Antigen	Adjuvant	Mechanism		Route	Phase	Animal models	Clinical Trial 1	Clinical Trial 2		Results
GSK RSV F	GSK	Pre-F produced in CHO cells	Pre-F	With or without aluminum hydroxide	Pre-F antigen induces neutralizing antibodies which are transferred to infant	M	IM	II	Mice, cotton rats, guinea pigs, cows	Dec 2014 –Mar 2017 NCT02298179 (n=288); Jul 2013-Mar 2015 NCT01905215 (n=128) (102)	Mar 2015-June 2016 NCT02360475 (n=507); Apr 2016 – Jun 2016 NCT02753413 (n=102); Nov 2016 – Mar 2018 NCT02956837 (n=406); Jul 2017 – Jan 2021 NCT03191383 Halted due to instability of pre-F antigen during manufacturing	N/A	PhI: safe, RSV-A neutralizing Ab titers increased 3.2-4.9x; remained high to day 60, decreased on day 180 & 360 in healthy men (n=128)(102); Ph II: Increased RSV-A neutralizing Ab 30 days post-vaccination in healthy non-pregnant women (142)
DPX-RSV	Dalhousie University	DepovaxTM delivery in 100% oil-based platform preventing release at injection site	SHe	DepovaxTM or aluminum hydroxide	Depovax gives controlled and prolonged exposure of antigen and adjuvant	O	IM	I	Mice, cotton rats	May 2015-June 2017 NCT02472548 (n=40)	N/A	N/A	PhI: Well-tolerated, Antigen-specific Ab response durable to day 421, low immunogenicity with alum adjuvant in healthy older adults(143)
RSV F DS-Cav1	NIH/NIAID/VRC	Prefusion stabilitized trimeric RSVF	Pre-F	Alum/ TLR4 agonist (E)	Pre-F antigen elicits highly neutralizing	M & O	IM	I	Cotton rats, mice, calves(14	Feb 2017-Jan 2020 NCT0304	N/A	N/A	Preclinical: Induction of high neutralizing Abs and differential adjuvant-induced

		expressed in CHO cell line			antibodies against pre-F epitopes				4), macaques (52)	9488 (n=100)			enhancement(144) Immunization of mice and macaques induces RSV-neutralizing Ab many times Protective threshold(52)

LIVE-ATTENUATED/CHIMERIC

Name	Institution	Description	Based on	Adjuvant	Rationale		Route	Phase	Model	Dates/NCT			Outcomes
rBCG-N-hRSV	Pontificia Universidad Catolica de Chile	Recombinant BCG expressing N antigen	N	none	Paired BCG and RSV vaccine induces Th1 response	P	ID	I	Mice (107–109,145)	Jun 2017-May 2018 NCT03213405 (n=24)	N/A	N/A	Preclinical: Protective T cell immune response and recruitment of Th1 cells (107,108)
RSV D46 cpΔM2-2	Sanofi Pasteur/LID/N IAID/NIH	M2-2 deletion via reverse genetics and 5 aa substitutions in 3 proteins called the "cp" mutations, originally identified in a cold-passaged vaccine candidate cpRSV	native RSV	none	Deletion of regulatory factor M2.2 causes inefficient replication but high immunogenicity, further attenuation with cp mutations	P	IN	I	African green monkeys	Oct 2015-May 2018 NCT02601612 (n=45)	N/A	N/A	N/A
RSV LID ΔM2-2 1030s	Sanofi Pasteur/LID/N IAID/NIH	M2-2 deletion via reverse genetics and temperature sensitivity mutation 1030s	native RSV	none	Deletion of regulator factor M2-2causes inefficient replication but high immunogenicity; temperature sensitive mutation at position 1030 of L gene	P	IN	I	Mice, African green monkeys	Jun 2016-Jul 2017 NCT02794870, NCT02952339 (n=33)	N/A	N/A	N/A
RSV ΔNS2 Δ1313 I1314L	Sanofi Pasteur/LID/N IAID/NIH	NS2 and 1313 deletion via reverse geneticsm I1314L substation.	native RSV	none	NS2 deletion bolsters innate response. Deletion at position 1313 of L protein, and I1314L substation confers moderate temperature sensitivity	P	IN	I	Mice and chimpanzees	Jun 2013-May 2017 NCT01893554 (n=75) Aug 2017-May 2019 NCT03227029 (n=80)	N/A	N/A	N/A
RSV D46/NS2/N ΔM2-2 HindIII	Sanofi Pasteur/LID/N IAID/NIH	LID backbone without deletions or substitutions in SH gene, point mutation in NS2 and N proteins, modified M2-2 deletion, based on RSV MEDI ΔM2-2.	native RSV	none	Deletion of regulatory factor M2.2 causes inefficient replication but high immunogenicity	P	IN	I	African green monkeys	Mar 2017-April 2019 NCT03102034, NCT03099291 (n=33)	N/A	N/A	N/A
RSV LID cp ΔM2-2	Sanofi Pasteur/LID/N IAID/NIH	M2-2 deletion via reverse genetics, and cp mutation	native RSV	none	Deletion of regulatory factor M2.2 causes inefficient replication but high immunogenic	P	IN	I	African green monkeys	Sep 2016-Apr 2018 NCT02890381 (n=17)	N/A	N/A	N/A

					ity ; further attenuation with cp mutations									

MONOCLONAL ANTIBODY (mAb)

MEDI8897	MedImmune	In vitro-optimized human mAb with YTE mutation in Fc	N/A	N/A	Antibody targeting site Ø of the F protein of RSV with an extended half-life	P	IV/IM	II	Cotton rats, cynomolgus monkeys (66)	Apr 2014- Jun 2015 NCT02114268 (n=342) Jan 2015-Sep 2016 NCT02290340 (n=151)	Nov 2016-Nov 2018 NCT02878330 (n=1454)	N/A	PhI; well-tolerated,, Mean half-life 85-117d; time to max concentration 5-9 days; bioavailability 77% in healthy adults (n=136) (146)	

Legend: N/A: not applicable or not available, IM: intramuscular, ID: intradermal, IN: intranasal, IV: intravenous; ARD: acute respiratory disease, PCA: palivizumab-competing antibodies, P: pediatric, M: maternal, O: older adults, SHe: small hydrophobic protein ectodomain; RSV ARD: all symptomatic respiratory disease due to RSV; msLRTD: moderate-severe RSV-associated lower respiratory tract disease; NIAID: National Institutes of Allergy and Infectious Diseases; VRC: Vaccine Research Center; NIH: National Institute of Health, Ab: antibody, aa: amino acid.

Table 2 Overview of vaccines and mAbs by target population

Target Population	Vaccine	Vaccine type
Pregnant mothers		
Third trimester	RSV F nanoparticle (Novavax)	Nanoparticle
Third trimester	RSV F (GSK)	Subunit
	RSV F protein (NIH/NIAID/VRC)	Subunit
Pediatric		
6m-5y	RSV F nanoparticle (Novavax)	Nanoparticle
Start 2m	Adenovirus (GSK)	Vector
Start 2-3m	Adenovirus (Janssen)	Vector
	BCG/RSV (Pontificia Unversidad Catolica de Chile)	Chimeric
	RSV D46 cp ΔM2-2 (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
	RSV LID ΔM2-2 1030s (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
	RSV ΔNS2 Δ1313 I1314L (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
	RSV D46/NS2/ N/ΔM2-2-HindIII (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
	RSV LID cp ΔM2-2 (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
	MEDI8897 (MedImmune)	Monoclonal antibody
Older adults		
	RSV F nanoparticle (Novavax)	Nanoparticle
	RSV BLP (Mucosis)	Nanoparticle
	MVA (Bavarian Nordic)	Vector
	Adenovirus (Vaxart)	Vector
	Adenovirus (Janssen)	Vector
	DPX-RSV-SH Protein (Immunovaccine)	Subunit
	RSV F protein (NIH/NIAID/VRC)	Subunit

Legend: m: months; y: years

Table 3: Expected immune response and previous successes for vaccine candidates and monoclonal antibodies

Vaccine	Target Population	Pre-F Immunity* (86)	Immune response	Mucosal/Systemic
Nanoparticle				
RSV F Nanoparticle (Novavax)	M	Pre-F < post-F	Broadly neutralizing antibodies	systemic
RSV F Nanoparticle (Novavax)	O	Pre-F < post-F	Broadly neutralizing antibodies	systemic
RSV F Nanoparticle (Novavax)	P	Pre-F < post-F	Broadly neutralizing antibodies	systemic
RSV BLP (Mucosis)	O & P	unclear F confirmation	Activation of B & T cells; local secretion of neutralizing IgA in the nose; production of IgG neutralizing IgG in the blood	mucosal & systemic
Vector				
MVA (Bavarian Nordic)	O	Pre-F < post-F	B & T cell response; antibodies against 5 RSV antigens	systemic
Adenovirus (GSK)	O	Pre-F > post-F	B & T cell response; neutralizing antibodies against F antigen; CD8 T cells against F, N and M2.1 antigens	systemic
Adenovirus (Vaxart)	O	Pre-F < post-F	B & T cell immunity, protection at mucosal surface	mucosal > systemic
Adenovirus (Janssen)	P	Pre-F	B & T cells	systemic
Adenovirus (Janssen)	O	Pre-F	B & T cells	systemic
Subunit				
RSV F (GSK)	M	Pre-F	B & T cell response	systemic
DPX-RSV (Dalhousie University)	O	none		systemic
RSV F protein (NIH/NIAID/VRC)	O & M	Pre-F		systemic
Live-attenuated				
BCG/RSV (Pontificia Universidad Catolica de Chile)	P	Pre-F & post-F	B & T cell response; Th1 polarized response; antibodies against N, F, G	systemic
RSV D46 cp ΔM2-2 (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F & post-F	B & T cell response; enhanced antibody production due to increased antigen production from M2-2 deletion	mucosal & systemic
RSV LID ΔM2-2 1030s (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F & post-F	B & T cell response; enhanced antibody production due to increased antigen production from M2-2 deletion	mucosal & systemic
RSV ΔNS2 Δ1313/I1314L (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F & post-F	B & T cell response	mucosal & systemic
RSV D46 ΔNS2 N ΔM2-2 HindIII	P	Pre-F & post-F	B & T cell response; enhanced antibody	mucosal & systemic

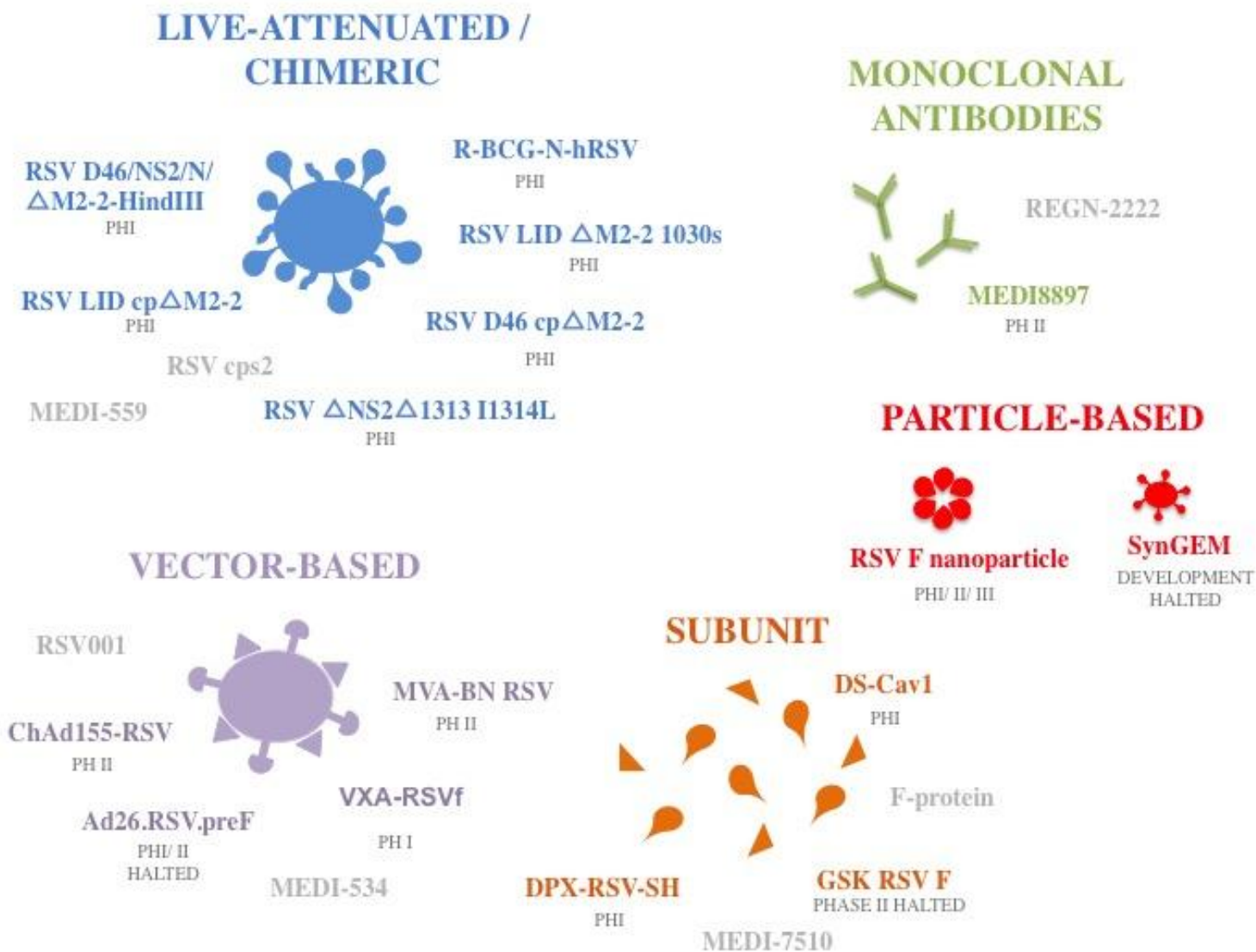|				
(Sanofi Pasteur/LID/NIAID/NIH)			production due to increased antigen production from M2-2 deletion	
RSV LID cp ΔM2-2 (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F & post-F	B & T cell response; enhanced antibody production due to increased antigen production from M2-2 deletion	mucosal & systemic
Monoclonal Antibody				
MEDI8897 (MedImmune)	P	N/A	N/A	N/A

Legend: Pre-F: prefusion conformation of the RSV F protein; Post-F: postfusion conformation of the RSV F protein; N: RSV nucleocapsid protein; F: RSV fusion protein; G: RSV attachment protein; O: older adults; M: maternal; P: pediatric.

Figure 1: RSV global burden of disease in children under 5 years of age: key facts and figures

Figure 1 Incidence is shown worldwide for children under 5 years of age unless otherwise stated. The hospital admission rate of 15.9 hospital admissions per 1000 neonates per year is in developing countries. The RSV ALRI hospitalization 63.9 among premature infants <1 year is reported per 1000 children per year globally. Legend: OR: odds ratio; LRTI: lower respiratory tract infection, RSV: respiratory syncytial virus, HIC: high income country, *: compared to children who survived RSV hospitalization and were mechanically ventilated. References: (a)(1) (b)(78) (c)(147) (d)(148) (e)(149) (f)(150)

Figure 2: Overview of Vaccine candidates and monoclonal antibodies in clinical trials per preventive approach including candidates for which development was recently halted

Legend: For vaccine candidate names listed in gray development has been halted since the last RSV therapeutics review performed in 2015(20). Abbreviations: PH I: phase I; PH II: phase II; PH III: phase III.

Supplemental Table 1: Data Collection Template

Type of Vaccine	Nano particle				Vector					Subunit			Live-attenuated/ Chimeric						Mabs/ biosimilars
PATH snapshot **Candidate**	RSV F nanoparticle (Novavax, M)	RSV F nanoparticle (Novavax, E)	RSV F nanoparticle (Novavax, P)	RSV BLP (Mucosis, E&P)	MVA (Bavarian Nordic, E)	Adenovirus (GSK, P)	Adneovirus (Vaxart, E)	Adenovirus (Janssen, E)	Adenovirus (Janssen, P)	RSV F (GSK, M)	DPX-RSV-SH Protein (Immunovaccine /VIB, E)	RSV F Protein (NIH/NIAID/VRC, M&E)	BCG/RSV (Pontificia Unversidad Catolica de Chile)	RSV D46 cp ΔM2-2 (Sanofi/LID/NIAID/NIH)	RSV LID ΔM2-2 1030s (Sanofi/LID/NIAID/NIH)	RSV ΔNS2 Δ1313 (Sanofi/LID/NIAID/NIH)	RSV D46/NS2/N/ΔM2-2-HindIII (Sanofi/LID/NIAID/NIH)	RSV LID cp ΔM2-2 (Sanofi/LID/NIAID/NIH)	MEDI8897
Person responsible																			
RSVVW related program items / names																			
Manufacturing process																			
Adjuvant																			
Animal models																			
Pre-F immunity																			

Immunity (general)																	
Antigens																	
Mechanism of action																	
Mode of administration																	
Target populations																	
Results clinical studies so far																	
Efficacy																	
Endpoints																	
PMID results																	
Timing Ph1																	
Timing Ph2																	
Timing Ph3																	
Current develo																	

pment status																	
Trial names																	
Expected herd immunity																	
Previous vaccine successes																	
Description current trial(s), register																	
Summary corporate website																	
LMIC target																	
Expected price																	
Important Links																	
Important Links																	

The Respiratory Syncytial Virus Vaccine Landscape

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Declaration of interest

The authors alone are responsible for the views expressed in this publication, which do not necessarily represent the decisions or the stated policy of any organization, institution or agency.

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Abstract (word count: ~~132~~167/~~150~~200)

The global burden of disease caused by respiratory syncytial virus (RSV) is increasingly recognized, not only in infants, but also in older adults. Advances in knowledge of the structural biology of the RSV surface fusion (F) glycoprotein have revolutionized RSV vaccine development by providing a new target for preventive interventions. The RSV vaccine landscape has rapidly expanded to include 19 vaccine candidates and ~~and~~ monoclonal antibodies (mAbs) in clinical trials, reflecting the urgency of reducing this global health problem and hence the prioritization of RSV vaccine development. The candidates include mAbs and vaccines using four approaches: (1) particle-based, (2) live-attenuated/chimeric, (3) subunit, (4) vector-based. Late phase RSV vaccine trial failures highlight gaps in knowledge regarding immunologic protection and provide lessons for future development. In this review we highlight promising new approaches to RSV vaccine design and provide a comprehensive overview of RSV vaccine candidates and mAbs currently in clinical development to prevent one of the most common and severe infectious diseases in young children and older adults worldwide.

Search strategy and selection criteria

References for this review were identified through a search of PubMed for clinical trials with "syncytial" in the title published after January 1, 2013 with no language restrictions, through April 3, 2018. We did not intend to do a systematic review of the literature. No inclusion or exclusion criteria were used. Instead, we selected articles that were most relevant to the subheadings used in this review. The PATH RSV vaccine and mAb Snapshot was used as a reference to identify all vaccine and mAb candidates in clinical trials. ClinicalTrials.gov as well as the WHO vaccine pipeline tracker for RSV were used to identify all relevant trials for these vaccine candidates and mAbs. Additional data was collected during the RSV Vaccines for the World Conference on November 29- December 1, 2017 and through pharmaceutical websites for the respective vaccine and mAb candidates.

13 Introduction

14 Respiratory syncytial virus (RSV) acute lower respiratory infection (ALRI) has
15 gained recognition as a global health problem with a high burden of disease and no
16 vaccine licensed for prevention. In children under 5 years, it is estimated that 33.1
17 million episodes of ALRI, 3.2 million hospital admissions and as many as 118,200
18 deaths were attributable to RSV worldwide in 2015(1) [Figure 1]. Although often
19 characterized as a pediatric disease, ~~the burden of~~ RSV in adults ~~is also~~represents a
20 significant health burden. ~~with~~ Mortality attributable to RSV in adults ≥65 years of
21 age is estimated to be 7.2 per 100,000 person years(2) and ~~a mortality rate of 7 to~~
22 ~~8%~~ of RSV ARLI among older hospitalized adults ~~hospitalized with RSV ALRI~~was
23 reported to result in death ~~in the United States~~(3) in the United States(US). The
24 ~~mortality attributable to RSV in adults ≥65 years of age is estimated to be 7.2 per~~
25 ~~100,000 person years (3)~~. RSV vaccine candidates aim to protect at least three
26 target populations that are at risk for severe RSV disease: (1) young infants through
27 passive immunization, (2) older infants and young children through active
28 immunization, and (3) older adults.

29 Development of effective RSV vaccines and monoclonal antibodies (mAbs)
30 presents both opportunities and challenges. First, concerns of enhanced respiratory
31 disease (ERD) following vaccination with the formalin-inactivated RSV (FI-RSV)
32 vaccine~~s~~ in the 1960s have complicated the design and testing of RSV vaccines(4).
33 ~~ERD occurred in RSV-naïve infants who experienced infection with community-~~
34 ~~acquired wild-type RSV following receipt of FI-RSV. Decades of research have~~
35 ~~revealed that in these FI-RSV primed infants, natural RSV infection triggered a~~
36 ~~strong but non-neutralizing antibody response(5), followed by a T helper 2 (Th2)~~
37 ~~skewed immunologic response(6). The failure to mount a protective cytotoxic T~~
38 ~~lymphocyte (CTL) response was coupled with excess lung eosinophilia and~~
39 ~~neutrophilia, monocytic infiltration, and immune complex deposition in the lungs(7).~~
40 Current vaccine candidates, especially those designed for RSV naïve infants and
41 children, must demonstrate safety by avoiding these immunologic hallmarks of ERD.
42 Second, an absolute correlate of protection against a clinically relevant RSV infection
43 remains elusive, although cell-mediated immunity(5), mucosal IgA(6) and potent
44 neutralizing antibodies(7) have been associated with decreased disease severity.

45 Recently, three phase IIb/III trials (two vaccine trials in older adults(8,9) and
46 one mAb trial in infants(10)) failed to meet clinical endpoints. ~~The~~ In addition to
47 possible inadequacies in trial design and implementation, the failure of these
48 ~~vaccine and mAb~~ candidates demonstrates the continued gaps in knowledge
49 regarding immunologic mechanisms of protection in the different target populations.
50 Another challenge to RSV vaccine design is the lack of consensus regarding clinical
51 endpoints ~~of vaccine trials~~ though attempts have been made to define these for RSV
52 ~~both~~ prevention trials(11–13) ~~and treatment trials(17)~~. Furthermore, these
53 endpoints may differ ~~based on~~according to the target population. Finally, a
54 consideration in RSV vaccine development is the limited protection conferred by
55 immune responses elicited by natural RSV infection. Natural immunity provides
56 only transient protection against subsequent infection and re-infection occurs
57 frequently(14) though the most severe RSV disease is usually observed during the
58 primary infection. Disease in older children and healthy younger adults is typically

8

mild. ~~Monoclonal antibodies circumvent the problem of transient immunity to RSV and an immature immune response to vaccination in young infants at risk of severe disease.~~ An ideal RSV vaccine candidate should prevent severe disease in at risk populations. Certain vaccines might also lessen person-to-person transmission and thereby provide secondary benefits in those who cannot benefit directly from vaccination(15).

