Safety, tolerability, and immunogenicity of the respiratory syncytial virus prefusion F subunit vaccine DS-Cav1: a phase 1, randomised, open-label, dose-escalation clinical trial

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Summary

Background Multiple active vaccination approaches have proven ineffective in reducing the substantial morbidity and mortality caused by respiratory syncytial virus (RSV) in infants and older adults (aged ≥65 years). A vaccine conferring a substantial and sustainable boost in neutralising activity is required to protect against severe RSV disease. To that end, we evaluated the safety and immunogenicity of DS-Cav1, a prefusion F subunit vaccine.

Methods In this randomised, open-label, phase 1 clinical trial, the stabilised prefusion F vaccine DS-Cav1 was evaluated for dose, safety, tolerability, and immunogenicity in healthy adults aged 18–50 years at a single US site. Participants were assigned to receive escalating doses of either 50 µg, 150 µg, or 500 µg DS-Cav1 at weeks 0 and 12, and were randomly allocated in a 1:1 ratio within each dose group to receive the vaccine with or without aluminium hydroxide (AlOH) adjuvant. After 71 participants had been randomised, the protocol was amended to allow some participants to receive a single vaccination at week 0. The primary objectives evaluated the safety and tolerability at every dose within 28 days following each injection. Neutralising activity and RSV F-binding antibodies were evaluated from week 0 to week 44 as secondary and exploratory objectives. Safety was assessed in all participants who received at least one vaccine dose; secondary and exploratory immunogenicity analysis included all participants with available data at a given visit. The trial is registered with ClinicalTrials.gov, NCT03049488, and is complete and no longer recruiting.

Findings Between Feb 21, 2017, and Nov 29, 2018, 244 participants were screened for eligibility and 95 were enrolled to receive DS-Cav1 at the 50 µg (n=30, of which n=15 with AlOH), 150 µg (n=35, of which n=15 with AlOH), or 500 µg (n=30, of which n=15 with AlOH) doses. DS-Cav1 was safe and well tolerated and no serious vaccine-associated adverse events deemed related to the vaccine were identified. DS-Cav1 vaccination elicited robust neutralising activity and binding antibodies by 4 weeks after a single vaccination ($p<0·0001$ for F-binding and neutralising antibodies). In analyses of exploratory endpoints at week 44, pre-F-binding IgG and neutralising activity were significantly increased compared with baseline in all groups. At week 44, RSV A neutralising activity was 3·1 fold above baseline in the 50 µg group, 3·8 fold in the 150 µg group, and 4·5 fold in the 500 µg group ($p<0·0001$). RSV B neutralising activity was 2·8 fold above baseline in the 50 µg group, 3·4 fold in the 150 µg group, and 3·7 fold in the 500 µg group ($p<0·0001$). Pre-F-binding IgG remained significantly 3·2 fold above baseline in the 50 µg group, 3·4 fold in the 150 µg group, and 4·0 fold in the 500 µg group ($p<0·0001$). Pre-F-binding serum IgA remained 4·1 fold above baseline in the 50 µg group, 4·3 fold in the 150 µg group, and 4·8 fold in the 500 µg group ($p<0·0001$). Although a higher vaccine dose or second immunisation elicited a transient advantage compared with lower doses or a single immunisation, neither significantly impacted long-term neutralisation. There was no long-term effect of dose, number of vaccinations, or adjuvant on neutralising activity.

Interpretation In this phase 1 study, DS-Cav1 vaccination was safe and well tolerated. DS-Cav1 vaccination elicited a robust boost in RSV F-specific antibodies and neutralising activity that was sustained above baseline for at least 44 weeks. A single low-dose of pre-F immunisation of antigen-experienced individuals might confer protection that extends throughout an entire RSV season.

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Or

Introduction

Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract infection in infants and older adults. Globally, RSV causes an estimated 33·1 million acute lower respiratory tract infections and 3·2 million hospitalisations in children under 5 years annually.[1] In adults aged 65 years or older, RSV contributed to 14·5% of hospital admissions in high-

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income countries and caused 8% of lower respiratory-related deaths in US hospitals.[2,3] Despite decades of effort, there is no licensed vaccine for RSV, and vaccine development has encountered several obstacles. In the 1960s, administration of formalin-inactivated RSV to antigen-naive children resulted in vaccine-enhanced respiratory disease upon subsequent RSV infection.[4–7] There is no defined correlate of protection from severe disease, complicating efforts to evaluate vaccine efficacy. Most RSV neutralising antibodies target the fusion (F) protein. RSV F exists in two major conformations: the active prefusion (pre-F), and the inactive postfusion (post-F) conformation adopted after triggering and rearrangement. Although several post-F-based vaccines have been shown to be immunogenic, they did not show efficacy in phase 2 and 3 clinical trials.[8] The DS-Cav1 subunit vaccine, which is stabilised in the pre-F conformation, maintains several neutralisation-sensitive antigenic sites on the trimer apex and elicits unprecedented levels of neutralising activity.[9,10]

A randomised, open-label, dose-escalation phase 1 clinical trial was done to evaluate the safety and immunogenicity of two DS-Cav1 immunisations in healthy adults aged 18–50 years. We previously reported interim data from 40 participants through 12 weeks following a single vaccination with 50 µg or 150 µg of DS-Cav1 with or without aluminium hydroxide (AlOH) adjuvant, showing that DS-Cav1 elicited robust neutralising activity and antibodies targeting the pre-F exclusive antigenic sites and sites present on both conformations of the F protein.[10] Here, we report the responses of all participants enrolled in the trial through completion of the study at week 44, showing that vaccination with DS-Cav1 is safe, well tolerated, and capable of eliciting sustained, high-potency neutralising activity against RSV.

Methods

Study design and participants

VRC 317 was a phase 1, open-label, randomised, domestic single-site clinical trial to evaluate dose, safety, tolerability, and immunogenicity of an investigational stabilised prefusion RSV F subunit protein vaccine, DS-Cav1, alone or with AlOH adjuvant. Eligible participants were healthy adults aged 18–50 years with body-mass index of 40 or less. Participants were recruited from the Washington, DC, metropolitan area by institutional review board-approved advertisements. Top recruitment sources include word of mouth and distribution of advertisements in person at the National Institutes of Health (NIH) and online at ResearchMatch.org. The trial was sponsored by the Vaccine Research Center (VRC) of the National Institute of Allergy and Infectious Diseases (NIAID) at the NIH in Bethesda, MD, USA and done at the NIH Clinical Center. The study was reviewed and approved by the institutional review board at NIAID. The protocol was subsequently amended to give participants the option to forgo the second immunisation with DS-Cav1 and complete the scheduled study visits to further