Despite these obstacles, there are several opportunities for RSV vaccine and mAb -development. First, ~~the~~ RSV disease burden has received increasing attention from international stakeholders such as the World Health Organization (WHO)(16) and the Bill & Melinda Gates Foundation based on better estimates of RSV-associated mortality worldwide(17). Second, the discovery and ~~of the structure and~~ stabilization of the prefusion (pre-F) conformation of the RSV F protein ~~has advanced the field~~provided a new target for vaccines and mAbs(18,19) as p ~~by showing that p~~re-F specific antibodies may be more potent ~~in protecting against RSV LRTI~~ than ~~antibodies that also bind the~~ postfusion (post-F) ~~conformation~~ antibodies in protecting against RSV ALRI. ~~and by thus providing a new target for vaccines and mAbs(21,22).~~ Third, pharmaceutical companies have recognized the urgent unmet need of RSV prevention and prioritized the development of RSV vaccines and mAbs.

In 2015, a review of ~~new~~ RSV prevention and therapeutic strategies was conducted which demonstrated that 10 vaccines were in clinical development(20). An update of that review is necessary in light of the recent failures and new ~~vaccine~~ candidates in the last ~~several~~ few years. In this review, we show that only 50% (5/10) of candidates from 2015 are currently continuing in clinical trials and 14 additional new ~~vaccine~~ candidates have entered clinical trials [Figure 2]. In the context of RSV as an increasingly recognized global health problem, these rapid changes and expansion show the prioritization of RSV vaccine and mAb development.

Methods

A data collection template was designed for all vaccines in clinical development according to the PATH RSV ~~v~~Vaccine and mAb ~~snapshot~~ Snapshot (updated November 2017 (21)) [Supplementary Table 1]. Gaps in knowledge were identified by searching PubMed, clinical trial registries, WHO, European Medicines Agency (EMA) and pharmaceutical websites for each vaccine candidate, with no ~~date or~~ language restrictions, ~~on January 31, 2018~~through April 3, 2018 (NM, ACL, NH, IR, EP, JS). We did not intend to do a systematic review of the literature. No inclusion or exclusion criteria were used. Instead, ~~we selected~~ articles were selected ~~that were most relevant~~based on relevance to the subheadings used in this review as well as each vaccine candidate or mAb in clinical development. ~~To supplement the data collected and the identified gaps in knowledge,~~Furthermore, data for this review were systematically collected using ~~a~~the data collection template [Supplemental Table 1] at the RSV Vaccines for the World conference organized by the Respiratory Syncytial Virus Network (ReSViNET) from November 29 - December 1, 2017 in Malaga, Spain. The goal of this meeting was to share scientific data and expertise on RSV vaccine development, and to connect stakeholders involved in RSV research. During the meeting information was collected (NM, ACL, NH, IR, EP, JS) from scientific presentations, posters and personal communications.

We included all vaccine candidates and mAbs in clinical development according to the PATH RSV ~~Vaccine~~ vaccine ~~snapshot~~ and mAb ~~snapshot~~Snapshot. Vaccines were divided into four major groups: particle-based, vector-based, live-attenuated/chimeric and subunit vaccines. Immunoprophylaxis with mAbs was included as a fifth category.

RSV Vaccine History

RSV vaccine development started shortly after the first identification of the virus in humans in 1957(22). However, ERD upon natural RSV infection after vaccination with a formalin-inactivated RSV (FI-RSV) candidate in a series of trials in the 1960s severely hindered inactivated virus and subunit vaccine development for many years. In the youngest age ~~cohort of RSV naïve infants,~~group, 20 of 31 of RSV naïve infants were infected with community-acquired wild-type RSV during the next RSV season and 16 (80%) required hospitalization including two deaths(4) in whom ERD was documented. ~~ERD occurred in RSV-naïve infants who experienced infection with community-acquired wild-type RSV following receipt of FI-RSV.~~ Decades of research have revealed that ~~in these~~priming with FI-RSV vaccine ~~primed infants, natural RSV infection~~ triggered a strong but non-neutralizing antibody response(23), followed by a T-helper 2 (Th2) skewed immunologic response(24) which led to ERD upon natural RSV infection. Other aspects of the immune response implicated in ERD include distinct subsets of CD4 T-cells(25) and memory CD8 T-cells(26). The failure to mount a protective cytotoxic T-lymphocyte (CTL) response was coupled with excess lung eosinophilia and neutrophilia, monocytic infiltration, and immune complex deposition in the lungs(27).

Nevertheless, work continued on development and human testing of live-attenuated RSV vaccine candidates. In the following 60 years, only two products were licensed for prevention of RSV~~.~~: The first product was RSV intravenous

134 immunoglobulin (RSV-IVIG), a polyclonal immunoglobulin preparation with high
135 titers of anti-RSV neutralizing activity , that was approved in the United StatesUS
136 and Canada and discontinued after 2003. when RSV-IVIG was replaced by the
137 second approved product palivizumab, a humanized mAb directed against the RSV F
138 glycoprotein(28,29). Since its initial approval in 1998, palivizumab remains the only
139 licensed preventive intervention against RSV after demonstrating a reduction of
140 39% to almost 80% reduction of RSV hospitalizations in preterm infants < 35 weeks
141 gestational age with and without chronic lung disease respectively(29). Palivizumab
142 has an excellent safety profile and is indicated for the prevention of severe RSV ALRI
143 in children born prematurely, with congenital heart disease, for with chronic lung
144 disease(30).
145   MotivazumabMotavizumab, a higher affinity variant of palivizumab, was
146 developed was developed in early 2000 but was withdrawn discontinued in
147 2010(31). In a non-inferiority head-to-head comparative trial designed to show
148 non-inferiority to palivizumab, motavizumab recipients had a slightly higher
149 frequency of mild skin reactions following administration when compared to
150 palivizumab(32). However, in a placebo-controlled trial, motavizumab was highly
151 efficacious against inpatient and outpatient RSV LRI in healthy term American
152 Indian infants (33). Without Nevertheless, without evidence of superiority
153 compared to palivizumab, for protection from RSV-related hospitalization, evidence
154 of slightly higher side effects, and no plan for dose reduction or cost-saving, the
155 product did not attain regulatory approval(34,35). However, in a placebo-controlled
156 trial, motavizumab was highly efficacious against inpatient and outpatient RSV LRI
157 in healthy term American Indian infants (35).
158   With respect to vaccines for active immunization, many approaches targeted
159 for RSV naïve children were evaluated pre-clinically over the years. Only LLive-
160 attenuated vaccine candidates were considered safe for clinical evaluation in these
161 children because these vaccines are not expected to cause ERD(36). Over the past 40
162 years, several biologically derived live-attenuated vaccine candidates with
163 attenuating temperature sensitivity or cold-passage mutations were evaluated
164 clinically, including in the pediatric population, but the appropriate balance of
165 attenuation and immunogenicity, suitable for RSV-naive children and infants,
166 remained elusive. After reverse genetics techniques became available in the 1990s,
167 it became possible to design vaccines candidates with the appropriate level of
168 attenuation, but with increased immunogenicity(37). While pediatric live-
169 attenuated RSV vaccine candidates were under continued evaluation since the
170 1970s there were relatively few trials of RSV subunit vaccines conducted before
171 2000, with the exception of the purified F protein (PFP) vaccines(38,39), and an RSV
172 fusion (F) attachment (G), and matrix (M) subunit vaccine(40).
173   Over the past 10 years development of preventive interventions for RSV has
174 rapidly expanded. Currently, 19 vaccine candidates and mAbs for different target
175 populations are in clinical trials, and many more are in preclinical development(21).
176
177 Lessons from the vaccine and mAb graveyard

178 While vaccine development has accelerated, there have been three recent late-phase
179 vaccine and mAb trial failures. It is important to distil lessons learned from these
180 results to inform future vaccine development.
181 1. A phase III double-blind, placebo-controlled trial (NURSERY) evaluating
182 REGN2222 (suptavumab), a mAb against antigenic site V on the RSV pre-F
183 protein, a major target for high-potency mAbs(41) was conducted at 250 sites
184 in 19 countries. REGN2222 was administered once or twice during the
185 respiratory season in to 1,149 healthy preterm infants < 6 months of age with
186 a gestational age ≤35 weeks who were not eligible to receive palivizumab
187 prophylaxis. and The trial did not meet its primary efficacy endpoint to
188 prevent medically-attended RSV infections through day 150 of life(42).
189 REGN2222 was accelerated from phase I to phase III due to promising results
190 and the United StatesUS Food and Drug Administration (FDA) granted Fast
191 Track designation in October 2015. A speculation for this failure may be
192 inadequate dosing schedule in regard to the antibody half-life. The Ultimately,
193 the basis for failing to meet the primary clinical endpoint is not known, as
194 analyses of this late-stage failure have not yet been made public.
195 2. The second candidate that failed to meet the predefined study endpoint in
196 phase III clinical trials was the RSV F nanoparticle vaccine candidate for older
197 adults, a candidate based on aggregates of full-length post-F. The results of the
198 preceding phase II RSV F nanoparticle trial suggested the candidate vaccine
199 might haveshowed modest efficacy(43) and promising immunogenicity
200 measures, as determined by rise in geometric mean titer for IgG antibodies
201 against the F protein and palivizumab competing antibodies (PCA), in the
202 phase II trial(44). In the phase III trial, 11,58506 subjects ≥60 years of age
203 were enrolled in 60 US sites in a double-blind placebo-controlled trial
204 (RESOLVE) over a single season starting November 2015 with 330 182 days
205 follow-up for the efficacy outcome. The trial was granted fast track designation
206 by the FDA in 2016. (45). Although the vaccine showed promising results in
207 phase II and comparable immunogenicity measures in the two phases as
208 determined by neutralizing and palivizumab competing antibody induction,
209 However, the vaccine candidate failed to show efficacy against RSV moderate–
210 severe lower respiratory tract disease (ms-LRTD) in phase III results(9).
211 Compared to the previous season, RSV acute respiratory disease (RSV-ARD)
212 and ms-LRTD attack rates were lower than expected in the 2015 – 2016
213 season (RSV-ARD: 2.0% versus 4.9% and RSV-msLRTD 0.4% versus 1.8%
214 during the vaccine and previous season respectively). The pharmaceutical
215 companyvaccine manufacturer speculates that the difference in vaccine
216 efficacy observed may in part be due to thise lower attack rate as well as high
217 pre-existing immunity in the study population(43). Another proposed
218 explanation for failure of this vaccine candidate is that the quantity of the
219 immune response to vaccination may not represent effective immunity. For
220 example, PCA titers may not correspond to effective immunity as non-
221 neutralizing antibodies can also bind the palivizumab binding site and can
222 interfere with the binding of neutralizing antibodies(46). In a post-hoc
223 subgroup analysis, the vaccine candidate showed efficacy against

224 hospitalizations for all-cause chronic obstructive pulmonary disease (COPD)
225 exacerbations ~~from all causes~~(43). Upon further analysis of the phase III
226 results, there was a non-statistically significant trend towards higher RSV
227 microneutralization titers in adults without RSV-ARD when compared to
228 adults with RSV-ARD, but this difference was not statistically significant. One
229 conclusion that can be drawn from this trial is that late-phase clinical research
230 for ~~an~~ RSV vaccine candidates should include evaluation across more than one
231 RSV season.
232 3. Development of the MEDI-7510 vaccine candidate, a subunit vaccine candidate
233 for older adults, was discontinued after a phase IIb trial in North America,
234 Europe, South Africa, and Chile. The vaccine candidate was evaluated in 1900
235 adults ≥60 years ~~after~~ and the study failed to meet its primary objective,
236 efficacy against RSV-associated respiratory illness between 14 days post
237 vaccination throughout the end of the surveillance period, approximately 7
238 months. MEDI-7510 was a subunit vaccine using soluble (unaggregated)
239 postfusion (post-F) conformation of the F protein with a TLR4 agonist
240 adjuvant. ~~that~~ The vaccine candidate showed safety and immunogenicity with
241 ~~elevated~~ increased B and T cell responses in the vaccine ~~group~~ compared to
242 the placebo group in a phase I clinical trial~~s~~(47) after safety and improved
243 immunogenicity with an adjuvant was demonstrated in a first-in-human
244 trial(48). The incidence of RSV-associated ~~ARI~~ respiratory illness as diagnosed
245 by PCR was 1.7% and 1.6% in the vaccine and placebo groups respectively, for
246 a vaccine efficacy (VE) of -7.1(47). No efficacy was found in secondary subset
247 analyses. On day 29, 93% of vaccinees had an anti-F IgG antibody
248 seroresponse and there was a 4.6 geometric mean fold rise in anti-F IgG titer at
249 the end of the RSV season in vaccine recipients compared to the placebo
250 group(47). One proposed explanation for the negative results may be that the
251 choice of a post-F antigen induced antibodies without appropriate epitope
252 specificity(49). Upon further analysis, other proposed explanations include a
253 low incidence of lab~~oratory~~-confirmed RSV in the study population, or ~~a~~
254 selection of the study population, which included high-risk and low-risk older
255 adults. Considerations for the future include selection of an older study
256 population at higher risk of RSV infection.
257
258 Vaccine antigens
259 Vaccine antigens included in RSV vaccine candidates are diverse. The majority of
260 vaccines in clinical trials (11/18) ~~utilize~~ use the F protein, a class I viral fusion
261 protein, as an antigenic target. The RSV F protein is highly conserved and facilitates
262 viral fusion with host cells. ~~An u~~Understanding ~~of~~ the structural differences between
263 pre-F and post-F conformations, as well as stabilization of the pre-F soluble forms,
264 has resulted in advance~~ment~~ in vaccine antigen design(19,50). Current vaccine
265 candidate~~s~~ use pre-F and post-F as vaccine antigens [Table 1]. Of note, the
266 predominant conformation displayed on the FI-RSV vaccine candidate was the post-
267 F conformation(51). ~~There is no consensus on the~~It remains unclear as to whether
268 there is a trigger for the pre-F to post-F conformational change, but it does occur
269 spontaneously, making it difficult to ensure that a wild-type F vaccine antigen

270 maintains a pre-F conformation. However, ~~but~~ stabilizing mutations have been
271 identified that can preserve the pre-F-specific epitopes(50,52). The antigenicity of
272 some stabilized pre-F constructs has not been rigorously investigated, and it
273 remains an open question as to whether certain stabilizing mutations affect the
274 conformation of antibody binding sites Assays to assess antigen conformation are
275 needed. Likewise there is no consensus on cellular receptors that determine viral
276 tropism(53).
277   Other less frequent~~ly~~ ~~utilized~~ vaccine antigens, used alone or in combination
278 with other antigens ~~in vaccine candidates~~, include the RSV envelope associated
279 glycoproteins G (1/18) and small hydrophobic (SH) protein ~~(SH)~~ (1/18) as well as
280 internal proteins: nucleocapsid (N) (3/18), M (1/18), and M2-1 (1/18). ~~Other~~
281 ~~than~~Besides the F protein, the G protein is the only other target for neutralizing
282 antibodies on the viral surface. The G protein is most important for viral attachment
283 ~~of RSV~~ and is less frequently utilized as a vaccine antigen due to high variability
284 across RSV strains(54), and limited knowledge of its surface structure(55). The G
285 protein exists as an oligomer on the surface of RSV particles and as a monomer
286 when secreted from infected cells ~~as a~~in soluble form(56). There is evidence that the
287 soluble form of the G protein can act as a decoy that helps the virus evade the
288 antibody response(57). Another possible vaccine target, the SH protein, is not well
289 understood, but ~~has been observed~~data suggest that it ~~to~~ plays a role in viral
290 replication *in vivo*(53) and inflammasome activation(58). The SH protein contains a
291 transmembrane and extracellular domains(59); the latter has been used as a
292 vaccine antigen(60). Internal proteins are particularly relevant to induce T cell-
293 mediated immunity(55). As such, three non-membrane RSV proteins have been
294 included in RSV vaccine design. The N protein is the major nucleocapsid protein that
295 encapsidates the RNA genome of the virus(61). The M2-1 and M2-2 proteins are
296 specific to RSV and other *Pneumoviridae*. M2-1 is ~~an~~ essential ~~protein~~ for viral
297 transcription (62), and ~~deletion of~~ M2-2 deletion is utilized in live vaccine
298 candidates for viral attenuation. Finally, the M protein is a membrane-associated
299 protein ~~which is important for formation of the viral envelope~~that gives virions their
300 filamentous shape(63,64). In summary, different viral proteins are being employed
301 as antigens in RSV vaccine design. Viral surface glycoproteins such as F and G are
302 known to induce antibodies with differing neutralization capacity. The SH protein
303 may be important for induction of antibody dependent cell-mediated cytotoxicity
304 (ADCC), whereas non-membrane proteins are especially important to induce a
305 robust T-cell response(55).
306
307 Target populations
308 RSV prophylactic interventions are designed to protect at least two populations
309 most vulnerable to severe RSV disease: RSV-naïve young infants and children, and
310 older adults, although other ~~important~~ high-risk populations are important to
311 consider. It is estimated that 45% of hospital admissions and in-hospital deaths due
312 to RSV-ALRI occur in infants younger than 6 months of age(1), an age at which
313 vaccines are generally less immunogenic. Older adults and adults with chronic
314 cardiopulmonary conditions have emerged as an important target for RSV

315 prevention due to an increased understanding of RSV burden in this population. An
316 overview of all RSV vaccine candidates per target population is shown in Table 2.
317   Maternal vaccination is utilized to provide passive immunity to young infants
318 by boosting maternal vaccine-specific antibody titers that are actively transferred
319 through the placenta~~to the infant~~, thereby extending the period of protection
320 conferred by maternal antibodies. Historically, epidemiologic studies have
321 demonstrated an association between higher maternal RSV antibody concentrations
322 and protection from ~~lower respiratory tract infection~~ALRI in infants(65). Passive
323 transfer of antibodies to infants has been shown to be protective against severe RSV
324 infection through the administration of high-titer polyclonal and monoclonal
325 antibodies (RSV-IVIG and palivizumab) (28,29). The duration of protection of
326 maternal vaccination is defined by the antibody half-life. Administration of mAbs is
327 an alternative form of passive vaccination that can circumvent this hurdle due to
328 extended antibody half-life through Fc alterations(66). The proof-of-principle of
329 maternal vaccination as a tool to prevent infant disease has been demonstrated by
330 the effective near-elimination of maternal and neonatal tetanus worldwide through
331 tetanus toxoid vaccination in pregnancy(67). Maternal vaccination may also play a
332 role in ~~prevention of~~preventing RSV infection in pregnant women and ~~prevention of~~
333 adverse birth outcomes, however data on the burden of RSV disease in pregnant
334 women and the effect of RSV infection during pregnancy on the fetus is limited(68–
335 71)
336   Premature infants, a population at high risk for severe RSV disease, may be
337 insufficiently protected by maternal vaccination given that the majority of IgG
338 transport ~~of IgG~~ occurs after 32 weeks gestational age(72). Globally 10% of children
339 are born preterm(73). The burden is especially relevant in low and middle-income
340 countries (LMICs) as more than 60% of preterm birth occurs in Sub-Saharan Africa
341 and South Asia(74). Thus, a maternal vaccination strategy may not be sufficient to
342 protect the high-risk preterm population if administered during the third trimester
343 of pregnancy. Tetanus-–diphtheria-–acellular pertussis (Tdap) immunization in the
344 second trimester is associated with higher cord-blood antibody titers ~~by time of~~
345 ~~birth~~ as compared to third trimester immunization(75). A strategy of earlier
346 vaccination could be considered for maternal RSV immunization to maximize
347 protection for~~to~~ preterm infants. Other populations in which impaired
348 transplacental antibody transfer may limit protection by maternal vaccination
349 include infants of mothers with chronic infection, hypergammaglobulinaemia,
350 malaria, and HIV infection(76). The ratio of transplacental antibody transfer and
351 antibody decay kinetics are currently considered the main parameters to assess
352 protection conferred via maternal vaccination. However, protection may also be
353 mediated by breast milk antibodies transferred postnatally.
354   A combined strategy that utilizes ~~maternal~~ passive ~~vaccination~~ immunization
355 to protect young infants, via maternal vaccination or mAbs, ~~–~~followed by pediatric
356 active ~~vaccination~~ immunization may be effective to prevent severe RSV infection in
357 young children(77). Th~~e combined~~is strategy is estimated to avert at least twice as
358 many admissions per 100 births and four times as many in-hospital deaths per 1000
359 births than maternal vaccination alone(77). ~~A combined~~This strategy will be
360 particularly relevant to prevent morbidity and mortality in children with

361 comorbidities who are at risk of severe RSV disease at older ages (78,79). A similar
362 maternal and pediatric combined passive and active immunization strategy is
363 currently employed for pertussis and influenza vaccination(76).
364   Although RSV is frequently considered a pediatric pathogen, it is important
365 to consider the older adult population with regard to prevention of severe RSV
366 disease. RSV has been identified as an important disease in older and high-risk
367 adults, with a disease burden similar to that of influenza(3). It is estimated that RSV
368 accounts for 10,000 – 14,000 deaths annually in adults over the age of 65 years in
369 the ~~United States~~US(2,3). In addition, older adults with comorbidities such as
370 underlying heart or lung disease are at elevated risk of severe RSV disease; 4-10% of
371 high-risk adults will develop acute RSV infection annually(3).
372
373 Immunologic endpoints
374 Antibodies are thought to be the key players in limiting RSV ALRI as evidenced by
375 proven protection in immunoprophylaxis trials in children (28,29,33). Recent
376 evidence from experimental human infection in adults shows a protective role for
377 nasal RSV-specific IgA against RSV infection(6), underscoring the importance of
378 mucosal immunity. A limited ability to generate memory IgA responses after RSV
379 infection may be in part responsible for incomplete immunity and subsequent RSV
380 re-infection. Antibodies directed against different antigenic sites of the F protein
381 display different neutralization capacities with the most neutralization-sensitive
382 epitopes exclusive to the pre-F conformation. Antibodies with specificity for
383 antigenic sites Ø and V show high neutralizing activity and are exclusive to the pre-F
384 conformation(41,80). Antigenic site Ø is located at the apex of the pre-F
385 conformation, the most variable region of the highly conserved F protein(19).
386 Antibodies against antigenic site III prefer the pre-F conformation and exhibit high
387 neutralizing activity(81). Antibodies directed against site II and IV, present on both
388 pre-F and post-F, exhibit medium to high neutralization potency(80,82). Finally,
389 antibodies against antigenic site I, present primarily on post-F, show weak or no
390 neutralization. Escape mutants of these antigenic sites have been identified, but
391 global RSV genetic data are needed to assess the molecular heterogeneity of RSV
392 and the subsequent susceptibility or resistance to mAbs targeting RSV among
393 circulating viruses.
394   The mechanisms of protection may differ according to ~~the type of
395 vaccine~~vaccine type, and therefore, many different immunologic assays are
396 employed in clinical trials. Neutralizing activity of serum is a frequent immunologic
397 endpoint of vaccine trials. A measure of functional antibody response can be
398 elucidated by the ratio of fold-increase in RSV-binding antibodies to fold-increase in
399 RSV-neutralizing antibodies (ELISA-to-neutralization response ratio). A ratio of <1
400 may be an important correlate of protection(83). Furthermore, rather than a
401 definitive protective threshold for antibodies, fold-rise in antibody titer may be a
402 relevant correlate of protection for live-attenuated vaccines, since that may be the
403 best indicator of B- cell priming. Recent efforts by PATH, the WHO, and the National
404 Institute for Biological Standards and Control (NIBSC) examined the variability of
405 RSV neutralization assays across laboratories and recommended steps for improved
406 standardization globally(84), resulting in the development of a new WHO

16

International Standard for Antiserum to RSV with 1000 International Units of RSV subtype A neutralizing activity per vial now available through the NIBSC(85). ~~For other~~Standardization of other frequently used immunologic assays such as ~~palivizumab-competing antibodies (~~PCA~~)~~, ELISA and T-_cell assays ~~such standardization~~ has not yet taken place.

Once infection of the lower airways is established, CD8 T-_cells play an important role in viral clearance(86). Th2-biased responses have been associated with animal models of RSV ERD and measurement of Th1 and Th2 responses are considered important to predict safety of vaccine candidates other than live-attenuated vaccines in clinical trials in young children.

Animal models are important for preclinical development of vaccine candidates and assessing the possibility of enhanced disease. Alveolitis in the cotton rat and priming of a Th2 response in mice are considered markers to assess possible ERD; there is no consensus on the ability to reproduce ERD in calves(87).

Although we discuss several potential immunological correlates of protection for vaccine trials, we considered cell-mediated immunity beyond the scope of the manuscript. However, w~~W~~e highlight the different aspects of the expected immune response for all 19 vaccine candidates and mAbs in clinical development in Table 3. A definitive threshold for protection against RSV disease remains elusive. So far no vaccine candidates have been tested in the experimental human infection model, but the model provides a unique opportunity to test vaccine candidates in the natural host despite practical and ethical challenges(88).Ultimately, the outcome of large-scale vaccine trials will inform which immunologic measures correspond to protection from clinical RSV disease.

Vaccine strategies

We have divided vaccines in clinical development into four categories in accordance with the PATH RSV vaccine and mAb snapshot: particle-based, vector-based, subunit and live-attenuated/chimeric vaccines(21). We have also included mAbs in clinical development for the prevention of RSV ALRI. In the snapshot there are 43 vaccines and 4 mAbs in development of which 19 are in clinical stage development. An important consideration for all vaccines is not only to prevent severe RSV disease, but also to avoid the risk of priming for RSV ERD. Based on our current understanding of the underlying mechanisms leading to RSV ERD, ~~this would suggest~~ caution should be taken in the use of protein-based vaccines in RSV naïve ~~infants and children~~individuals. Replication deficient vectors, engineered to induce CD8 T cell responses expressing RSV antigens intracellularly, are considered more similar to live-attenuated virus vaccines which have been shown ~~to be safe~~not to cause ERD in this population. In Table 1 we provide a comprehensive overview and more detailed comparison of all characteristics of the 19 vaccine candidates and mAbs in clinical development.

Particle-based vaccines

The RSV F nanoparticle-based vaccine platform is currently being evaluated for protection of three target populations: (1) infants through maternal vaccination, (2) children between 6 months and 5 years, and (3) older adults. These vaccine

candidates utilize aggregates of a modified stabilized F protein which exhibits the post-F morphology(89). The maternal RSV F nanoparticle vaccine candidate is farthest along in clinical development and the PREPARE trial has entered the third year of a phase III trial to enroll up to 8,618 pregnant women at 80 sites in 11 countries(43). In January 2018 an informational analysis of the phase III trial was announced in which the vaccine candidate successfully targeted an efficacy threshold against the primary endpoint in infants at day 90 of >40%(90). Second in clinical development is the RSV F nanoparticle vaccine for older adults. Despite lack of efficacy in a phase III trial (RESOLVE) with a non-adjuvanted vaccine candidate, development was continued in a phase II roll-over study initiated in January 2017 in Australia in 300 adults. The aim of this rollover trial is to determine whether 2 dose regimens with an adjuvant (Matrix-M, a saponin-based adjuvant, or aluminum-phosphate) may increase the magnitude and quality of the immune response in this population. The results from the RESOLVE trial in older adults suggested vaccine efficacy in adults with COPD, leading to considerations to initiate a future trial in this older adult population at high risk for severe RSV infection(43). Finally, the phase I trial was completed in young children 24-72 months of age in 2016, but no data have been published yet(91).

SynGEM is a particle-based needle-free vaccine candidate containing the RSV F protein attached to empty bacterial particles made from *Lactococcus lactis*. The In this vaccine platform an in which an antigen is presented by a bacterial particle. An influenza vaccine candidate in clinical trials which uses the same vaccine platform, has shown both local and systemic antibody responses for the influenza candidate in clinical trials using the same platform(92) but needs further optimization for RSV vaccination. The preliminary results of immunogenicity testing have been reported. The immunogenicity of this vaccine was evaluated after delivery as a nasal spray to healthy adult volunteers. Two intranasal doses of SynGEM were administered 28 days apart at low or high dose in 24 subjects per group (6 subjects in each group receiving placebo, double blinded). Assays of serum virus neutralization, RSV F-specific antibodies, palivizumab competing antibodiesPCA, and F-specific IgA indicated some immunogenicity, but the results did not reach the threshold set for continuation to viral challenge and the studies were suspended in 2017 (Openshaw and Chiu, personal communication).

Vector-based vaccines
There are five vector-based vaccines in clinical development. The first uses a modified vaccinia virus Ankara (MVA), a replication-defective smallpox viral vector, and the remaining four vaccine candidates employ an adenovirus vector to display viral antigens. The MVA vector has been safely used in vaccines for other infectious diseases(93). This vaccine candidate, MVA-BN-RSV, induces both a humoral and cell-mediated responses by displaying four vaccine antigens: F, G, N and M2-1. Phase II results in healthy older adults from this candidate will soon be announced.

The second vector-based vaccine candidate, VXA-RSV-f, uses an innovative platform with an adenovirus 5 based oral tablet delivery platform that is stable at room temperature. Using the same oral adenovirus vaccine delivery platform, a phase I trial for influenza has been conducted, which showed neutralizing antibody

responses against influenza and no interference of pre-existing vector immunity(94). ~~The results from P~~preclinical studies for the RSV vaccine candidate in the cotton rat model showed ~~that mucosal immunization with the oral vaccine candidate enhanced mucosal IgA in the upper airways~~an increase in anti-F antibodies and protection against RSV challenge(95). ~~Given that severe disease i~~In the older adult population ~~is thought to be mediated by~~ immunosenescence may be characterized by impaired T-cell response~~s~~ to RSV(96,97). ~~T~~this vaccine candidate~~,~~ which induces a humoral response~~,~~ may be a promising intervention in this population. ~~for the older adult population(98).~~

Third and fourth, Ad26.RSV.preF, is a vaccine candidate being developed for two populations: the older adult and the pediatric population. In this~~The~~ candidate ~~uses~~ pre-F antigen is expressed in the human adenovirus strain 26, a vector with a favorable safety profile when used for other infectious diseases(98,99). Previously, the vaccine candidate vector expressed post-F as antigen (FA2) but has now been changed to ~~the~~ stabilized pre-F conformation ~~as vaccine antigen~~. The stabilized pre-F protein has 5 amino acid changes from wild-type, and is stable at 4C and heat-stable(50). With the expectation that this vaccine candidate will induce highly neutralizing antibodies against pre-F, phase II trials will be ~~initiated~~ conducted in RSV-seropositive children. In December 2017 a phase II trial ~~has been~~was initiated comparing concomitant administration of ~~influenza vaccination with~~ RSV vaccine and seasonal influenza vaccine versus seasonal influenza vaccine alone ~~co-administration~~ in healthy older adults(100).

Fifth, ChAd155-RSV, a replication-incompetent chimpanzee adenovirus 155 has been used as a vector for the F, N and M2.1 proteins. The anticipated use for this pediatric vaccine is to start immunization at two months of age, and to use two ~~to three~~ doses alongside the normal pediatric vaccination schedule, instead of seasonally(101). This vaccine candidate is currently being evaluated in 12-23 month old RSV seropositive children. In the future, there are plans to conduct clinical trials in seronegative children sequentially from older to younger ages (12-24 months followed by 6-12 months and subsequently 2-6 months of age) to ensure safety in RSV-naïve populations. Results of phase II trials are expected to be announced in 2020.

In summary, vector-based vaccines are used to display various RSV viral proteins and three of these vaccine candidates are in phase II trials.

Subunit vaccines

Due to concerns of ERD associated with protein-based vaccines, subunit vaccines are only in development for pregnant women and older adult populations. One subunit vaccine in development is the GSK RSV F vaccine candidate, which uses a version of soluble secreted F protein empirically engineered to maintain the Pre-F conformation. Phase I results demonstrated safety and immunogenicity as evidenced by RSV neutralizing antibody response in healthy men(102). However, a phase II trial scheduled for 2017 was halted due to instability of the pre-F antigen during manufacturing.

Structure-guided stabilization of the pre-F conformation has yielded a subunit vaccine candidate, DS-Cav1. The stabilization includes a foldon

trimer~~ization domain~~, the introduction of cysteine residues to form a disulfide bond, and cavity-filling hydrophobic residues(52). The vaccine is able to preserve neutralization-sensitive epitopes on a functional pre-F form of the viral surface protein. In preclinical studies the subunit vaccine induced high levels of RSV-neutralizing antibodies in mice and non-human primates(52). Preliminary results from the phase I trial, VRC 317, are promising and ~~will soon be published.~~ are expected to be published soon.

DPX-RSV is a vaccine candidate with a unique choice of vaccine antigen~~;~~ the extracellular domain of the SH protein of RSV(60). The DepoVax technology allows for a prolonged exposure of antigen and adjuvant, and aims to induce ADCC using a liposome and oil-based depot(103). The antigen and adjuvant are encapsulated in a liposome, lyophilized and suspended in oil and the process is expected to produce vaccines with long shelf-life stability(104). Phase I results on safety and immunogenicity in the older adult population ~~will soon~~have been released and are expected to be ~~be~~ published from ~~this~~an investigator-initiated study.

Live-attenuated and chimeric vaccines
In the context of historical concerns for enhanced RSV disease, live-attenuated vaccines can be considered safe for RSV naïve infants, based on consistent clinical study results showing that these candidates do not prime for ERD following subsequent exposure to wild-type RSV after vaccination(105). Another benefit of live-attenuated vaccines against RSV in ~~the pediatric population~~ young infants is their ability to ~~generate an immune response~~replicate in the respiratory tract despite the presence of maternally-acquired antibodies, and to elicit a ~~more~~ broad ~~antibody~~ humoral and cellular response(106). Live-attenuated vaccines are likely limited to the pediatric population under two years of age, as pre-existing immunity in older populations might not permit sufficient replication to generate protective immune responses. Safety could be a concern for intranasal live-attenuated vaccines, in particular if attenuation is insufficient. However, evaluation of current vaccines has not shown evidence of increased rates of vaccine-associated ALRI or fever, though there may be increased rates of rhinorrhea, similar to what has been observed with the live-attenuated influenza vaccines.

Five live-attenuated vaccine candidates in phase I clinical trials are being developed in partnership with the National Institutes of Health ~~(NIH)~~. Live-attenuated vaccines face the challenge of achieving sufficient attenuation to be safe while remaining immunogenic enough to induce a protective immune response. ~~but~~ An improved understanding of the RSV viral genome has informed the development of new vaccine candidates that may overcome this challenge. Two main modifications to the RSV genome have been engineered through reverse genetics: the ΔM2-2 deletion which attenuates viral replication and upregulates antigen expression(37) as well as the ΔNS2 deletion which reduces viral suppression of host interferon thereby boosting the innate immune response. RSV MEDI ΔM2-2 ~~strongly~~ reduced viral replication while inducing a strong primary serum neutralizing antibody as well as potent anamnestic response in RSV-seronegative infants and children(37). Further results from phase I clinical trials with the other live-attenuated vaccine candidates are expected.

20

591   The only chimeric vaccine candidate, rBCG-N-hRSV, currently in clinical
592 development is delivered via a BCG strain. BCG has a safe profile in newborns and
593 infants, induces a Th1 response(107,108), and allows for combined vaccination
594 against two major respiratory pathogens: *Mycobacterium tuberculosis* and RSV. Not
595 only is the Th1 cellular response important in protecting against lung pathology,
596 inflammation and viral replication(109) but the candidate also induces a humoral
597 response. The antigen presented by this vaccine candidate is the RSV N protein(110).
598 ~~Importantly~~Presently, this candidate is the only vaccine candidate intended for
599 administration to newborn infants(110).
600
601 Monoclonal antibodies
602 A promising highly potent monoclonal antibody has emerged as a passive
603 administration strategy to prevent severe RSV infection. MEDI8897, also known as
604 nirsevimab, was optimized from the human antibody D25 that targets antigenic site
605 Ø on the pre-F conformation, which is more neutralization sensitive than the
606 palivizumab epitope, antigenic site II. Using the YTE technology ~~for extending~~which
607 extends antibody half-life as well as modulates ADCC(111), the three-fold increase
608 in half-life of MEDI8897(112) compared to palivizumab offers the possibility of
609 passive protection for all infants for an entire season through a single intramuscular
610 injection. The intended use is for both term and preterm infants entering their first
611 RSV season. Passive vaccination with an extended half-life antibody offers an
612 approach to protecting infants that is safe and may be reasonably priced.
613 Representatives of the pharmaceutical company have indicated that they expect
614 vaccine-like pricing ~~of~~ for MEDI8897. Given the increased potency, the extended
615 half-life, and the required dose, it is expected that the cost to protect an infant
616 during the RSV season can be kept relatively low(66).
617
618 Other approaches not in clinical development
619 Other emerging approaches not yet in clinical development include nucleic acid-
620 based vaccines(113). Importantly these vaccines induce a T-~~-~~cell response
621 mimicking the response to live virus infection. Both DNA and messenger RNA
622 (mRNA) vaccines against RSV have shown promising results in preclinical
623 studies(113). Notably, through a collaboration with the Bill & Melinda Gates
624 Foundation, an mRNA technology vaccine platform for HIV and rotavirus has also
625 expanded to include RSV. Another vaccine approach in preclinical development is a
626 whole-inactivated vaccine to be delivered intranasally via a nanoemulsion
627 technology, for which development has been supported by the Bill & Melinda Gates
628 Foundation(114). Furthermore, with the first of the palivizumab patents expiring in
629 October 2015 and the last in 2022, there has been active development to produce a
630 biosimilar in order to provide a low-cost RSV preventive intervention.~~-~~.
631
632 Considerations by regulatory agencies and the World Health Organization
633 The FDA has articulated that differences between high income countries (HICs) and
634 LMICs are not particularly relevant to regulatory decisions, though a bridging study
635 in the US must be performed if all clinical trials have been performed outside of the
636 US(115). The EMA does not require that trials intended to support a regulatory

637 decision are conducted in the European Union. Other considerations in population
638 selection for vaccine trials mentioned by EMA include: first testing a vaccine
639 candidate in a seropositive before testing in a seronegative population, testing a
640 maternal vaccine in non-pregnant women of child-bearing age before testing in
641 pregnant women, and including older adults with comorbidities in vaccine trials. No
642 particular considerations were mentioned for population selection in studies for
643 mAbs. In October 2017 the EMA released draft guidelines for the clinical evaluation
644 of RSV prophylactic interventions which included guidance regarding trial design,
645 assessment of efficacy, and safety(116). The draft guidelines will be revised after a
646 period of public consultation based on comments and new publications.
647   The WHO has recognized the importance of RSV as a global health problem
648 and has identified the development of RSV vaccines as a priority for the WHO
649 Initiative for Vaccine Research and for Biological Standardization. WHO recently
650 developed RSV vaccines preferred product characteristics and research and
651 development technical roadmap documents(117,118). Further guidance for
652 development will contribute to adequate policy-making. WHO standardization
653 activities led to the development and establishment of the first international
654 standard for antiserum to RSV. Development of guidelines for evaluation of quality,
655 safety and efficacy of RSV vaccines has been initiated and will be part of consultation
656 with regulators, manufacturers and academia in 2018 with the aim of finalizing it in
657 2019. Further discussion on guiding principles for mAbs is needed before
658 proceeding with the development of the WHO Guidelines. These and other WHO
659 standards serve as a basis for setting national regulatory requirements as well as
660 WHO prequalification.
661   Finally, the WHO is now performing a surveillance pilot study in 14 countries
662 to test the feasibility of using the Global Influenza Surveillance and Response System
663 platform for RSV surveillance and it is expected that this pilot will contribute to our
664 understanding of the RSV disease burden and seasonality in different geographical
665 regions(119).
666
667 <u>Discussion</u>
668 Challenges in RSV vaccine design include concerns of ERD post-vaccination, lack of
669 definitive immunologic correlates of protection, lack of consensus regarding clinical
670 endpoints, and limited natural immunity following RSV infection. Despite these
671 challenges, recent developments such as an understanding of the structural biology
672 of the RSV fusion protein as well as lessons learned from late-phase vaccine trial
673 failures have informed the field as it moves forward.
674   We attempted to collect data regarding expected plans for access to a
675 preventive intervention in LMICs and expected pricing for all vaccine candidates,
676 however this information iwas not publicly available. The only information obtained
677 regarding expected pricing was for MEDI8897, though a more specific estimate than
678 vaccine-like pricing was not available. Given that the most severe RSV infection
679 occurs in LMICs(17), information regarding LMIC target countries and potential
680 pricing for vaccine candidates will be essential to facilitate access to vaccines
681 worldwide, especially in areas where the mortality burden is highest. In LMICs the
682 most important target for vaccine candidates is young children(120). A mechanism

22

683 should be introduced to ensure that information regarding expected pricing and
684 access to interventions is transparent and available in the public domain. RSV
685 vaccines and mAbs will be considered in the development of the Vaccine Investment
686 Strategy ~~(VIS)~~ by GAVI, the Vaccine Alliance in 2018(121).
687   A vaccine trial may be considered a probe study to determine whether a
688 causal relationship exists between RSV infection and asthma, a longstanding
689 question in the field. If long-term follow-up had been undertaken during the pivotal
690 RSV prevention trials using palivizumab, these trials would now have provided 20
691 years of follow-up on respiratory morbidity after RSV prevention in high-risk
692 infants. Lack of long-term surveillance for airway morbidity in vaccine trials are
693 missed opportunities to provide novel scientific insights important not only to
694 understand the ~~pathophysiology~~ pathogenesis but also the long-term vaccine
695 efficacy against airway morbidity following RSV infection. In addition to wheeze,
696 objective outcomes, such as lung function measurements including demonstration
697 of bronchial hyperreactivity and IgE measurements will ideally be incorporated in
698 vaccine trials to fully understand the impact of RSV prevention on asthma
699 development.
700   Viral interference, in which RSV inhibits infection by other viruses, is
701 becoming an increasingly important concept to understand in the context of an
702 approved RSV vaccine. RSV vaccination may conceivably result in an increased ~~or~~
703 ~~decreased~~ prevalence of other respiratory viruses. There is evidence supporting
704 viral interference for influenza vaccination(122,123), for RSV prevention(124,125),
705 and during the RSV season in the absence of RSV (126). It is important for vaccine
706 trials to examine this phenomenon by evaluating the incidence of all-cause ALRI, as
707 well as RSV-specific ALRI, to better understand the implications of viral interference
708 for an RSV vaccine.
709   This review provides an extensive overview of the 19 vaccine candidates and
710 mAbs in clinical trials to prevent RSV infection. RSV vaccine development is moving
711 rapidly and shows promise to address an unmet global health problem. Vaccines for
712 various target populations are in clinical development. One vaccine candidate and
713 one mAb are in late phase trials (IIb/III) and aim to prevent the disease burden in
714 young infants. Despite some recent failures, RSV vaccine candidates and mAbs, in
715 clinical development hold the promise that a preventive intervention for RSV is on
716 the horizon.
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Tables/Figures
Table 1: Overview of RSV vaccines and mAbs in clinical development

Vaccine	Company/ Sponsor	Manufacturing Process	Antigen	Adjuvant	Mechanism of Action	Target Population	Route of Administration	Clinical Phase	Animal Models	Phase I	Phase II	Phase III	Result Summary
PARTICLE-BASED													
RSV F Nanoparticle	Novavax	Sf9/BV recombinant technology	Stabilized F protein exhibiting post-F morphology	Aluminum phosphate	F forms nanoparticle in multimeric micelle format	M	IM	III	Cotton rats (127,128), baboons(129) Guinea pigs(130)	Dec 2010-Dec 2011 NCT01290419 (n=150)	Oct 2012 – May 2013 NCT01704365 (n=330) Oct 2013 – April 2014 NCT01960686 (n=720) Sep 2014 – Jul 2016 NCT02247726 (n=50)	Dec 2015-Jun 2020 NCT02624947 (n=8618)	PhII: all formulations well-tolerated and immunogenic; most robust Ab response with 120ug and 0.4mg aluminum formulation, peak d14 and persistence through d91; RSV infection measured by Western blot was reduced by 52% (p=0.009) in healthy women of childbearing age (n=720)(131,132) Vaccine safe, immunogenic and reduced RSV infection in healthy women of childbearing age (n=330)(133)
RSV F Nanoparticle	Novavax	Sf9/BV recombinant technology	Stabilized F protein exhibiting post-F morphology	Aluminum phosphate & Matrix M	F forms nanoparticle in multimeric micelle format	O	IM	II	Cotton rats (127,128), baboons(129)	Oct 2012-Mar 2014 NCT01709019 (n=220)(134)	Oct 2014 – Mar 2016 NCT02266628 (n=1599) Oct 2015 – Nov 2016 NCT02593071 (n=1330) Rollover: Jan 2017 – Jul 2018 NCT03026348 (n=1329)	Nov 2015 – Dec 2016 NCT02608502 (n=11850)	PhII: safe, VE: 41% against RSV-ARD, 64% VE against RSV-msLRTD(135) PhIII: safe, no efficacy v. RSV-ARD &RSV-msLRTD;; post-hoc efficacy v all-cause hospitalization (n=11850) PhII rollover: no residual protection in 2nd year; second immunization protective against RSV-ARD and msLRTD (n=1329)(131,136)
RSV F Nanoparticle	Novavax	Sf9/BV recombinant technology	Stabilized F protein exhibiting post-F morphology	Aluminum phosphate/ Matrix M-1	F forms nanoparticle in multimeric micelle format	P	IM	I	Cotton rats, (127,128) baboons (129)	Nov 2014 – Apr 2016 NCT02296463 (n=32)	N/A	N/A	PhI: well-tolerates;; Anti-F IgG & PCA increase d14, Peak d28, elevated to d56; 10-fold increase PCA & anti-F IgA adjuvanted 6-fold increase in unadjuvanted(135) (n=32)
SynGEM	Mucosis	Bacterium-like-particle (BLP) mimopath technology carrying F proteins	F protein, unclear which conformation	BLP	BLP allows presentation of F protein and elicits mucosal IgA	O & P	IN	I	Mice	July 2016 – Dec 2017 NCT02958540 (n=48)	N/A	N/A	PhI: some immunogenicity in healthy adults but did not meet threshold; development suspended.
VECTOR-BASED													
MVA-BN RSV	Bavarian Nordic	MVA-BN technology (antigens expressed in attenuated modified vaccinia Ankara)	F, G (subtype A & B), N, M2	none	Virus replication blocked at a late stage	O	IM/IN	II	Cotton rats, BALB/c mice(137)	IM: Aug 2015-May 2016 NCT02419391 (n=63) IN: Sep 2018 – Aug 2019 NCT02864628	Sep 2016 – Aug 2018 NCT02873286 (n=400)	N/A	PhI; safe, 2x increase IgG & IgA; 3-5x increase In T cell responses (n=63)(137) PhII interim results: well-tolerated; broad Ab & T cell response in older adults after single vaccination (n=421) (138)

										(n=96)			
VXA-RSVf oral	Vaxart	antigen and adjuvant expressed in non-replicating adenovirus vector (Ad5)	F	dsDNA that activates TLR3 receptor	Vector delivers directly to gut (ileum)	O	Oral	I	Cotton rat	Jun 2016-Dec 2017 NCT0283 0932 (n=66)	2018?	N/A	Preclinical: Systemic Anti-F Ab's and protection against RSV infectio in cotton rat model(95)
Ad26.RSV.preF	Janssen	Antigen expressed in human adenovirus type 26 produced in PER.C6 human cell line	Pre-F (previous ly FA2)	none	Ad26 vector is replication incompetent but expresses immunogenic F antigen	O	IM	II	Mice, cotton rats(139)	Nov 2016 – Dec 2018 NCT0292 6430 (n-73)	Dec 2017 – Jul 2018 NCT033397 13 (n=180)	N/A	PhI: well-tolerated; durable humoral and cellular immune response for FA2 candidate; comparable or higher for preF candidate in older adults (140)
Ad26.RSV.preF	Janssen	Antigen expressed in human adenovirus type 26 produced in PER.C6 human cell line	Pre-F (previous ly FA2)	none	Ad26 vector is replication incompetent and expresses immunogenic F antigen	P	IM	I	Mice, cotton rats(139)	Nov 2017-Mar 2019 NCT0330 3625 (n=60)	Nov 2017-Mar 2019 NCT033036 25 (n=60)	N/A	N/A
ChAd155-RSV	GSK	Chimpanzee adenovirus ChAd155-RSV with F, N, M2.1 insert and E1 deletion	F, N, M2.1	none	Intracellular RSV antigen expression; replication incompetent vector	P	IM	II	Mouse, cotton rat, calves (101)	Jul 2015 – Feb 2017 NCT0249 1463 (n=73)	Jan 2017 – Sep 2020 NCT029278 73 (n=96)	Plan to start post 2020 with age de-escalation seronegative infants	PhI: safe, B cell and RSV-neutralizing antibodies in RSV-seropositive adults (n=73)(141)
SUBUNIT													
GSK RSV F	GSK	Pre-F produced in CHO cells	Pre-F	With or without aluminum hydroxide	Pre-F antigen induces neutralizing antibodies which are transferred to infant	M	IM	II	Mice, cotton rats, guinea pigs, cows	Dec 2014 –Mar 2017 NCT0229 8179 (n=288) Jul 2013-Mar 2015 NCT0190 5215 (n=128) (102)	Mar 2015-June 2016 NCT023604 75 (n=507) Apr 2016 – Jun 2016 NCT027534 13 (n=102) Nov 2016 – Mar 2018 NCT029568 37 (n=406) Jul 2017 – Jan 2021 NCT031913 83 Halted due to instability of pre-F antigen during manufacturi ng	N/A	PhI: safe, RSV-A neutralizing Ab titers increased 3.2-4.9x; remained high to day 60, decreased on day 180 & 360 in healthy men (n=128)(102) Ph II: Increased RSV-A neutralizing Ab 30 days post-vaccination in healthy non-pregnant women (142)
DPX-RSV	Dalhousie University	DepovaxTM delivery in 100% oil-based platform preventing release at injection site	SHe	DepovaxTM or aluminum hydroxide	Depovax gives controlled and prolonged exposure of antigen and adjuvant	O	IM	I	Mice, cotton rats	May 2015-June 2017 NCT0247 2548 (n=40)	N/A	N/A	PhI: Well-tolerated, Antigen-specific Ab response durable to day 421, low immunogenicity with alum adjuvant in healthy older adults(143)
RSV F DS-Cav1	NIH/NIAID/V RC	Prefusion stabilitized trimeric RSVF	Pre-F	Alum/ TLR4 agonist (E)	Pre-F antigen elicits highly neutralizing	M & O	IM	I	Cotton rats, mice, calves(14	Feb 2017-Jan 2020 NCT0304	N/A	N/A	Preclinical: Induction of high neutralizing Abs and differential adjuvant-induced

		expressed in CHO cell line			antibodies against pre-F epitopes				4), macaques (52)	9488 (n=100)			enhancement(144) Immunization of mice and macaques induces RSV-neutralizing Ab many times Protective threshold(52)

LIVE-ATTENUATED/CHIMERIC

rBCG-N-hRSV	Pontificia Universidad Catolica de Chile	Recombinant BCG expressing N antigen	N	none	Paired BCG and RSV vaccine induces Th1 response	P	ID	I	Mice (107–109,145)	Jun 2017-May 2018 NCT0321 3405 (n=24)	N/A	N/A	Preclinical: Protective T cell immune response and recruitment of Th1 cells (107,108)
RSV D46 cpΔM2-2	Sanofi Pasteur/LID/N IAID/NIH	M2-2 deletion via reverse genetics and 5 aa substitutions in 3 proteins called the "cp" mutations, originally identified in a cold-passaged vaccine candidate cpRSV	native RSV	none	Deletion of regulatory factor M2.2 causes inefficient replication but high immunogenic ity, further attenuation with cp mutations	P	IN	I	African green monkeys	Oct 2015-May 2018 NCT0260 1612 (n=45)	N/A	N/A	N/A
RSV LID ΔM2-2 1030s	Sanofi Pasteur/LID/N IAID/NIH	M2-2 deletion via reverse genetics and temperature sensitivity mutation 1030s	native RSV	none	Deletion of regulator factor M2-2causes inefficient replication but high immunogenic ity; temperature sensitive mutation at position 1030 of L gene	P	IN	I	Mice, African green monkeys	Jun 2016-Jul 2017 NCT0279 4870, NCT0295 2339 (n=33)	N/A	N/A	N/A
RSV ΔNS2 Δ1313 I1314L	Sanofi Pasteur/LID/N IAID/NIH	NS2 and 1313 deletion via reverse geneticsm I1314L substation.	native RSV	none	NS2 deletion bolsters innate response. Deletion at position 1313 of L protein, and I1314L substation confers moderate temperature sensitivity	P	IN	I	Mice and chimpanz ees	Jun 2013-May 2017 NCT0189 3554 (n=75) Aug 2017-May 2019 NCT0322 7029 (n=80)	N/A	N/A	N/A
RSV D46/NS2/N ΔM2-2 HindIII	Sanofi Pasteur/LID/N IAID/NIH	LID backbone without deletions or substitutions in SH gene, point mutation in NS2 and N proteins, modified M2-2 deletion, based on RSV MEDI ΔM2-2.	native RSV	none	Deletion of regulatory factor M2.2 causes inefficient replication but high immunogenic ity	P	IN	I	African green monkeys	Mar 2017-April 2019 NCT0310 2034, NCT0309 9291 (n=33)	N/A	N/A	N/A
RSV LID cp ΔM2-2	Sanofi Pasteur/LID/N IAID/NIH	M2-2 deletion via reverse genetics, and cp mutation	native RSV	none	Deletion of regulatory factor M2.2 causes inefficient replication but high immunogenic	P	IN	I	African green monkeys	Sep 2016-Apr 2018 NCT0289 0381 (n=17)	N/A	N/A	N/A

					ity ; further attenuation with cp mutations								
MONOCLONAL ANTIBODY (mAb)													
MEDI8897	MedImmune	In vitro-optimized human mAb with YTE mutation in Fc	N/A	N/A	Antibody targeting site Ø of the F protein of RSV with an extended half-life	P	IV/IM	II	Cotton rats, cynomolgus monkeys (66)	Apr 2014- Jun 2015 NCT0211 4268 (n=342) Jan 2015- Sep 2016 NCT0229 0340 (n=151)	Nov 2016- Nov 2018 NCT028783 30 (n=1454)	N/A	PhI; well-tolerated,, Mean half-life 85-117d; time to max concentration 5-9 days; bioavailability 77% in healthy adults (n=136) (146)

Legend: N/A: not applicable or not available, IM: intramuscular, ID: intradermal, IN: intranasal, IV: intravenous; ARD: acute respiratory disease, PCA: palivizumab-competing antibodies, P: pediatric, M: maternal, O: older adults, SHe: small hydrophobic protein ectodomiain; RSV ARD: all symptomatic respiratory disease due to RSV; msLRTD: moderate-severe RSV-associated lower respiratory tract disease; NIAID: National Institutes of Allergy and Infectious Diseases; VRC: Vaccine Research Center; NIH: National Institute of Health, Ab: antibody, aa: amino acid.

Table 2 Overview of vaccines and mAbs by target population

Target Population	Vaccine	Vaccine type
Pregnant mothers		
Third trimester	RSV F nanoparticle (Novavax)	Nanoparticle
Third trimester	RSV F (GSK)	Subunit
	RSV F protein (NIH/NIAID/VRC)	Subunit
Pediatric		
6m-5y	RSV F nanoparticle (Novavax)	Nanoparticle
Start 2m	Adenovirus (GSK)	Vector
Start 2-3m	Adenovirus (Janssen)	Vector
	BCG/RSV (Pontificia Unversidad Catolica de Chile)	Chimeric
	RSV D46 cp ΔM2-2 (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
	RSV LID ΔM2-2 1030s (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
	RSV ΔNS2 Δ1313 I1314L (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
	RSV D46/NS2/ N/ΔM2-2-HindIII (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
	RSV LID cp ΔM2-2 (Sanofi Pasteur/LID/NIAID/NIH)	Live-attenuated
	MEDI8897 (MedImmune)	Monoclonal antibody
Older adults		
	RSV F nanoparticle (Novavax)	Nanoparticle
	RSV BLP (Mucosis)	Nanoparticle
	MVA (Bavarian Nordic)	Vector
	Adenovirus (Vaxart)	Vector
	Adenovirus (Janssen)	Vector
	DPX-RSV-SH Protein (Immunovaccine)	Subunit
	RSV F protein (NIH/NIAID/VRC)	Subunit

Legend: m: months; y: years

Table 3: Expected immune response and previous successes for vaccine candidates and monoclonal antibodies

Vaccine	Target Population	Pre-F Immunity* (86)	Immune response	Mucosal/Systemic
Nanoparticle				
RSV F Nanoparticle (Novavax)	M	Pre-F < post-F	Broadly neutralizing antibodies	systemic
RSV F Nanoparticle (Novavax)	O	Pre-F < post-F	Broadly neutralizing antibodies	systemic
RSV F Nanoparticle (Novavax)	P	Pre-F < post-F	Broadly neutralizing antibodies	systemic
RSV BLP (Mucosis)	O & P	unclear F confirmation	Activation of B & T cells; local secretion of neutralizing IgA in the nose; production of IgG neutralizing IgG in the blood	mucosal & systemic
Vector				
MVA (Bavarian Nordic)	O	Pre-F < post-F	B & T cell response; antibodies against 5 RSV antigens	systemic
Adenovirus (GSK)	O	Pre-F > post-F	B & T cell response; neutralizing antibodies against F antigen; CD8 T cells against F, N and M2.1 antigens	systemic
Adenovirus (Vaxart)	O	Pre-F < post-F	B & T cell immunity, protection at mucosal surface	mucosal > systemic
Adenovirus (Janssen)	P	Pre-F	B & T cells	systemic
Adenovirus (Janssen)	O	Pre-F	B & T cells	systemic
Subunit				
RSV F (GSK)	M	Pre-F	B & T cell response	systemic
DPX-RSV (Dalhousie University)	O	none		systemic
RSV F protein (NIH/NIAID/VRC)	O & M	Pre-F		systemic
Live-attenuated				
BCG/RSV s(Pontificia Universidad Catolica de Chile)	P	Pre-F & post-F	B & T cell response; Th1 polarized response; antibodies against N, F, G	systemic
RSV D46 cp ΔM2-2 (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F & post-F	B & T cell response; enhanced antibody production due to increased antigen production from M2-2 deletion	mucosal & systemic
RSV LID ΔM2-2 1030s (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F & post-F	B & T cell response; enhanced antibody production due to increased antigen production from M2-2 deletion	mucosal & systemic
RSV ΔNS2 Δ1313/I1314L (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F & post-F	B & T cell response	mucosal & systemic
RSV D46 ΔNS2 N ΔM2-2 HindIII	P	Pre-F & post-F	B & T cell response; enhanced antibody	mucosal & systemic

(Sanofi Pasteur/LID/NIAID/NIH)			production due to increased antigen production from M2-2 deletion	
RSV LID cp ΔM2-2 (Sanofi Pasteur/LID/NIAID/NIH)	P	Pre-F & post-F	B & T cell response; enhanced antibody production due to increased antigen production from M2-2 deletion	mucosal & systemic
Monoclonal Antibody				
MEDI8897 (MedImmune)	P	N/A	N/A	N/A

Legend: Pre-F: prefusion conformation of the RSV F protein; Post-F: postfusion conformation of the RSV F protein; N: RSV nucleocapsid protein; F: RSV fusion protein; G: RSV attachment protein; O: older adults; M: maternal; P: pediatric.

Figure 1: RSV global burden of disease in children under 5 years of age: key facts and figures

Figure 1 Incidence is shown worldwide for children under 5 years of age unless otherwise stated. The hospital admission rate of 15.9 hospital admissions per 1000 neonates per year is in developing countries. The RSV ALRI hospitalization 63.9 among premature infants <1 year is reported per 1000 children per year globally. Legend: OR: odds ratio; LRTI: lower respiratory tract infection, RSV: respiratory syncytial virus, HIC: high income country, *: compared to children who survived RSV hospitalization and were mechanically ventilated. References: (a)(1) (b)(78) (c)(147) (d)(148) (e)(149) (f)(150)

Figure 2: Overview of Vaccine candidates and monoclonal antibodies in clinical trials per preventive approach including candidates for which development was recently halted

LIVE-ATTENUATED / CHIMERIC

RSV D46/NS2/N/ΔM2-2-HindIII
PHI

R-BCG-N-hRSV
PHI

RSV LID ΔM2-2 1030s
PHI

RSV LID cpΔM2-2
PHI

RSV D46 cpΔM2-2
PHI

RSV cps2

MEDI-559

RSV ΔNS2Δ1313 I1314L
PHI

MONOCLONAL ANTIBODIES

REGN-2222

MEDI8897
PH II

PARTICLE-BASED

RSV F nanoparticle
PHI/ II/ III

SynGEM
DEVELOPMENT HALTED

VECTOR-BASED

RSV001

ChAd155-RSV
PH II

Ad26.RSV.preF
PHI/ II
HALTED

MVA-BN RSV
PH II

VXA-RSVf
PH I

MEDI-534

SUBUNIT

DS-Cav1
PHI

F-protein

DPX-RSV-SH
PHI

GSK RSV F
PHASE II HALTED

MEDI-7510

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LIVE-ATTENUATED / CHIMERIC

RSV D46/NS2/N/ΔM2-2-HindIII
PH I

RSV LID cpΔM2-2
PH I

MEDI-559

RSV BCG

R-BCG-N-hRSV
PH I

RSV LID ΔM2-2 1030s
PH I

RSV D46 cpΔM2-2
PH I

RSV ΔNS2Δ1313 I1314L
PH I

RSV cps2

MONOCLONAL ANTIBODIES

MEDI8897
PH II

PARTICLE-BASED

RSV F nanoparticle
PH I/ II/ III

SynGEM
DEVELOPMENT HALTED

VECTOR-BASED

RSV001

Adv26/5 ChAd155 MVA

ChAd155-RSV
PH II

MVA-BN RSV
PH II

Ad26.RSV.preF
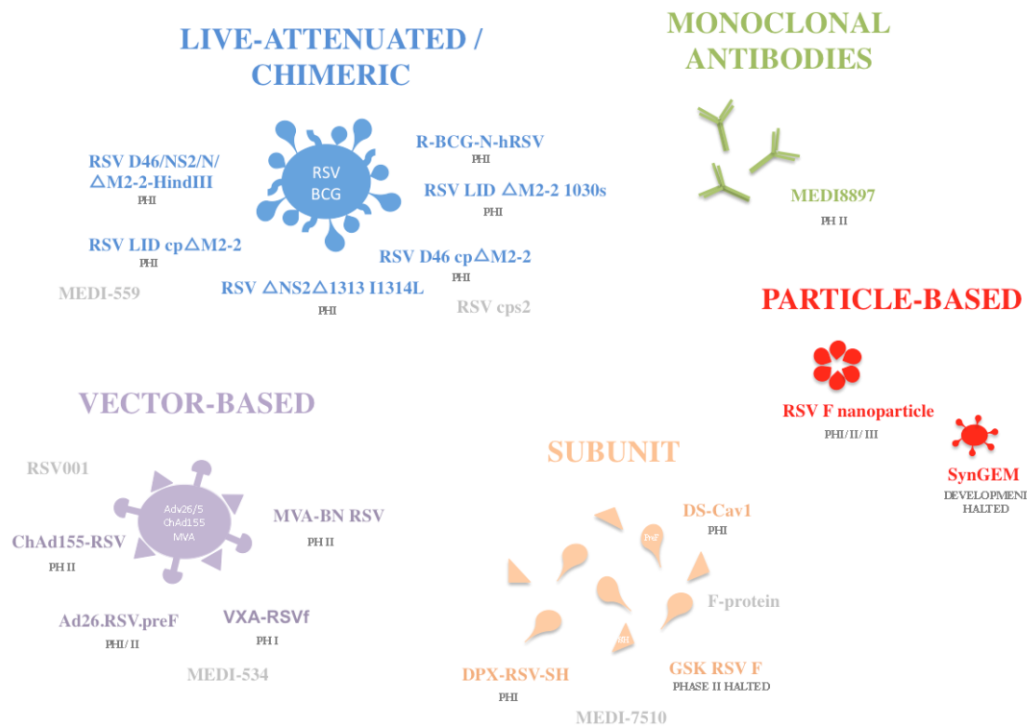PH I/ II

VXA-RSVf
PH I

MEDI-534

SUBUNIT

DS-Cav1
PH I

F-protein

DPX-RSV-SH
PH I

GSK RSV F
PHASE II HALTED

MEDI-7510

Legend: For vaccine candidate names listed in gray development has been halted since the last RSV therapeutics review performed in 2015(20). Abbreviations: PH I: phase I; PH II: phase II; PH III: phase III.

Supplemental Table 1: Data Collection Template

Type of Vaccine	Nanoparticle				Vector					Subunit			Live-attenuated/ Chimeric						Mabs/ biosimilars
PATH snapshot Candidate	RSV F nanoparticle (Novavax, M)	RSV F nanoparticle (Novavax, E)	RSV F nanoparticle (Novavax, P)	RSV BLP (Mucosis, E&P)	MVA (Bavarian Nordic, E)	Adenovirus (GSK, P)	Adenovirus (Vaxart, E)	Adenovirus (Janssen, E)	Adenovirus (Janssen, P)	RSV F (GSK, M)	DPX-RSV-SH Protein (Immunovaccine /VIB, E)	RSV F Protein (NIH/NIAID/VRC, M&E)	BCG/RSV (Pontificia Unversidad Catolica de Chile)	RSV D46 cp ΔM2-2 (Sanofi/LID/NIAID/NIH)	RSV LID ΔM2-2 1030s (Sanofi/LID/NIAID/NIH)	RSV ΔNS2 Δ1313 (Sanofi/LID/NIAID/NIH)	RSV D46/NS2/N/ΔM2-2-HindIII (Sanofi/LID/NIAID/NIH)	RSV LID cp ΔM2-2 (Sanofi/LID/NIAID/NIH)	MEDI8897
Person responsible																			
RSVVW related program items / names																			
Manufacturing process																			
Adjuvants																			
Animal models																			
Pre-F immunity																			

Immunity (general)																
Antigens																
Mechanism of action																
Mode of administration																
Target populations																
Results clinical studies so far																
Efficacy																
Endpoints																
PMID results																
Timing Ph1																
Timing Ph2																
Timing Ph3																
Current development status																

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Trial names																
Expected herd immunity																
Previous vaccine successes																
Description current trial(s), register																
Summary corporate website																
LMIC target																
Expected price																
Important Links																
Important Links																

Response to comments

Dear editor,

We would like to thank you for the extensive comments, which have given us the chance to clarify and improve the manuscript. The manuscript has been revised addressing each of the recommended changes made by reviewers point-by-point. We hope the length of the manuscript is acceptable as we cover a broad range of issues related to RSV vaccine development. Actually, the reviewers encouraged us to expand on a few topics. Should you decide it needs shortening, we would welcome any editorial suggestion.

Kind regards,
Natalie Mazur, also on behalf of Louis Bont

Editor's comments:

Comment 1 [General]: Please be aware that the limit for the word count is 4500 words.
Response: The current word count is 7,109 words. If the word count is absolute, we can consider moving part of the manuscript to supplemental materials. Please advise whether this is necessary.

Comment 2 [General]: Please be aware that the limit for the number of references in 150.
Response: We are aware of this limit and the manuscript currently contains 150 references.

Comment 3 [General]: Please include, at the end of the main text, a "Contributors" section detailing the role of each author in the preparation of your paper.
Response: We moved the "authors contributions" to the end of the main text and renamed it "contributors."

Comment 4 [General]: Please include, at the end of the main text, a "Conflicts of interest" statement summarising key conflicts from the ICMJE forms. The standard wording, if there are no conflicts, is "We declare that we have no conflicts of interest."
Response: We have summarized all relevant conflicts of interest in the manuscript using the conflict of interest forms sent in by all authors.
Revised text: LJB and NIM were involved in the design and plan for this review.
ACL and NH were involved in data collection.
All authors contributed to the final manuscript.

Comment 5 [General]: Please consider the possibility of having a study group name. When a paper includes a study group name in the byline, we're now required to supply a separate list of the group members in a specific format if we want these names to be shown on PubMed. (This is in addition to the list of names and affiliations required by the journal to be listed at the end of the paper or in the appendix.)
To ensure that the information we supply to PubMed is accurate and complete, please email me a list of the study group members whose names should appear on PubMed in

Word table format, as follows:

First names (will be abbreviated on Pubmed)Surnames (not abbreviated)

David Villa Sanchez

Jackie Henrietta   Davidson

This list will not be included in the paper itself - it's simply used to make sure that PubMed adds the names correctly. Please include the names of all study group members who need to be listed on PubMed. Names that do not need to be shown on PubMed do not need to be included.

Response: We have added "on behalf of ReSViNET" as a study group. Please find the list of names to be shown on PubMed as you suggest.

First names	Surnames
Natalie I	Mazur
Deborah	Higgins
Marta C	Nunes
José A.	Melero*
Annefleur C	Langedijk
Nicole	Horsley
Ursula J	Buchholz
Peter J	Openshaw
Jason S	McLellan
Janet A	Englund
Asuncion	Mejias
Ruth A	Karron
Eric AF	Simões
Ivana	Knezevic
Octavio	Ramilo
Pedro A	Piedra
Helen Y	Chu
Ann R	Falsey
Harish	Nair
Leyla	Kragten-Tabatabaie
Anne	Greenough
Eugenio	Baraldi
Nikolaos G	Papadopoulos
Johan	Vekemans
Fernando	Polack
Mair	Powell
Ashish	Satav
Edward E	Walsh
Renato T	Stein
Barney S	Graham
Louis J	Bont

José Melero has passed away since the original submission of the manuscript.

Comment 6 [General]: Please also include written consent of any cited individual(s) noted in acknowledgments or cited as personal communications.
Response: We have obtained written consent from all individuals listed in the acknowledgments section and as personal communications.

Comment 7 [General]: Reviews should include a brief section entitled "Search strategy and selection criteria" stating the sources (including databases, MeSH and free text search terms and filters, and reference lists from journals or books) of the material covered, and the criteria used to include or exclude studies. Citations to papers published in non-peer reviewed supplements are discouraged. Since these papers should be comprehensive, we encourage citation of publications in non-English languages.
Response: Search strategy and selection criteria
References for this review were identified through a search of PubMed for clinical trials with "syncytial" in the title published after January 1, 2013 with no language restrictions, through April 3, 2018. We did not intend to do a systematic review of the literature. No inclusion or exclusion criteria were used. Instead, we selected articles that were most relevant to the subheadings used in this review. The PATH RSV vaccine and mAb Snapshot was used as a reference to identify all vaccine and mAb candidates in clinical trials. ClinicalTrials.gov as well as the WHO vaccine pipeline tracker for RSV were used to identify all relevant trials for these vaccine candidates and mAbs. Additional data was collected during the RSV Vaccines for the World Conference on November 29-December 1, 2017 and through pharmaceutical websites for the respective vaccine and mAb candidates.

Comment 8 [General]: Please ensure that you provide your figures in an editable format and as separate files. For trial profiles a word file made of editable text boxes is the preferred format. For any statistical images (histograms, survival or time-to-even curves, line graphs, scatter graphs, forest plots, etc) you should provide editable vector files (ie, the original artwork generated by the statistical package used to make the image). Our preferred formats for these files are .ai, .eps, or .pdf. We cannot guarantee accurate reproduction of images without these files.
Response: We have sent all files in the correct format.

General editorial points that apply to all pieces in the Green section (please check each point):

Comment 9 [General]: Please see the end of this email for a list of signed statements from authors and people named in your paper that we will need before we can consider your paper further. Please scan and upload signed author statements and ICMJE conflict of interest forms for all authors with your revised submission.
Response: We have obtained all signed statements from authors and added them to the submission.

Comment 10 [General]: You will need to provide permission from the relevant publisher to include any material previously published that you wish to include in your paper.
Response: We understand this but have not reproduced any material for which we need permission.

Comment 11 [General]: Please provide: one preferred degree qualification per author and indicate any full professors; affiliation details (department, institute, city, state, country) for each author; full institutional correspondence address for corresponding author.
Response: We have provided a preferred degree for each author as well as affiliation details. We have marked full professors in the manuscript with an asterisk (*).

Comment 12 [General]: Please include, at the end of the main text, a "Contributors" section detailing the role of each author in the preparation of your paper.
Response: Please see comment 3 above

Comment 13 [General]: Please include, at the end of the main text, a "Conflicts of interest" statement summarising key conflicts from the ICMJE forms. The standard wording, if there are no conflicts is "We declare that we have no conflicts of interest."
Response: Please see comment 4 above.

Comment 14 [General]: For Reviews, Personal Views, Historical Reviews, and Grand Rounds please supply a 150-200 word unstructured summary of your manuscript. References should not be cited in the summary.
Response: We have added some information to the abstract (as summary) so it is now 160 words.
Revised text: The global burden of disease caused by respiratory syncytial virus (RSV) is increasingly recognized, not only in infants, but also in older adults. Advances in knowledge of the structural biology of the RSV surface fusion (F) glycoprotein have revolutionized RSV vaccine development by providing a new target for preventive interventions. The RSV vaccine landscape has rapidly expanded to include 19 vaccine candidates, including four approaches: (1)particle-based, (2)live-attenuated/chimeric, (3)subunit, (4)vector-based, as well as monoclonal antibodies (mAbs) in clinical trials, reflecting the urgency of reducing this global health problem and hence the prioritization of RSV vaccine development. Late phase RSV vaccine trial failures highlight gaps in knowledge regarding immunologic protection and provide lessons for future development. In this review we highlight promising new approaches to RSV vaccine design and provide a comprehensive overview of RSV vaccine candidates and mAbs currently in clinical development to prevent one of the most common and severe infectious diseases in young children and older adults worldwide.

Comment 15 [General]: Images that have been published previously should be accompanied by a statement indicating permission to reproduce the image. If required, further assistance can be obtained from the editorial team. If you have borrowed published images from colleagues, you must obtain permission from the publisher of the paper, not just from the authors. If all the figures are your own and have not been published before then this requirement does not apply.
Response: We do not have any relevant images or figures.

Comment 16 [General]: Figure titles should be a maximum of 30 words.
Response: We have kept our figure titles <30 words.

Comment 17 [General]: References should be in the Vancouver style and numbered in the order in which they first appear in the manuscript. References in figures, panels, and tables should be numbered in sequence with the references in the text where that figure, panel, or table is cited. Please ensure tables and figures are cited correctly in the body text to prevent the need for renumbering of references should the table and figure citations subsequently move.
Response: We have kept references in Vancouver style and adhered to other guidelines regarding references as mentioned above.

Comment 18 [General]: For papers listed in references that are "in press" we need to see a galley proof and letter from the publisher stating that it is 'in press' as well as the full expected citation (ie, publication date/volume/issue etc).
Response: We have included no references in press.

Comment 19 [General]: Please ensure that references are not inserted as Footnotes.
Response: We have no references as footnotes.

Comment 20 [General]: For Reviews, Historical Reviews, and Grand Rounds, please supply a section entitled "Search strategy and selection criteria". This should state clearly the sources (databases, journals, or book reference lists, etc) of the material covered and the criteria used to include or exclude studies. Please state which search terms, languages and date ranges were used.
Response: Please see comment 7 above.

Comment 21 [General]: Please include the signatures of any people whose names were on a previous version of this manuscript as an author but have now been deleted, or reclassified as an acknowledgment. Ex-authors should declare that they have agreed to have their names deleted or reclassified.
Response: There has been no change of authors since the last version of the manuscript. One author (Dr. José Melero) has passed away since the previous submission. Is it possible to add a posthumous note in the manuscript?

Comment 22 [General]: If you have added to or changed the order of existing authors, we require signed statements from ALL authors that they are happy with these changes.
Response: There is no change to author order since the previous manuscript.

Comment 23 [General]: Guidelines on electronic submission of text and figures are available at: http://ees.elsevier.com/thelancetid/. Please read these carefully; to ensure efficient prpreparation for publication, the text and figures should conform to these guidelines.
Response: We have adhered to your guidelines.

Comment 24 [General]: All authors are required to provide a Conflict of Interest Statement and should complete a standard form, which is available at http://download.thelancet.com/flatcontentassets/authors/icmje-coi-form.pdf. This form can be uploaded with the manuscript at submission. The form has been modified by the ICMJE following consultation with authors and editors. Further information is available in a joint ICMJE statement published on July 1, 2010. For more information see Lancet 2009; 374: 1395-96.
In summary, the signed statements we require are:
* Signed conflict of interest statements for ALL authors
Response: We have made sure that all authors have submitted conflict of interest forms.

Comment 25 [General]: All authors should complete and sign the author statement form and upload the signed copy. The form can be downloaded from the page (http://www.thelancet.com/lancet-infectious-diseases-information-for-authors/statements-permissions-signatures#conflicts-of-interest) from the fourth line of "Authors and contributions". The corresponding author must countersign manually the forms at the bottom of the page and send us the scanned version; electronic signatures are not accepted.
In summary, the signed statements we require are:
* Authors' contributions - signed by yourself and your co-authors indicating that you have all seen and approved the paper
Response: We have made sure that all authors have author statement forms, which have been countersigned by the corresponding author.

Reviewers' comments:

Reviewer #1:

Comment 1 [General]: The manuscript entitled "The Respiratory Syncytial Virus Vaccine Landscape" is an comprehensive review of vaccine and mABs candidates in clinical trials to prevent hRSV infection. They review from lessons of the failure of the first vaccines, explaining the different vaccines undergoing clinical trials that are reported according to PATH, the antigens use for vaccines against RSV and the target populations. The article describes the preventive strategies under clinical development addressing the problem caused by the RSV infection globally. It intends to provide updated information about the prophylactic and active immunization strategies currently under evaluation to prevent RSV infection. The manuscript includes bibliographic information, communications performed in the last RSV meeting in 2017 and information made public through several websites, such as clinicaltrials.gov. It also discussed strategies that have failed to meet clinical endpoints with the aim of providing information that should be considered for the current strategies under evaluation and future candidates moving to clinical evaluation. The manuscript is well organized and meets

the aim to provide the complete landscape of RSV vaccines and antibodies under clinical evaluation. However, there are several corrections that the authors need to perform before the manuscript be accepted for publication.

Response: Many thanks for thoroughly revising the manuscript. We have done our best to address all concerns mentioned.

Major comments:

Comment 2 [Reference]: Lack of references to support some statements throughout the text (For example, lines 108, 113, 153, 156, 280, 396, 430, 455, 463, 500, 520, 578, 600).

Referring to: 108, Motivazumab, a higher affinity variant of palivizumab, was developed in early 2000 but was withdrawn in 2010
Response: We have added a reference for this statement.
Revised text:
Mazur NI, van Delden JJ, Bont LJ. Respiratory syncytial virus trials and beyond. Lancet Infect Dis. 2015 Dec;15(12):1363–5.

Referring to: 113, Without evidence of superiority for protection from RSV-related hospitalization, evidence of slightly higher side effects, and no plan for dose reduction or cost-saving, the product did not attain regulatory approval.
Response: We have added a reference for this statement.
Revised text:
1. Carbonell-Estrany X, Simoes EAF, Dagan R, Hall CB, Harris B, Hultquist M, et al. Motavizumab for Prophylaxis of Respiratory Syncytial Virus in High-Risk Children: A Noninferiority Trial. Pediatrics. 2010 Jan 1;125(1):e35–51.
2. Feltes TF, Sondheimer HM, Tulloh RMR, Harris BS, Jensen KM, Losonsky GA, et al. A Randomized Controlled Trial of Motavizumab Versus Palivizumab for the Prophylaxis of Serious Respiratory Syncytial Virus Disease in Children With Hemodynamically Significant Congenital Heart Disease. Pediatr Res. 2011 Aug;70(2):186–91.

Referring to: 153, The results of the preceding phase II RSV F nanoparticle trial suggested the candidate vaccine might have modest efficacy.
Response: We have added a reference for this statement.
Revised text:
Novavax. Positive Topline Data from Phase 2 Older Adult Trial and Path Forward for RSV F Vaccine Programs [Internet]. Investor Slide Deck. 2017 [cited 2018 Jan 16]. Available from: http://novavax.com/download/files/presentation/Novavax_RSV_Analyst_Day_7-24-17_PDF2.pdf

Referring to: 156, In the phase III trial, 11,586 subjects ≥60 years of age were enrolled in 60 US sites in a double-blind placebo-controlled trial (RESOLVE) over a single season starting November 2015 with 330 days follow-up.
Response: We have added a reference for this statement.
Revised text:

ClinicalTrials.gov. A Study to Evaluate the Efficacy of an RSV F Vaccine in Older Adults [Internet]. NIH: US National Library of Medicine. 2017 [cited 2018 Mar 26]. Available from: https://clinicaltrials.gov/ct2/show/study/NCT02608502?show_locs=Y

Referring to: 280, A combined strategy that utilizes maternal vaccination to protect young infants followed by pediatric vaccination may be effective to prevent severe RSV infection in young children.
Response: We have added a reference for this statement.
Revised text:
Cromer D, van Hoek AJ, Newall AT, Pollard AJ, Jit M. 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. Elsevier; 2017 Aug 1;2(8):e367–74.

Referring to: 396, Assays of serum virus neutralization, RSV F-specific antibodies, palivizumab-competing antibodies and F-specific IgA indicated some immunogenicity, but the results did not reach the threshold set for continuation to viral challenge and the studies were suspended in 2017 (Openshaw and Chiu, personal communication).
Response: Unfortunately there is no published material to support this statement but we feel it is valuable to include. We have received written consent from both authors for this personal communication to publish these results.

Referring to: 430, The anticipated use for this pediatric vaccine is to start immunization at two months of age, and to use two to three doses alongside the normal pediatric vaccination schedule, instead of seasonally. This vaccine candidate is currently being evaluated in 12-23 month old RSV seropositive children.
Response: We have added a reference for this statement.
Revised text:
Dieussaert I. GSK's Pediatric RSV Vaccine Program. In: Presentation at Food and Drug Administration (FDA) 150th Meeting of the Vaccines and Related Biological Products Advisory Committee Meeting (VRBPAC). Silver Spring; 2017.

Referring to: 455, Preliminary results from the phase I trial, VRC 317, are promising and will soon be published.
Response: As this is also unpublished, we have softened the statement, as we cannot provide published sources to support it. However, we expect these results to be published based on their release at the RSV Vaccines meeting.
Revised text: Preliminary results from the phase I trial, VRC 317, are promising and are expected to be published soon.

Referring to: 463, Phase I results on safety and immunogenicity in the older adult population will soon be published from an investigator-initiated study.
Response: There is no published citation to support this statement. Since the phase I study has been completed and the data presented in a poster at the RSV Vaccines for the World meeting in Malaga, we expect that the data will soon be published. We have changed the phrasing to say that we expect a publication of this data soon. We hope this is acceptable.

Revised text: Phase I results on safety and immunogenicity in the older adult population have been released and are expected to be published from this investigator-initiated study.

Referring to: 500, Importantly, this candidate is the only vaccine candidate intended for administration to newborn infants.
Response: We have added a reference for this statement.
Revised text:
Rey-Jurado E, Soto J, Gálvez N, Kalergis AM. A safe and efficient BCG vectored vaccine to prevent the disease caused by the human Respiratory Syncytial Virus. Hum Vaccin Immunother. Taylor & Francis; 2017 Sep 2;13(9):2092–7.

Referring to: 520, Other emerging approaches not yet in clinical development include nucleic acid-based vaccines.
Response: We have added a reference for this statement.
Revised text:
Smith TRF, Schultheis K, Broderick KE. Nucleic acid-based vaccines targeting respiratory syncytial virus: Delivering the goods. Hum Vaccin Immunother. 2017 Nov 2;13(11):2626–9.

Referring to: 578, Given that the most severe RSV infection occurs in LMICs, information regarding LMIC target countries and potential pricing for vaccine candidates will be essential to facilitate access to vaccines worldwide, especially in areas where the mortality burden is highest.
Response: We have added a reference for this statement.
Revised text:
Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi S a, Singleton RJ, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet. Elsevier Ltd; 2010 May 1;375(9725):1545–55.

Referring to: 600,Viral interference, in which RSV inhibits infection by other viruses, is becoming an increasingly important concept to understand in the context of an approved RSV vaccine. RSV vaccination may conceivably result in an increased or decreased prevalence of other respiratory viruses.
Response: This sentence is used to introduce the following sentences, which mention evidence of viral interference after influenza vaccination, after RSV prevention and cross-sectionally in the absence of RSV during the RSV season. We have cited 5 peer-reviewed articles to support that viral interference exists and our interpretation is that this is an important concept in the context of RSV vaccination and could conceivable result in increased prevalence of other viruses.

Comment 3 [Reference]: References should be original articles or review but not oral communications (Lines 455 and 396)
Referring to:
396, The preliminary results of immunogenicity testing have been reported. The immunogenicity of this vaccine was evaluated after delivery as a nasal spray to healthy adult volunteers. Two intranasal doses of SynGEM were administered 28 days apart at low or high dose in 24 subjects per group (6 subjects in each group receiving placebo, double blinded). Assays of serum RSV F-specific antibodies, palivizumab-competing antibodies and F-specific IgA indicated some

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immunogenicity, but the results did not reach the threshold set for continuation to viral challenge and the studies were suspended in 2017 (Openshaw and Chiu, personal communication).

455, Preliminary results from the phase I trial, VRC 317, are promising and will soon be published (Graham, personal communication).
Response: Unfortunately there is no published material to support this statement but we feel it is valuable to include. We have received written consent from both authors for this personal communication to publish these results.

Comment 4 [Other approaches]: The section "Other approaches not in clinical development" is too short and is not taking into account all the vaccine and Ab candidates that are reported under preclinical development in the PATH report (Line 520).
Response: The aim of the manuscript is to give a detailed overview of RSV vaccine candidates in clinical development. We have decided to mention other promising approaches to RSV vaccine development, however this is not an exhaustive coverage of all candidates in preclinical development as this is outside of the scope of the manuscript. We have mentioned only approaches that are not mentioned earlier in the rest of the manuscript to give a sense of other new approaches to developing an RSV vaccine: namely nucleic-acid based vaccines and biosimilars. The only approach we have not added that is not mentioned elsewhere is whole-inactivated vaccines but this is not a "new approach" which is why we had not mentioned it. However, we have now added whole-inactivated vaccines to this section so that all vaccine approaches in clinical and pre-clinical development are covered.
Revised text: Finally, another vaccine approach in preclinical development is a whole-inactivated vaccine to be delivered intranasally via a nanoemulsion technology for which development has been supported by the Bill & Melinda Gates Foundation(99).

Comment 5 [Table 1]: Some data are cited on the table 1 but not throughout the text (For example Line 500).
Referring to: Importantly, this candidate is the only vaccine candidate intended for administration to newborn infants.
Response: We have added the citations from the table into the text for the rBCG-N-hRSV vaccine candidate. However, table 1 provides a comprehensive overview of all vaccine candidates while the text highlights key elements. The length of the review does not allow us to mention every element of the table in the text. Please let us know if there are specific elements you feel are underrepresented.
Revised text:
1. Bueno SM, Gonzalez PA, Cautivo KM, Mora JE, Leiva ED, Tobar HE, et al. Protective T cell immunity against respiratory syncytial virus is efficiently induced by recombinant BCG. Proc Natl Acad Sci. 2008 Dec 30;105(52):20822–7.
2. Cautivo KM, Bueno SM, Cortes CM, Wozniak A, Riedel CA, Kalergis AM. Efficient lung recruitment of respiratory syncytial virus-specific Th1 cells induced by recombinant bacillus Calmette-Guérin promotes virus clearance and protects from infection. J Immunol. 2010 Dec 15;185(12):7633–45.
3. Céspedes PF, Rey-Jurado E, Espinoza JA, Rivera CA, Canedo-Marroquín G, Bueno SM, et al. A single, low dose of a cGMP recombinant BCG vaccine elicits protective T cell immunity

against the human respiratory syncytial virus infection and prevents lung pathology in mice. Vaccine. 2017 Feb 1;35(5):757–66.

Comment 6 [Table 1]: The status for clinical evaluation is outdated for some of the vaccines described. For example the following trial was omitted NCT03213405 (from www.clinicaltrials.gov) for a vaccine described in these references that should be added: Vaccine. 2017 Feb 1;35(5):757-766. doi: 10.1016/j.vaccine.2016.12.048. Proc Natl Acad Sci U S A. 2008 Dec 30;105(52):20822-7. doi: 10.1073/pnas.0806244105.
Response: The trial you mention was already in the table and the correct phase has been listed for this vaccine candidate (phase I). Both publications mentioned have also already been cited both in the text and in the table (please refer to comment 4 above, we have added these citations to the text as they were already in the table. The phases we list for all trials are up to date according to the PATH vaccine snapshot.

Comment 7 [General]: Authors failed to cite some recent papers on RSV vaccines and should include: Vaccine. 2017 Jan 11;35(3):489-495. doi: 10.1016/j.vaccine.2016.08.086. Vaccine. 2017 Jan 11;35(3):496-502. doi: 10.1016/j.vaccine.2016.09.026. Vaccine. 2016 May 27;34(25):2847-54. doi: 10.1016/j.vaccine.2016.04.002.
Controlled human infection with RSV: The opportunities of experimental challenge.
Response: Many thanks for these suggestions. We have added a sentence in immunologic endpoints regarding the opportunities and challenges with controlled human infection as well as the reference you mention:
So far no vaccine candidates have been tested with experimental human infection model, but the model provides a unique opportunity to test vaccine candidates in the natural host despite practical and ethical challenges(81).
We have also added the first-in-human trial for MEDI7510:
The vaccine candidate showed safety and immunogenicity with elevated B and T cell responses in the vaccine group compared to the placebo group in phase I clinical trials(43) after safety and improved immunogenicity with an adjuvant was demonstrated in a first-in-human trial(44).
The last reference you mention is a review of novel antibodies for the prevention and treatment of RSV. We have used this as a resource to cross-reference our own manuscript but have not cited this reference in the text as no information used primarily came from this manuscript.

Comment 8 [Introduction, Methods, RSV Vaccine History]: Some parts of the manuscript required edition for writing, gramma and spelling. For instance, from pages 5 to 8, most of the sentences are too long and some of them are not clear: line 12 "young infants passive immunization". The sentence is not clear. Should be "young infants through passive immunization"?. Lines 34-36, the sentence is too long. Lines 50-54. Again, the sentence is too long, authors should either add colons or break the sentence in two.
Response: Thank you for bringing this to our attention. Line 12 was a typo and has been changed to read "young infants through passive immunization" as you suggest. We agree that the sentence for lines 34-36 was too long and not clear and have shortened and clarified it. We have also condensed the sentence in lines 50-54 and hope you will now find it reads more clearly.
Revised text:

Lines 34-36: Another challenge to RSV vaccine design is the lack of consensus regarding vaccine trial clinical endpoints though attempts have been made to define these for RSV prevention trials(14–16).
Referring to: Second, the discovery of the structure and stabilization of the prefusion (pre-F) conformation of the RSV F protein has advanced the field by showing that pre-F specific antibodies may be more potent in protecting against RSV LRTI than antibodies that also bind the postfusion (post-F) conformation and by thus providing a new target for vaccines and mAbs(21,22).
Lines 50-54: Second, the discovery and stabilization of the prefusion (pre-F) conformation of the RSV F protein provided a new target for vaccines and mAbs(21,22) as pre-F specific antibodies may be more potent than postfusion (post-F) antibodies in protecting against RSV LRTI.

Comment 9 [RSV Vaccine History]: Line 107. "Motivazumab" should be replaced for for "Motavizumab".
Response: We have changed this as suggested, thank you for pointing out this typo.

Comment 10 [RSV Vaccine History]: Line 125. For better fluidity and coherence, please add a brief comment about the clinical development of these live attenuated vaccines and a sentence indicating that additional information will be discussed in a following chapter.
Referring to: After reverse genetics techniques became available in the 1990s, it became possible to design vaccineswith the appropriate level of attenuation, but with increased immunogenicity(32).
Response: To prevent the manuscript becoming too lengthy we have left the paper as is. Please let us know if additions are required.

Comment 11 [RSV Vaccine History]: Lines 9-8 "The mortality attributable to RSV in adults > 65 year of age is estimated to be 7.2 per 100,000 person year". Authors should clarify whether this is a global estimate or refers to data from the US.
Response: We agree this needs to be clarified and have added that this is an estimate from the US.
Revised text: The mortality attributable to RSV in adults ≥65 years of age is estimated to be 7.2 per 100,000 person years in the United States(3).

Comment 12 [Introduction]: Lines 18-24. The information provided here should be moved to "RSV vaccine history", line 96, to avoid redundancy through the text.
Referring to: ERD occurred in RSV-naïve infants who experienced infection with community-acquired wild-type RSV following receipt of FI-RSV. Decades of research have revealed that in these FI-RSV primed infants, natural RSV infection triggered a strong but non-neutralizing antibody response(5), followed by a T helper 2 (Th2) skewed immunologic response(6). The failure to mount a protective cytotoxic T lymphocyte (CTL) response was coupled with excess lung eosinophilia and neutrophilia, monocytic infiltration, and immune complex deposition in the lungs(7).
Response: We agree and have moved this section to RSV vaccine history as suggested.

Comment 13 [Introduction]: Lines 385-387. These sentences are not clear, it seems that some information is missing.

Referring to: The platform in which an antigen is presented by a bacterial particle has shown both local and systemic antibody responses for the influenza candidate in clinical trials using the same platform(79)

Response: We agree that this sentence was not clearly worded. We hope you will find the revision acceptable.

Revised text: The influenza vaccine candidate in clinical trials which uses the same vaccine platform, has shown both local and systemic antibody responses(85).

Comment 14 [References]: Some of the references are not properly cited and should be amended:

Several the links for the websites cited in the manuscript are not working (Ref 11, 74). These should be amended.

11 http://www.ema.europa.eu/docs/en_GB/document_library/Other/2016/11/WC500216809.pdf
17 http://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=16/284

Referring to: 11

http://www.ema.europa.eu/docs/en_GB/document_library/Other/2016/11/WC500216809.pdf

Response: The link to this website works, could you please clarify your comment? We have cited everything according to Vancouver citation style as a website.

Referring to: 17

http://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=16/284

Response: We believe you are referring to citation 74 instead of 17. In this case, the link works as well so we have undertaken no action. The link goes to the website at which one can download the NIBSC Antiserum to RSV WHO 1st international standard instructions for use.

Comment 15 [General]: Authors should include a brief discussion about the advantages and disadvantages of the enhanced disease models currently available as a pre-clinical test required for candidates to advance into clinical trials.

Response: We agree that this would be a valuable addition to the manuscript and have added a brief discussion as suggested to the section on immunologic endpoints.

Revised text:

Animal models are important for preclinical development of vaccine candidates and assessing the possibility of enhanced disease. Alveolitis in the cotton rat and priming of a Th2 response in mice are considered markers to assess ERD; there is no consensus on the ability to reproduce ERD in calves(80). So far no vaccine candidates have been tested with experimental human infection model, but the model provides a unique opportunity to test vaccine candidates in the natural host despite practical and ethical challenges(81).

Reviewer #2:

Comment 1 [General]: This review article provides a good overview of the current state of the RSV vaccine field including an comprehensive overview of the vaccine candidates currently undergoing clinical trials. Overall, the review is well written and fairly comprehensive. It could benefit from an expanded discussion regarding what is known about the deficits in immunity following a natural RSV infection as this is critical for trying to develop an efficacious vaccine

as well as more discussion regarding the potential importance of developing vaccine approaches that will elicit both cell mediated immunity in addition to humoral immunity.

Response: We thank the reviewer for the comprehensive review of the manuscript. The aim of the manuscript is to focus on vaccine candidates in clinical development with a short overview of vaccine development history, important vaccine failures, target populations and immunologic endpoints. Unfortunately discussing cell-mediated immunity and deficits in immunity in detail is beyond the scope of this manuscript, as this would require a separate review. We hope the reviewer will find this acceptable. We realize this is a limitation of the manuscript and have mentioned this in the section on immunologic endpoints.

Revised text: Although we discuss several potential immunological correlates of protection for vaccine trials, we considered cell-mediated immunity beyond the scope of the manuscript.

Specific comments:

Comment 2 [General]: The discussion of ERD caused by the FI-RSV vaccine should include the study by Knudson et al PLoS Pathogens 2015 11(3):e1004757 as it demonstrates the critical role of CD4 T cells in mediating ERD as well as calling into question the implication that eosinophils contributed to the pathology.

Response: We have added this reference in the section on ERD as suggested.

Revised text: Other aspects of the immune response implicated in ERD include distinct subsets of CD4 cells(24) and memory CD8 T cells(25).

Comment 3 [General]: The section on the failure to license Motivazumab on page 7 does not really add to the review and could easily be cut to allow for additional focus on other areas.

Response: Thank you for this comment. Although we understand your objection, we believe the failure of motavizumab to gain FDA approval after 3 phase III trials is an important part of RSV vaccine history given the large investment, demonstrated efficacy and late-stage failure and have therefore decided to leave this part in.

Comment 4 [Immunologic endpoints]: The immunologic endpoints section should include an enhanced discussion of the potential importance of the induction of cell mediated immunity in combination with humoral immunity and how one may measure cell mediated immunity endpoints.

Response: Please refer to response to comment 1 above.

Comment 5 [General]: The authors should consider recent papers examining the role of T cell responses to RSV including Schmidt et al PLoS Pathogens 2018 14(1):e1006810, Scheible et al JCI Insight 2018 3(4) pit: 96724 [Epub ahead of print], and Mariani et al J Infect Dis 2017 216(8): 1027-1037.

Referring to: Schmidt et al PLoS Pathogens 2018 14(1):e1006810

Response: Thank you for this excellent suggestion, we have included this reference in the section on immunologic mechanisms of ERD.

Revised text: Other aspects of the immune response implicated in ERD include distinct subsets of CD4 cells(24) and memory CD8 T cells(25).

Referring to: Scheible et al JCI Insight 2018 3(4) pit: 96724 [Epub ahead of print]
Response: Although this reference is an important contribution to the body of knowledge regarding T cell development and abnormal health outcomes for infants, we feel that it does not fit the scope of the manuscript and have decided not to include it.

Referring to: Mariani et al J Infect Dis 2017 216(8): 1027-1037.
Response: Please see response above.

Reviewer #3:

General Comments to Authors:

Comment 1 [General]: This manuscript represents a review of an extremely important topic for the health of the worlds pediatric and adult populations.   The topic of advances in the prevention of respiratory syncytial virus is timely as well. Furthermore, the authors represent an august group with International experience and reputation on this particular subject.
Response: We are happy the reviewers agree that the manuscript is timely and written by a wide range of experts on the subject.

Comment 2 [General]: The manuscript could be improved in two important areas: first, the manuscript needs to have more attention devoted to RSV in adults, especially because this is the likely first population group for which vaccines will be developed.
Response: Thank you for this suggestion. Following the comments below we tried to devote more attention to RSV in adults as the reviewer suggests.

Comment 3 [General]: Second, the manuscript has a potentially very valuable section devoted to recent lessons Learned from recent clinical trial failures. This section needs to be updated and expanded using all available publicly accessible evidence.
Response: Although, we understand the reviewers comments, we have tried to be complete. We welcome any missing information as we have indeed attempted to use all available information.

Comment 4 [General]: Third, the manuscript needs more attention devoted to an integrated discussion of monoclonal antibodies. More detailed line - specific comments are provided below.
Response: We have followed the reviewer's suggestions below to devote more attention to the discussion of monoclonal antibodies.

Detailed Comments to Authors:

Comment 5 [Introduction]: Lines 14-15 This paragraph starts out lumping RSV vaccines and monoclonal antibodies together in the title sentence (which is a good idea). However, the discussion which follows is solely focused on vaccines and the problems encountered in their development. It is important to include a statement that monoclonal antibodies have avoided these issues.
Referring to: Development of effective RSV vaccines and monoclonal antibodies (mAbs) presents both opportunities and challenges.

Response: We agree that the introduction does not give a balanced introduction of both vaccine candidates and monoclonal antibodies and have revised the introduction to now include both.
Revised text:
We have added the following two sentences:
Despite these obstacles, there are several opportunities for RSV vaccine and monoclonal antibody development.
Monoclonal antibodies circumvent the problem of transient immunity to RSV and an immature immune response to vaccination in young infants at risk of severe disease.

Comment 6 [Introduction]: Lines 20-24 I believe that the authors may be inadvertently mischaracterizing the data from all of these referenced papers. The authors must make a clear delineation as to what they are talking about. Are they talking about the vaccine failing to do these things, or are they talking about the natural RSV infection after the vaccine failing to do these things.? The way it is written, the authors are talking about the natural infection (following the vaccine) which failed to do these things. This is a very important part of this review, and it needs to be explained correctly and well.
Referring to: Decades of research have revealed that in these FI-RSV primed infants, natural RSV infection triggered a strong but non-neutralizing antibody response(5), followed by a T helper 2 (Th2) skewed immunologic response(6). The failure to mount a protective cytotoxic T lymphocyte (CTL) response was coupled with excess lung eosinophilia and neutrophilia, monocytic infiltration, and immune complex deposition in the lungs(7).
Response: The intention of this statement is to talk about the response to natural RSV infection following priming with a FI-RSV However, we agree that it is unclear as written that the priming with FI-RSV results in low avidity antibodies and skews the immune system towards a Th2 response upon natural RSV infection. The 2010 Nature Medicine paper by Polack et al describes FI-RSV induction of low affinity antibodies which led to severe disease upon exposure to RSV. The 2006 Nature Medicine paper by Moghaddam et al boosts Th2 responses in mice. We hope the reviewer feels that the revision is clearer.
Revised text: ERD occurred in RSV-naïve infants who experienced infection with community-acquired wild-type RSV following receipt of FI-RSV. Decades of research have revealed that priming with FI-RSV triggered a strong but non-neutralizing antibody response(22), followed by a T helper 2 (Th2) skewed immunologic response(23) which may lead to ERD upon natural RSV infection

Comment 7 [Introduction]: Lines 30-33 The failure of these referenced trials includes inadequacies in the study design, Logistics, and implementation. It is not necessarily the fault of gap in knowledge but rather the fault in implementation that drove the studies to fail. This statement needs to be softened so as to make it clear that in certain regards a Failure may just be logistic. This reviewer has personally evaluated the data sets for two of these three reference trials, and this evaluation is the basis for this reviewer's comment. Additionally, putting all of the blame on gaps in knowledge tends to inappropriately paralyze the vaccine and monoclonal antibody development pathways, which need to be open and active.
Referring to: Recently, three phase IIb/III trials (two vaccine trials in older adults(11,12) and one mAb trial in infants(13)) failed to meet clinical endpoints. The failure of these vaccine and mAb candidates demonstrates the continued gaps in knowledge regarding immunologic mechanisms of protection in the different target populations.

Response: We have rephrased this sentence to soften it and also acknowledge the importance of inadequacies of trial design.

Revised text: In addition to possible inadequacies in trial design and implementation, the failure of these vaccine and mAb candidates demonstrates the continued gaps in knowledge regarding immunologic mechanisms of protection in the different target populations

Comment 8 [Introduction]: Lines 34-36 It is not clear what is meant buy endpoints of vaccine treatment trials. I think that the authors should limit the discussion to RSV prevention strategies, rather than therapeutic vaccines which are highly controversial.

Referring to: Another challenge to RSV vaccine design is the lack of consensus regarding clinical endpoints of vaccine trials though attempts have been made to define these for both prevention(14–16) and treatment trials(17).

Response: We agree with the reviewer and have revised the text so that only prevention is mentioned and not treatment trials.

Revised text: Another challenge to RSV vaccine design is the lack of consensus regarding vaccine trial clinical endpoints though attempts have been made to define these for RSV prevention trials(11–13).

Comment 9 [Introduction]: Lines 43 This is an ideal place to start talking about the advantages of passive antibody prophylaxis with monoclonal antibodies. In general, the discussion has been far too RSV vaccine focused and has neglected monoclonal antibodies in the discussion. The authors need to add a brief section here on the advantages of monoclonal antibodies which overcome this problem of in infants.

Referring to: An ideal RSV vaccine candidate should prevent severe disease in at risk populations.

Response: We agree that this is a good place to add a section on the advantages of monoclonal antibodies and have added this as per suggestion.

Revised text: Monoclonal antibodies circumvent the problem transient immunity to RSV and an immature immune response to vaccination in young infants at risk of severe disease.

Comment 10 [Introduction]: Lines 45 The authors need a reference to this statement. The prospect of herd immunity to RSV provided by a putative RSV vaccine has been modeled. I believe the paper is found in Proceedings of the National Academy of Sciences, Senior author: Galvani.

Referring to: Certain vaccines might also lessen person-to-person transmission and thereby provide secondary benefits in those who cannot benefit directly from vaccination.

Response: Thank you for this excellent suggestion, we have added the reference you suggest.

Revised text:

Yamin D, Jones FK, DeVincenzo JP, Gertler S, Kobiler O, Townsend JP, et al. Vaccination strategies against respiratory syncytial virus. Proc Natl Acad Sci. 2016 Nov 15;113(46):13239–44.

Comment 11 [Introduction]: Lines 64 This introduction continues to be too focused on vaccine to the exclusion of a discussion of monoclonal antibodies. Also, the authors focused too much on pediatrics to the relative amount of the discussion devoted to adult issues

In the context of RSV as an increasingly recognized global health problem, these rapid changes and expansion show the prioritization of RSV vaccine development.

Response: We agree and have rephrased vaccine to vaccine and mAb for the "opportunities" paragraph at the end of the introduction. Although we only wrote vaccine, these opportunities are equally relevant for mAbs in clinical development. For the rest, we do not believe the introduction to be more focused on pediatric populations than adults as the focus of the introduction is to lay out the challenges and opportunities to RSV vaccine and mAb development. Please let us know if you find this acceptable as now written.

Revised text: In the context of RSV as an increasingly recognized global health problem, these rapid changes and expansion show the prioritization of RSV vaccine and mAb development.

Comment 12 [RSV Vaccine history]: Lines 104 The authors need to include the polyclonal RSV immune globulin manufactured by ADMA Biologics (RI-002) (ADMA Biologics, Ramsey, NJ, USA). This is-FDA approved as an immune globulin, and has been manufactured to mimic Respigam, but it did not receive the "FDA indication" of RSV preventing because the trials were not performed for that purpose (due to immense const). However, it is approved for use as a replacement for Primary Immune Deficiencies.

Referring to: Since its initial approval in 1998, palivizumab remains the only licensed preventive intervention against RSV(27).

Response: Although we understand that RI-002 is an approved polyclonal antibody with high RSV-neutralizing antibodies, we believe that RI-002 falls outside the scope of this manuscript as it was not developed as a strategy for RSV prevention specifically (but immunoglobulin supplementation in PIDD patients) and is not included in the PATH vaccine snapshot. The Phase III trial showed efficacy against serious bacterial infections but not against RSV. As a review of novel antibodies (Mejias et al, Vaccine 2017) mentioned "The role of RI-002 in preventing RSV infection in this population has not been reported." The aim of this manuscript is to focus on prevention strategies in clinical development on the basis of the PATH snapshot. We have therefore decided not to include it in the manuscript. We hope the editor finds this acceptable.

Comment 13 [RSV Vaccine history]: Lines 106 The authors need to mention the efficacy of palivizumab in the various populations evaluated (including mentioning the approximately 80% reduction in RSV-hospitalizations in infants with milder degrees' prematurity and without chronic lung disease.

Referring to: Palivizumab has an excellent safety profile and is indicated for the prevention of severe RSV ALRI in children born prematurely, with congenital heart disease, or with chronic lung disease(28).

Response: We agree that adding some information about the efficacy of palivizumab IMPACT-RSV trial is informative and have mentioned the efficacy from this trial in premature children without BPD.

Revised text: Since its initial approval in 1998, palivizumab remains the only licensed preventive intervention against RSV after demonstrating a reduction of 39% to almost 80% reduction of RSV hospitalization in preterm infants < 35 weeks gestational age with and without chronic lung disease respectively(29).

Comment 14 [RSV Vaccine history]: Lines 111 The authors need to replace the word evidence with the words "sufficient evidence".

Referring to: Without evidence of superiority for protection from RSV-related hospitalization, evidence of slightly higher side effects, and no plan for dose reduction or cost-saving, the product did not attain regulatory approval.

Response: We have made the revision as suggested.

Revised text: Without sufficient evidence of superiority for protection from RSV-related hospitalization, evidence of slightly higher side effects, and no plan for dose reduction or cost-saving, the product did not attain regulatory approval.

Comment 15 [RSV Vaccine history]: Lines 118 I think it is important to mention that the other vaccine strategies were not allowed to be tried in infants (rather than they were tried and found to be unsafe) needs to be expanded. It was the regulators who haven't allowed this. Not necessarily the investigators. If I am mistaken on this point, please help me and the reader understand.

Referring to: With respect to vaccines for active immunization, many approaches targeted for RSV naïve children were evaluated pre-clinically over the years. Only live-attenuated vaccine candidates were considered safe for clinical evaluation in these children(31).

Response: We have decided to remove this sentence.

Comment 16 [RSV Vaccine history]: Lines 139 So what is the lesson learned from this? The reasons for failure? Is there a new set of information from the Regeneron website? What about clin trials.gov? What about the half-life of the antibody with respect to the target dosing interval? Was it a failure of efficacy or a failure as safety? All these questions need to be addressed. We need to be able to learn a lesson, rather than have a bunch of questions still hanging.

Referring to: A phase III double-blind, placebo-controlled trial (NURSERY) evaluating REGN2222, a mAb against antigenic s V on the RSV pre-F protein, a major target for high-potency mAbs(37) was conducted.

Response: We agree upon the importance of learning a lesson from these large late-phase vaccine trial failures. Unfortunately, the lack of information available in the public domain (and lack of any peer-reviewed publication) make it very difficult to distil any lessons learned. This is exactly why we emphasize the importance of publishing these results and analyzing them in order to benefit future vaccine trials. Nevertheless, we have revisited clinicaltrials.gov, the Regeneron website, and any other published information to determine if we have missed any information. To address the question on whether it was a failure of efficacy or safety, we mentioned in the manuscript that the NURSERY trial did not meet its primary outcome to prevent medically-attended RSV infections which indicates that it was a failure of efficacy, not safety. The expected half-life of the antibody was not published. Only one or two doses were administered depending on the treatment arm. When two doses were administered, these were administered 8 weeks apart. It may be that this is not sufficient given the average half-life of antibodies to be was described in the poster as 32.0+/-8.79 and 34.4+/-11.9 days following IM administration of 3 mg/kg and 10 mg/kg doses, indicating the half life is longer than that of palivizumab. Unfortunately no new study results have been posted on the Regeneron website nor on clinicaltrials.gov. The only information in the public domain is a poster presentation from ID Week in 2015 on the phase 1 trial results (http://files.shareholder.com/downloads/REGN/0x0x853993/048D5B21-4FAD-4254-8299-6547853FCAC6/REGN2222_IDWeek_2015_poster_HIGH_RES.PDF). We have also written to Regeneron to inquire whether there were further analyses of this late-stage failure performed and whether there is a plan for publication of these results. Unfortunately, given the lack of

information we have only been able to speculate regarding the dosing interval and otherwise give the lack of information have called upon the company to analyze and publish phase III results.
Revised text: A proposed explanation for the failure of this trial may be inadequate dosing schedule in regard to the antibody half-life. Ultimately, the basis for failing to meet the primary clinical endpoint is not known, as analyses of this late-stage failure have not yet been made public.

Comment 17 [Lesson learned]: Lines 142 The authors need to add that these infants had to have been rejected from receiving palivizumab. (Full list of inclusion and exclusion criteria can be obtained at the Clintrials.gov website.
Referring to: REGN2222 was administered once or twice during the respiratory season in 1,149 healthy preterm infants < 6 months of age with a gestational age ≤35 weeks and did not meet its primary endpoint to prevent medically-attended RSV infections through day 150 of life.
Response: We agree that this is important information and have added this to the manuscript.
Revised text: REGN2222 was administered once or twice during the respiratory season in 1,149 healthy preterm infants < 6 months of age with a gestational age ≤35 weeks who were not eligible to receive palivizumab prophylaxis

Comment 18 [Lesson learned]: Lines 149. 2. The discussion of the second candidate is inadequate. Additionally, the manufacturer/ sponsor (Novavax) (and the product name itself), needs to be identified, so as to be parallel to the discussion of the first candidate, (Regeneron).
Referring to: The second candidate that failed to meet the predefined study endpoint in phase III clinical trials was the RSV F nanoparticle vaccine candidate for older adults, a candidate based on aggregates of full-length post-F.
Response: Throughout the entire manuscript text we have not mentioned the names of pharmaceutical companies. The section on REGN2222 does not refer to Regeneron specifically even though the name of the mAb includes "REGN." The only section of the manuscript where pharmaceutical names are mentioned is Table 1 under "Company/sponsor." Furthermore, we have addressed comments 18-20 and hope that this has led to a more adequate discussion of this vaccine candidate.

Comment 19 [Lesson learned]: Lines 158 The authors might consider a brief discussion of the appropriateness or inappropriateness of the controversial assay "palivizumab competing antibody".
Referring to: Although the vaccine showed promising results in phase II and comparable immunogenicity measures in the two phases as determined by neutralizing and palivizumab-competing antibody induction, the vaccine candidate failed to show efficacy against RSV moderate–severe lower respiratory tract disease (ms-LRTD) in phase III results(12).
Response: We agree that this is important to understand the Novavax phase III failure and have added a sentence on PCA.
Revised text: For example, PCA titers may not correspond to effective immunity as non-neutralizing antibodies also bind the palivizumab binding site and can interfere with the binding of neutralizing antibodies(48).

Comment 20 [Lesson learned]: In this reviewer's recollection, the neutralizing antibodies were not encouraging, But the PCA antibodies (Whatever that means) we're astoundingly high.

Referring to: Although the vaccine showed promising results in phase II and comparable immunogenicity measures in the two phases as determined by neutralizing and palivizumab-competing antibody induction, the vaccine candidate failed to show efficacy against RSV moderate–severe lower respiratory tract disease (ms-LRTD) in phase III results(12).
Response: We agree that in the public domain there is no clear data on increase of MN in the vaccination group compared to placebo (see for example:
http://novavax.com/download/files/presentation/Novavax_RSV_Analyst_Day_7-24-17_PDF2.pdf). Reported immunogenicity measures include anti-F IgG as well as PCA. We have clarified this in the text.
Revised text: Another proposed explanation for failure of this vaccine candidate is that the quantity of the immune response to vaccination may not represent effective immunity. For example, PCA titers may not correspond to effective immunity as non-neutralizing antibodies also bind the palivizumab binding site and can interfere with the binding of neutralizing antibodies(48).

Comment 21 [Lesson learned]: Lines 170-173 This was the company's stated reason. However, it appears that the lack of a sufficient number of endpoints (RSV-MS- LR TD) was not the sole reason for the vaccine failure. Because the vaccine affect size was also shown to be too low. This needs to be brought out. Also, it needs to be pointed out and appropriately referenced that low micro neutralization responses may have been achieved (If this information is attainable in published form).
Response: Unfortunately, as stated earlier the MN titers for phase II and phase III are nowhere to be found in the public domain. Only the phase I results for the older vaccine candidate have been published (PMC5389002) which describes a 1.3-1.7 fold rise in neutralizing antibody titers in response to vaccination (see figure from manuscript included below).

21
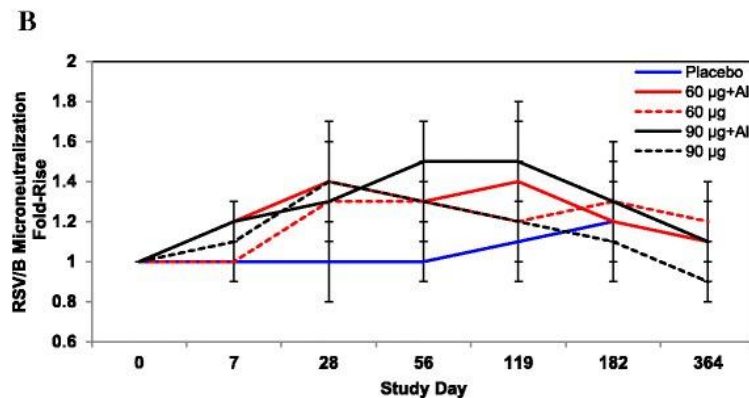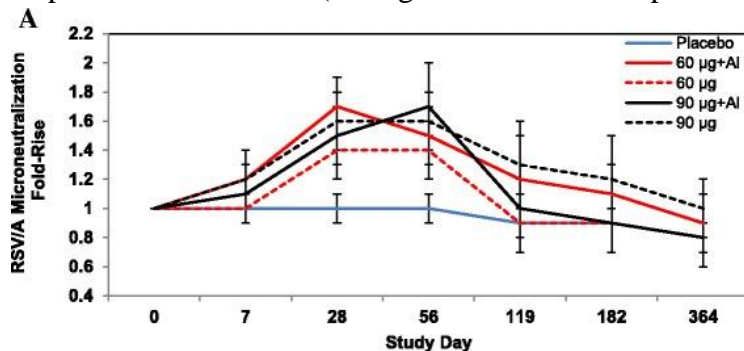
The increase in RSV microneutralizion response in vaccines compared to placebo is not available in the public domain for the phase III trial so it is difficult to draw a conclusion, but in the phase I trial there was only a modest (1.3-1.7 fold) increase in neutralizing antibody titers in response to vaccination(49).

Comment 22 [Lesson learned]: Lines 177 What was its primary objective? Phase 2 clinical trials are not usually having a primary objective of vaccine efficacy. Rather they usually have a primary objective of immunogenicity. What was the immunogenicity? The statement that the authors make that "93% of VAX recipients in these had an anti-F antibody seroresponse" is inadequate for the reader to understand what that quantitative level of seroresponse was.
Referring to: Development of the MEDI-7510 vaccine candidate, a subunit vaccine candidate for older adults, was discontinued after a phase IIb trial in North America, Europe, South Africa, and Chile in 1900 adults ≥60 years after the study failed to meet its primary objective.
Response: The primary outcome specified on clinicaltrials.gov (NCT02508194) was the percentage of participant who had a first episode of acute RSV-associated respiratory illness (ARA-RI) during the RSV season in season 1 for day 14 through the end of the surveillance period (approximately 7 months). Immunogenicity measures (GM fold change in Anti-F IgG, RSV microneutralization post dose geometric mean fold change, PCA post-dose GMC) were only included as secondary outcomes. We have clarified that this was the specified primary outcome of the phase IIb trial. Furthermore, we agree that the immunogenicity measures were not reported in enough detail and have now added this to the manuscript.
Revised text:
Development of the MEDI-7510 vaccine candidate, a subunit vaccine candidate for older adults, was discontinued after a phase IIb trial in North America, Europe, South Africa, and Chile in 1900 adults ≥60 years after the study failed to meet its primary objective, efficacy against RSV-associated respiratory illness between 14 days post-vaccination throughout the end of the surveillance period, approximately 7 months.
No efficacy was found in secondary subset analyses. On day 29, 93% of vaccinees had an anti-F IgG antibody seroresponse and there was a geometric mean fold rise in anti-F IgG titer of 4.6 at the end of the RSV season in vaccine recipients compared to the placebo group(50).

Comment 23 [Lesson learned]: Lines 187 The authors need to add information regarding the neutralizing antibody concentrations that were induced by the vaccine. This allows the reader to understand things about epitope specificity.
Referring to: One proposed explanation for the negative results may be that the choice of a post-F antigen induced antibodies without appropriate epitope specificity(40).
Response: We agree that this would be helpful to interpret whether epitope specificity played an important role in phase IIb failure. However, unfortunately the only data published show MN response at baseline and on day 29 after dosing in subjects who met the primary end point or were selected to match them (in a 1:6 ratio) or to match the sample size of a group that received the same formulation in the Phase Ib study. Thus, the MN data is not available for vaccine v placebo groups.

Comment 24 [Lesson learned]: Lines 191 Where are the authors going to mention the exciting preliminary results just announced for the maternal vaccination trial using nova VAX vaccine?

It may be appropriate to mention this here using proper soft and reserved language (beware of company-spin).

<u>Referring to:</u> Considerations for the future include selection of an older study population at higher risk of RSV infection.

<u>Response:</u> In accordance with the PATH vaccine snapshot we have considered the Novavax vaccine candidate for maternal immunization and older adults as separate candidates. Therefore we have decided to mention the results for the maternal vaccine in the section on particle-based vaccines and not together with this vaccine failure. The main aim of the section on the vaccine graveyard is to distil lessons learned from large late-phase RSV vaccine trial failures.

<u>Comment 25 [Vaccine antigens]:</u> Lines 201-203 Shouldn't the authors simply state that the energy of activation allowing pre-EF to change into post F is quite small, thus allowing "Spontaneous" conversion from pre-F to post F.

<u>Referring to</u>: There is no consensus on the trigger for the pre-F to post-F conformational change making it difficult to ensure a wild-type F vaccine antigen maintains a pre-F conformation, but stabilizing mutations have been identified that can preserve the pre-F-specific epitopes(41,43).

<u>Response:</u> We are not aware that the energy of activation is "quite small." We are left the statement as is which states that there is not yet consensus on a trigger for RSV F protein. A proposed mechanism that has been published is a reduction in buffer molarity (Chaiwatpongsakorn, J Virol 2011). Other cellular receptors implicated in this triggering include TLR4 (Haynes et al, 2001), nucelolin (Tayyari et al, 2011), and ICAM-1 (Behera et al, 2001). Thus, ultimatey there is no consensus as published in a review on the "Structure and function of RSV surface glycoproteins" (McLellan et al, Curr Top Microbiol Immunol, 2014). However we have consulted coauthors and rephrased the sentence to reflect this uncertainty.

<u>Revised text:</u> It remains unclear as to whether there is a trigger for the pre-F to post-F conformational change, but it does occurs spontaneously, making it difficult to ensure a wild-type F vaccine antigen maintains a pre-F conformation. However, stabilizing mutations have been identified that can preserve the pre-F-specific epitopes(53,55).

<u>Comment 26 [Vaccine antigens]:</u> Lines 205 Is it certain that these stabilizing mutations do not affect the conformation of the antibody binding sites? Is this still an open question? This reviewer does not know the answer to this but some of the authors probably do, and it would be appropriate to insert a simple sentence here describing the answer.

<u>Referring to:</u> There is no consensus on the trigger for the pre-F to post-F conformational change making it difficult to ensure a wild-type F vaccine antigen maintains a pre-F conformation, but stabilizing mutations have been identified that can preserve the pre-F-specific epitopes(41,43).

<u>Response:</u> We have consulted co-authors and added a sentence as the reviewer suggests.

<u>Revised text:</u> The antigenicity of some stabilized pre-F constructs has not been rigorously investigated, and it remains an open question as to whether certain stabilizing mutations affect the conformation of antibody binding sites

<u>Comment 27 [Vaccine antigens]:</u> Lines 231-233 This reviewer was expecting a brief discussion of the relevance of ADCC to RSV prevention, as well as the evidence or lack of evidence that T cell responses are important.

Referring to: The SH protein may be important for induction of antibody dependent cell-mediated cytotoxicity (ADCC), whereas non-membrane proteins are especially important to induce a robust T cell response(46)

Response: A more detailed discussion of ADCC and T cell immunity are unfortunately beyond the scope of this manuscript. This section focuses on vaccine antigens that are used in candidates in clinical developments. We only briefly highlight there relevance for different immune responses but do not provide an in depth discussion of the evidence and lack of evidence of importance of these immune responses in this section. In the section on immunologic endpoints we do briefly discuss the importance of T cell immunity as a marker for protection from clinical disease. We hope the reviewer understands these limitations.

Comment 28 [Target populations]: Lines 253 The authors need to mention at least one of the major limitations of maternal vaccination strategy to protect newborn infants: namely, that the elimination half-life of transplacental antibodies is naturally short, thus limiting the duration of protection even if passive antibody is transmitted to the infant in sufficient quantities.

Referring to: Passive transfer of antibodies to infants has been shown to be protective against severe RSV infection through the administration of high-titer polyclonal and monoclonal antibodies (RSV-IVIG and palivizumab) (26,27).

Response: We agree that we did not sufficiently highlight the limitations and have added this limitation.

Revised text: The duration of protection of maternal vaccination is defined by the antibody half-life.

Comment 29 [Target populations]: Lines 263 The authors need to define for the readers what the Word preterm means.

Referring to: Globally 10% of children are born preterm(62).

Response: We agree with this suggestion to clarify definition of preterm for the data from the systematic review we are referring to. However, in this systematic review, although there was a general consensus across the studies on a definition of less than 37 complete weeks of gestational age (75/92), some studies did not report a definition (14/92) and some had a different definition (3/92). Therefore, it would not be accurate to list a definition as different definitions were included in this systematic review.

Revised text: No change

Comment 30 [Target populations]: This review would be improved if specifics were given with respect to relative amounts of antibody transferred at different gestational age is. References do exist for this data. If preterm means less then 37 weeks, what percent of naturally transferred term antibody is present in these infants?

Response: We understand that this additional level of detail is interesting, however we also feel that it is beyond the scope of this review. We mention that the majority of IgG transfer occurs before 32 weeks gestational age to give an indication of the effect of preterm birth on the efficacy of maternal vaccination. Further detail goes beyond the scope of this manuscript. We hope the reviewer finds this acceptable.

Comment 31 [Target populations]: Lines 266 This reviewer believes that the reason that the premature infants do not receive passive antibody from mother is not because they are born

prematurely and therefore their umbilical cords are severed (thus cutting off flow). Rather it is because the maturation of the placenta does not occur to allow transfer of antibodies prior to being near-term. The wording here implies the different mechanism. And the concept is important with respect to the statements regarding improved efficacy of transfer if maternal vaccination occurs early or late within gestation.

Lines 268-271 See my previous comment.

Referring to: Thus, a maternal vaccination strategy may not be sufficient to protect the high-risk preterm population if administered during the third trimester of pregnancy. Tetanus diphtheria acellular pertussis (Tdap) immunization in the second trimester is associated with higher antibody titers by time of birth as compared to third trimester immunization(64). A strategy of earlier vaccination could be considered for maternal RSV immunization to maximize protection to preterm infants.

Response: There is limited data on gestational age-related antibody transfer, the most relevant information is from Malek (Am J Reprod Immunol, 1996). To our knowledge, it has been shown that prolonged maternofetal transfer cumulatively results in higher transferred IgG than exposure at maximum transfer efficiency which occurs at 32-33 weeks gestation age (Eberhardt, CID 2016). We are not familiar with the statement above, that transfer of antibodies is "not allowed prior to being near-term. IgG transfer, although limited, is known to begin as early as 13 weeks gestational age, with transfer increasing in a linear fashion as pregnancy progresses (Palmeira et al, Clin Dev Immunol 2012). Regardless, the statement as now written does not indicate which mechanism underlies higher antibody titers associated with second trimester vaccination in comparison with third trimester vaccination. We have therefore decided to keep this section as is and hope the reviewer agrees. If this is not acceptable please let us know how this should be changed.

Comment 32 [Target populations]: Lines 278-286 This entire paragraph would greatly benefit from the addition of a perspective of monoclonal antibody administration to infants' afterbirth,

Referring to: A combined strategy that utilizes maternal vaccination to protect young infants followed by pediatric vaccination may be effective to prevent severe RSV infection in young children. This strategy is estimated to avert at least twice as many admissions per 100 births and four times as many in-hospital deaths per 1000 births than maternal vaccination alone(66). A combined strategy will be particularly relevant to prevent morbidity and mortality in children with comorbidities who are at risk of severe RSV disease at older ages (67,68). A similar maternal and pediatric combined passive and active immunization strategy is currently employed for pertussis and influenza vaccination(65).

Response: We agree with the reviewer. We are trying to highlight a combination strategy in which young infants are protected via passive immunization (through maternal immunization or administration of mAbs) followed by pediatric active immunization. We have clarified this so that mAb administration after birth is equally well represented.

Revised text: A combined strategy that utilizes passive immunization to protect young infants, via maternal vaccination or mAbs, followed by pediatric active immunization may be effective to prevent severe RSV infection in young children(78).

Comment 33 [Target populations]: The extended half-life allowable by FC antibody alterations is a major potential improvement and needs appropriate coverage in this review. I have not seen it

discussed in this review yet. Information on these clinical trials which are ongoing, can be found in a few peer-reviewed publications, and clintrials.gov website.

Response: We discuss FC alterations to extend antibody half-life in the section about MEDI-8897 as this is mAb candidate with the YTE technology. We agree on the importance of this technology and have therefore also added it earlier in the manuscript when we discuss the limitations the duration of protection due to antibody half-life in passive vaccination.

Revised text: The duration of protection of maternal vaccination is defined by the antibody half-life. Administration of mAbs is an alternative form of passive vaccination that can circumvent this hurdle due to extended antibody half-life through Fc alterations(68).

Comment 34 [Immunologic endpoints]: Lines 317 The authors need to mention the well-defined serologic correlate of protection which has been repeatedly defined in phase 3 clinical trials in infants using both polyclonal and monoclonal antibodies. The actual level of protection in micro neutralization units needs to be mentioned in this review (immune experienced adults). This is a unique and important feature of RSV which can inform future vaccine development greatly.

Referring to: The mechanisms of protection may differ according to the type of vaccine, and therefore, many different immunologic assays are employed in clinical trials.

Response: To our knowledge there is no well-defined serologic correlate of protection, nor is there an "actual level" of protection in micro neutralization units. All authors on this manuscript have read and approved this section which argues that there is no definitive immunologic correlate of protection and that there is no consensus in the field in this regard. If there is specific evidence on vaccine-specific correlates of protection, we will gladly add this to our manuscript.

Comment 33 [Immunologic endpoints]: Lines 341 See my comment above. Is it really "elusive"?

Response: Please see response to comment 32 above.

Comment 35 [Immunologic endpoints]: 357 some of them have been shown to be safe, but others have definitely NOT been shown to be safe. The authors need to modify this statement. Accordingly. This reviewer believes they did not show vaccine enhanced disease, but that is a separate issue than safety.

Replication deficient vectors, engineered to induce CD8 T cell

356 responses expressing RSV antigens intracellularly, are considered more similar to

357 live-attenuated virus vaccines which have been shown to be safe in this population.

Referring to: Replication deficient vectors, engineered to induce CD8 T cell responses expressing RSV antigens intracellularly, are considered more similar to live-attenuated virus vaccines which have been shown to be safe in this population.

Response: We agree that it has been shown that these are shown not to be associated in ERD. We have rephrased the wording as the reviewer suggests.

Revised text: Replication deficient vectors, engineered to induce CD8 T cell responses expressing RSV antigens intracellularly, are considered more similar to live-attenuated virus vaccines which have been shown not to cause ERD in this population.

Comment 36 [Particle-based]: 393-396 Authors need to verify that this information is publicly releasable, or has already been released which necessitates a reference being placed here.

Referring to: Assays of serum virus neutralization, RSV F-specific antibodies, palivizumab-competing antibodies and F-specific IgA indicated some immunogenicity, but the results did not

reach the threshold set for continuation to viral challenge and the studies were suspended in 2017 (Openshaw and Chiu, personal communication).
Response: We have obtained written consent from the two authors mentioned to release this information. It is otherwise not yet available in the public domain.

Comment 37 [Vector-based]: 410-413 This statement is confusing. How does a vaccine - induction of a humoral response correct the impaired T cell immunity supposedly encountered in the elderly? Do the authors mean "cell mediated response rather than "humoral response"?
Referring to: Given that severe disease in the older adult population is thought to be mediated by immunosenescence characterized by impaired T cell response, this vaccine candidate, which induces a humoral response, may be a promising intervention for the older adult population(81).
Response: In this case we do not mean humoral response. We expect a vaccine candidate not to be able to induce an adequate T-cell response but to be able to induce an adequate humoral response. For this reason, we expect this candidate to be able to induce a strong and effective immune response. However, we agree that the wording was confusing because we also wrote that severe RSV disease was mediated by impaired T-cell response. For this reason, we have rephrased this sentence for clarity.
Revised text: In the older adult population, immunosenescence may be characterized by impaired T cell responses to RSV(97,98). Thus, this vaccine candidate which induces a humoral response may be a promising intervention in this population.

Comment 38 [Subunit]: 461-463 Can this statement be updated with the reference now? What about a presentation at a publicly disclosed meeting?
Referring to: Phase I results on safety and immunogenicity in the older adult population will soon be published from an investigator-initiated study.
Response: Unfortunately these results are not yet in the public domain so we cannot update this with a reference. However, we have reworded the sentence because we agree that it can otherwise not stand without a reference.
Revised text: Phase I results on safety and immunogenicity in the older adult population have been released and are expected to be published from this investigator-initiated study.

Comment 39 [mAbs]: 509   The authors should briefly mention the effect of this YTE mutation on the functionality of the antibody with respect to antibody dependent Cellular cytotoxicity and other signaling pathways.
Referring to: Using the YTE technology for extending antibody half-life, the three-fold increase in half-life of MEDI8897(91) compared to palivizumab offers the possibility of passive protection for all infants for an entire season through a single intramuscular injection.
Response: We have added this statement as the reviewers suggest with an appropriate citation.
Revised text: Using the YTE technology which extends antibody half-life as well as modulates ADCC(111), the three-fold increase in half-life of MEDI8897(112) compared to palivizumab offers the possibility of passive protection for all infants for an entire season through a single intramuscular injection.

Comment 40 [mAbs]: 516 This section needs to be expanded to review the clinical development progress of this antibody as mentioned by Clintrials.gov
Section on monoclonal antibodies

Referring to: Representatives of the pharmaceutical company have indicated that they expect vaccine-like pricing of MEDI8897. Given the increased potency, the extended half-life, and the required dose, it is expected that the cost to protect an infant during the RSV season can be kept relatively low(92).

Response: As far as we know the section on mAbs is up-to-date with clinicaltrials.gov. Please also see the mAbs in clinical development on the PATH snapshot we we used to define the scope of this manuscript. Please let us know if additional information is needed for the mAb paragraph.

Comment 41 [mAbs]: 517 The authors should also mention the major effect of improved manufacturing techniques of monoclonal antibodies which have been developed over the past decade which allows significantly less expensive production.

Referring to: Passive vaccination with an extended half-life antibody offers an approach to protecting infants that is safe and may be reasonably priced. Representatives of the pharmaceutical company have indicated that they expect vaccine-like pricing of MEDI8897. Given the increased potency, the extended half-life, and the required dose, it is expected that the cost to protect an infant during the RSV season can be kept relatively low(68).

Response: Although we agree with the reviewer, we felt that further analysis cost-related issues was beyond the scope of this review.

Comment 42 [References]: 1.there needs to be a mention of Galvani (sr. author) PNAS paper
Response: Please see response to comment 9 above.

Comment 43 [Table 2]: Overview of vaccines and MAbs by target population:
1.where are the MAbs mentioned?
Response: mAbs is the last category in the table.

Comment 44 [Table 3]: Expected immune response and precious successes for vaccine….1.do you need to mention RGN-2222? (MAb)?
Response: This table only contains mAbs that are in clinical development according to the PATH vaccine snapshot, which is why we have not included REGN-2222.

Comment 45 [Figure 1]: RSV global burden of disease in children: key facts and figures
This figure needs a lot of work and a greatly expanded footnote to add more detail to the various statements made. For example:

Comment 46 [Figure 1]: 1. is the 3.2 million hospitalization number from developed countries? If so, how does this fit with the fact that there are only 33.1 million RSV LRT eyes worldwide? A 10% hospitalization rate for RSP L RTI is quite hi depending on what age range are being evaluated. Likewise, the blue circle needs greater granularity also. I.e., which age range are we talking about?
Response: This concerns the hospitalization worldwide, we have clarified this in the legend.

Comment 47 [Figure 1]: 2. authors should explain the numerator and denominator of the 15.9 divided by 1000 new units per year. They also need to define the other rates mentioned in the green circles.

Response: 15.9 is the rate of neonates per year and this is also written inside of the green circle. We have added a clarifying sentence in the legend

Revised text: The hospital admission rate of 15.9 hospital admissions per 1000 neonates per year is in developing countries.

The RSV ALRI hospitalization 63.9 among premature infants <1 year is reported per 1000 children per year globally.

Comment 48 [Figure 1]: 3. might it be better to have a separate set of circles for infants in the developed world and another set for infants in the developing world? Might it be better to also include adults in this figure? As I understand, the review is not focused solely on children.

Response: We have decided to limit this figure to include only children. All rates have been specified inside of the green circles. We have also clarified in the legend which figures are global and which are for the developing world.

Comment 49 [Figure 2]: Overview of Vaccine candidates…(heading)
1.   This figure seems to be limited to those that are in clinical trials rather than those that are in clinical development. There are a lot more of these in clinical development. I suggest renaming the title to show that you are listing only those Advanced into clinical trials

Response: Figure 2, just as table 1 and table 2, and the entire manuscript focuses on vaccine candidates and mAbs for RSV in clinical trials only, not in preclinical testing. The only thing that has been added to this figure is candidates that are no longer in development since the review we published in the Lancet Respiratory Medicine. Nevertheless, we have changed the heading for this figure so that it is clearer what the scope of the figure entails.

Revised text: Figure 2: Overview of vaccine candidates and monoclonal antibodies in clinical trials per preventive approach including candidates for which development was recently halted

Comment 50 [Figure 2]: Monoclonal:
The regeneron molecule needs to be placed here. Also, the polyclonal product respimmune. Also, palivizumab needs to be placed here.

Response: We agree that REGN-2222 needs to be added and have done so. However respimmune and palivizumab fall outside the scope of this figure as they are no longer in clinical development and we have therefore not added them.

Revised text: Addition of REGN-2222

Comment 51 [Figure 2]: Vector-Based:
The Sendai virus backbone based vaccines need to be mentioned too since other phase ones have been mentioned

Response: The vaccine candidate with a Sendai backbone, we believe you are referring to (PMID: 28250126) is not yet in clinical development and has therefore not been included in this figure.

Comment 52 [Figure 2]:I believe there are some G - protein-based subunits vaccines. If so, they should be mentioned. The black label F- protein stands out, but there is no balanced label for SH.

Response: Currently there are no G protein-based subunit vaccines in clinical development, which is why we have not included them in this figure. Please refer to the most recent PATH

vaccine snapshot for an overview of vaccines and mAbs for RSV in clinical development. We hope we have addressed the reviewer's concerns adequately.

Comment 53 [Figure 2]:I don't understand what the label inside the purple circle means. It looks like this label needs to be removed.
Referring to: Ad26/5; ChAd155, MVA
Response: The label inside the graphic represent vector-based vaccines are the vectors that are used for these vector-based vaccines. The idea was to give an overview of all vectors employed for this preventive approach. However, we agree that this is more confusing than it is helpful and have removed this label as the reviewer suggests.

Comment 54 [Figure 2]:It also looks like the label inside the blue circle needs to be removed (there are other live attenuated chimeric's other than BCG - based.
Referring to: RSV/BCG
Response: At this moment, there are no other chimeric vaccines in clinical development than the BCG vaccine. However, we agree that the figure is clearer with this removed and have done so. Please see response to comment 51 above.

Comment 55 [Figure 2]: Subunit: Colors need to be altered to allow better visualization. This is especially true of the lavender and peach colored approaches.
Response: We have changed the color to a darker color to allow for better visualization as the reviewer suggests.

Comment 56 [Figure 2]: Particle Based: (RSV F nanoparticle)
If this is the novaVAX vaccine, the company needs to be identified within the graphic. (in harmony with the other parts of this graphic.
Response: We understand the reviewer's suggestion. However, we have not mentioned the company names anywhere in the manuscript besides Table 1 under the column "company/sponsor." Toa void any commercial biases, we have decided to not use any pharmaceutical names but instead the index name of the vaccine candidate or mAb. Sometimes this includes an abbreviation of the company name but never the entire company name. For consistency, we have not added in "Novavax" in this figure.

Comment 57 [Figure 2]: Particle-Based: (SynGEM)
Since this particle based vaccine has been halted, shouldn't it be in gray?
Response: We understand your comment. Only vaccine candidates or mAbs that were previously halted have been made gray. All vaccine candidates and mAbs considered in clinical development according to the PATH snapshot are still in color. Likewise, the GSK adenovirus 26 preF vaccine has PhII has been halted and it is unclear whether development will continue. For SynGEM, this is the first publication, which will contain information in the public domain that mentions development being halted. For consistency, we have kept all 19 candidates in development according to the PATH snapshot in color in this figure and older candidates in grey. Please let us know if you feel a change is necessary.

Reviewer #4:

General comments:

Comment 1 [General]: This is a well-written review of the RSV vaccine candidates and Mabs currently in clinical development. In view of the considerable global burden of RSV and the urgent need for efficacious vaccines for the populations most severely affected by RSV, this review is very timely. In addition, with so many vaccine candidates in clinical development, and many more in pre-clinical stages, this provides an excellent reference.
Response: We thank the reviewer for recognizing the importance of this manuscript to the field of RSV vaccine development.

Specific Comments

Comment 2 [Lessons learned]: Line 178: What was the TLR4 agonist adjuvant and is this an optimal adjuvant based on pre-clinical studies, or could stronger ones be used that might promote higher levels of immunity?
Referring to: MEDI-7510 was a subunit vaccine using soluble (unaggregated) postfusion (post-F) conformation of the F protein with a TLR4 agonist adjuvant that showed safety and immunogenicity with elevated B and T cell responses in the vaccine group compared to the placebo group in phase I clinical trials(39).
Response: The adjuvant is a glucopyranosyl lipid adjuvant (GLA) which was administered in a squalene-based 2% emulsion (GLA-SE). Phase I clinical testing provided support for inclusion of the adjuvant in the vaccine candidate (Falloon et al, Vaccine 2016 and Falloon et al Clin Vaccine Immunol 2017). In the first-in-man trial the vaccine was tested with the adjuvant and unadjuvanted, no other adjuvants were tested. The adjuvant was found to increase both humoral and cellular immune responses. Thus, given available evidence the best possible adjuvant was selected to continue into phase II clinical trials. We hope this answers the reviewer's question sufficiently.

Comment 3 [Lessons learned]: On line 179 the authors refer to induction of B and T cell responses in the group vaccinated with MEDI-7510; it is important to provide information about induction of VN antibodies, which are correlated to protection. Were they measured and if so, what were the levels?
Referring to: MEDI-7510 was a subunit vaccine using soluble (unaggregated) postfusion (post-F) conformation of the F protein with a TLR4 agonist adjuvant that showed safety and immunogenicity with elevated B and T cell responses in the vaccine group compared to the placebo group in phase I clinical trials(39).
Response: We agree that virus neutralization titers are most informative regarding the ability of the candidate to induce an effective immune response. VN titers were not reported for the IIb trial for vaccine v placebo groups. We have made this explicit in the manuscript.
Revised text: (50). Microneutralization, PCA and cell-mediated immunogenicity responses were only reported in subset analyses and therefore there is no data on microneutralization activity in the vaccine group versus the placebo group for this trial.

Comment 4 [Lesson learned]: Line 191: An alternative might be to increase the study population.
Referring to: Considerations for the future include selection of an older study population at higher risk of RSV infection

Response: Increasing the study population would have been necessary if this trial failure was due to an underpowered study. However, based on available knowledge from phase II the phase III trial was adequately powered. We mention extending enrolment by an additional RSV season since the pharmaceutical company has attributed the failure to a low attack rate and performing a trial over several seasons would have allowed for a more even distribution of attack rates from season to season, especially for a pivotal phase III trial. We hope this addresses the question.

Comment 5 [Immunologic endpoints]: Line 322: Would a ratio of fold-increase in RSV-binding antibodies to RSV neutralizing antibodies of 1 not be as effective?
Referring to: A measure of functional antibody response can be elucidated by the ratio of fold-increase in RSV-binding antibodies to fold-increase in RSV-neutralizing antibodies (ELISA-to-neutralization response ratio).
Response: This statement described total antibodies to functional antibodies. The higher the amount of functional antibodies in proportion to total antibodies (when this ratio is <1), the greater the neutralizing activity. However, there is no consensus on this measure nor is there an exact cut off for optimal neutralizing activity. However, the lower the ratio the more effective so the answer is yes, a ratio of 1 would not be as effective.

Comment 6 [Vector-based]: Lines 406-413: This section should be deleted. The VXA-RSV-f is described under "five vector-based vaccines in clinical development" (line 399), but this vaccine candidate is not in clinical trials and thus does not fit within this manuscript - if the authors want to include RSV vaccines in pre-clinical development, there are many other promising candidates that should be discussed. More importantly, the information on results from the pre-clinical studies on VXA-RSV-f is not useful at all, as "enhanced IgA in the upper airways" does not mean anything unless supported by protection data. Furthermore, no reference is provided to support this statement. Reference 81 refers to a 2013 study on RSV viral shedding in adults, totally unrelated to this vaccine candidate.
Referring to: The second vector-based vaccine candidate, VXA-RSV-f, uses an innovative platform with an adenovirus 5 based oral tablet delivery platform that is stable at room temperature. The results from preclinical studies show that mucosal immunization with the oral vaccine candidate enhanced mucosal IgA in the upper airways. Given that severe disease in the older adult population is thought to be mediated by immunosenescence characterized by impaired T cell response, this vaccine candidate, which induces a humoral response, may be a promising intervention for the older adult population(81).
Response: The Vaxart RSV vaccine candidate has entered phase I clinical trials in June 2016, please refer to the PATH Vaccine snapshot as well as clinicaltrials.gov (NCT02830932). In the manuscript text, we have focused only on candidates in clinical trials including this candidate. Unfortunately, since phase I is recruiting we can only mention data from preclinical testing. The reference to the 2013 paper was included in reference to immunosenescence mediated by impaired T cell function and was not supposed to be related to this vaccine candidate. We agree with the reviewer that references need to be added into this section regarding the vaccine candidate in question and have done so. The enhanced mucosal IgA in the upper airways was presented at the 2017 RSV vaccines for the World conference but is as of yet unpublished in a peer-reviewed journal. Therefore, we have decided to cite the phase I data from the influenza vaccine candidate using the same oral platform and vector. Furthermore, we have included the preclinical data which are mentioned in a press release on the company website.

Revised text: Using the same oral adenovirus vaccine delivery platform, a phase I trial for influenza has been conducted, which showed a neutralizing antibody responses against influenza in the vaccine group and no interference of pre-existing vector immunity(96). Preclinical studies for the RSV vaccine candidate in the cotton rat model showed an increase in anti-F antibodies and protection against RSV challenge(97).

Comment 7 [Vector-based]: Line 415: The phrase "The candidate uses pre-F antigen" is unclear; the authors likely mean: "In this vaccine candidate, pre-F antigen is expressed in… etc".
Referring to: The candidate uses pre-F antigen expressed in the human adenovirus strain 26, a vector with a favorable safety profile when used for other infectious diseases(82,83).
Response: We have revised this according to reviewer's comments and agree that this is clearer.
Revised text: In this candidate pre-F antigen is expressed in the human adenovirus strain 26, a vector with a favorable safety profile when used for other infectious diseases(100,101).

Comment 8 [Live-attenuated]: Lines 469-474: There is ample evidence that live-attenuated vaccines are often inhibited by maternal/ circulating antibodies. What is the evidence that live-attenuated RSV vaccines generate a strong enough immune response in the presence of maternal antibodies (how robust were those responses), and if so, why would such a vaccine then be expected to be inhibited by the presence of RSV-specific circulating antibodies in older adults?
Referring to: Another benefit of live-attenuated vaccines in the pediatric population is their ability to generate an immune response despite the presence of maternally-acquired antibodies, and to elicit a more broad antibody and cellular response(89). Live-attenuated vaccines are likely limited to the pediatric population under two years of age, as pre-existing immunity in older populations might not permit sufficient replication to generate protective immune responses.
Response: We agree that this is not accurately written as there is evidence of interference due to pre-existing immunity for live-attenuated vaccines. We have rephrased this sentence to clarify that there is empirical evidence for live-attenuated RSV vaccines that they are able to replicate in the upper respiratory tract of young infants despite pre-existing maternally acquired antibodies. This is indeed not true of ALL live-attenuated vaccines in ALL populations. We hope the reviewer will find this modification acceptable. The evidence for this statement comes from two RSV live-attenuated vaccine candidates which have been tested in 1-2 month old infants in which viral peak titers in nasal wash specimens demonstrated equal or higher viral replication when compared to seronegative 6-24 month children (presumed to have no residual maternally-acquired antibodies).
Revised text: Another benefit of live-attenuated vaccines against RSV in young infants is their ability to replicate in the respiratory tract despite the presence of maternally-acquired antibodies, and to elicit a broad humoral and cellular response(108).

Comment 9 [Live-attenuated]: Lines 480-493: Of the five live-attenuated vaccine candidates in Phase I clinical trials, the results for only one, MEDI <DELTA>M2-2, are provided. What is the developmental stage of the other four and what are they?
Referring to: Five live-attenuated vaccine candidates in phase I clinical trials are being developed in partnership with the National Institutes of Health (NIH). Live-attenuated vaccines face the challenge of achieving sufficient attenuation to be safe while remaining immunogenic enough to induce a protective immune response, but improved understanding of the RSV viral genome has

informed the development of new vaccine candidates that may overcome this challenge. Two main modifications to the RSV genome have been engineered through reverse genetics: the ΔM2-2 deletion which attenuates viral replication and upregulates antigen expression(32) as well as the ΔNS2 deletion which reduces viral suppression of host interferon thereby boosting the innate immune response. RSV MEDI ΔM2-2 strongly reduced viral replication while inducing a strong primary serum neutralizing antibody as well as potent anamnestic response in RSV-seronegative infants and children(32). Further results from phase I clinical trials with live-attenuated vaccines are expected.

Response: The other four candidates are in Phase I clinical trials. We have not provided the results as they are not yet available in the public domain, this is also why we write "further results from phase I clinical trials with live attenuate vaccines are expected." We have rephrased this for clarity.

Revised text: Further results from phase I clinical trials with the other live-attenuated vaccine candidates are expected.

Comment 10 [Discussion]: Line 573: Typo: plans
Referring to: We attempted to collect data regarding expected plan for access to a preventive intervention in LMICs and expected pricing for all vaccine candidates, however this information was not publicly available.
Response: We have changed this as suggested by the reviewer.
Revised text: We attempted to collect data regarding expected plans for access to a preventive intervention in LMICs and expected pricing for all vaccine candidates, however this information is not publicly available.

Comment 11 [Table 1]: Page 32: Typo: ectodomain
Referring to: SHe: small hydrophobic protein ectodomiain
Response: Thank you for the observant correction. We have fixed the typo.
Revised text: SHe: small hydrophobic protein ectodomain

Comment 12 [Figure 1]: Page 36, Fig 1: Add ALRI explanation to legend.
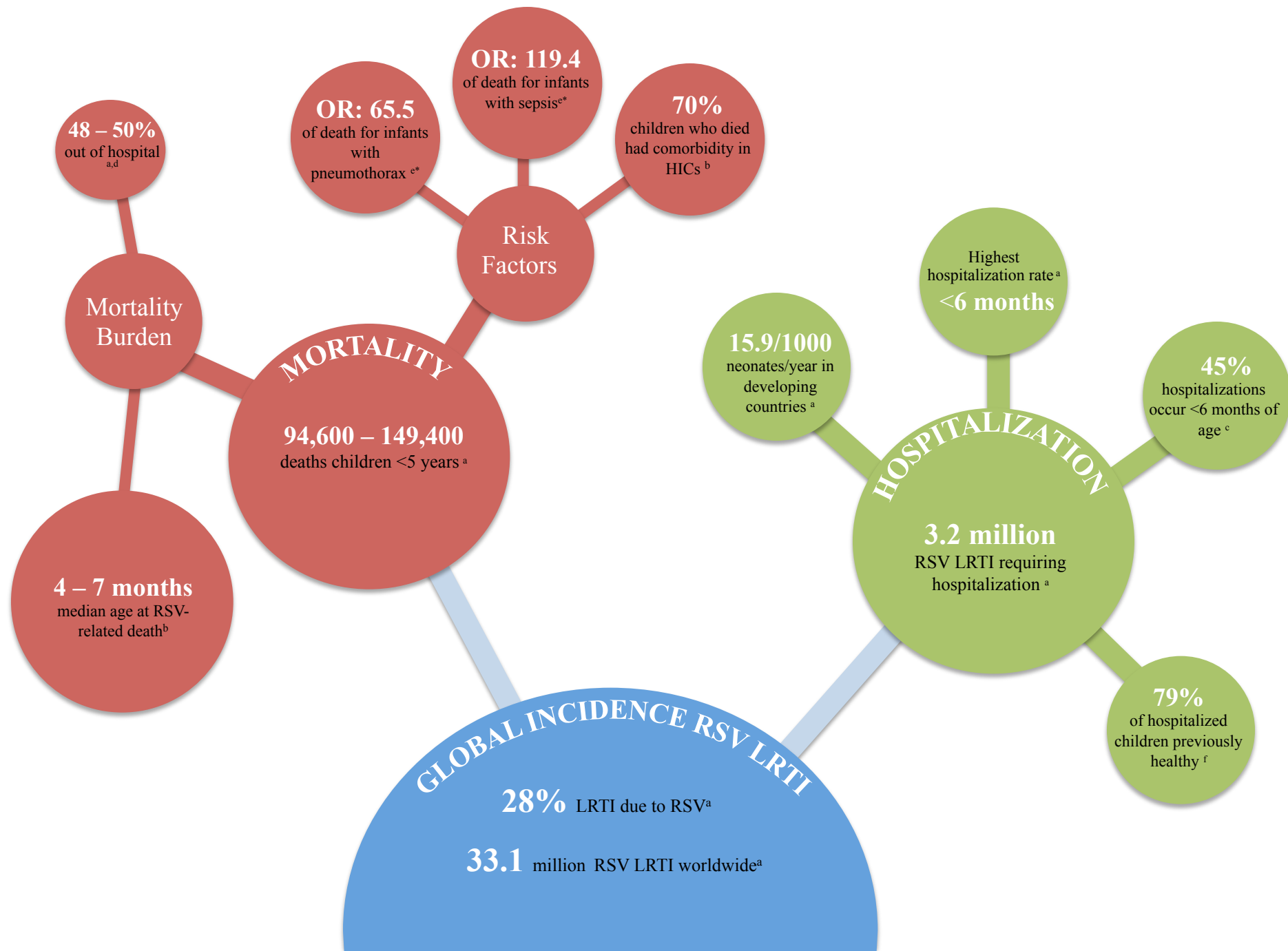Referring to: all ALRI mortality
Response: Instead of adding ALRI to the legend we have decided to consistently use LRTI throughout the figure.
Revised text: all LRTI mortality

Comment 13 [General]: Supplementary Table 1: only the first column is useful, the rest contains no heading or information, so can be deleted. Typo: Adjuvants
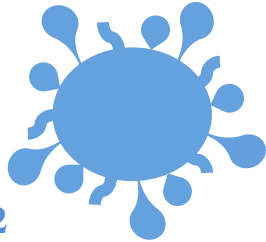Response: We agree that the rest of the template contains no useful information. However, we have added the column headings so that it is clear which template was used for data collection as this is key to the systematic collection of data for this manuscript. We have also fixed the typo you mention, many thanks for the observant correction.

Figure 1 & 2

Figure 1 & 2

- **48 – 50%** out of hospital [a,d]
- **Mortality Burden**
- **4 – 7 months** median age at RSV-related death [b]
- **OR: 65.5** of death for infants with pneumothorax [e*]
- **OR: 119.4** of death for infants with sepsis [e*]
- **70%** children who died had comorbidity in HICs [b]
- **Risk Factors**
- **MORTALITY** 94,600 – 149,400 deaths children <5 years [a]

HOSPITALIZATION
- **15.9/1000** neonates/year in developing countries [a]
- Highest hospitalization rate [a] **<6 months**
- **45%** hospitalizations occur <6 months of age [c]
- **3.2 million** RSV LRTI requiring hospitalization [a]
- **79%** of hospitalized children previously healthy [f]

GLOBAL INCIDENCE RSV LRTI
- **28%** LRTI due to RSV [a]
- **33.1** million RSV LRTI worldwide [a]

LIVE-ATTENUATED / CHIMERIC

R-BCG-N-hRSV
PHI

RSV D46/NS2/N/ △M2-2-HindIII
PHI

RSV LID △M2-2 1030s
PHI

RSV LID cp△M2-2
PHI

RSV cps2

RSV D46 cp△M2-2
PHI

MEDI-559

RSV △NS2△1313 I1314L
PHI

MONOCLONAL ANTIBODIES
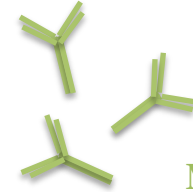
REGN-2222

MEDI8897
PH II

PARTICLE-BASED

RSV F nanoparticle
PHI/ II/ III

SynGEM
DEVELOPMENT HALTED

VECTOR-BASED

RSV001
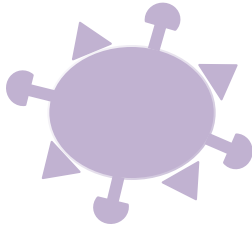
ChAd155-RSV
PH II

MVA-BN RSV
PH II

Ad26.RSV.preF
PHI/ II
HALTED

VXA-RSVf
PH I

MEDI-534

SUBUNIT

DS-Cav1
PHI

F-protein

DPX-RSV-SH
PHI

GSK RSV F
PHASE II HALTED

MEDI-7510

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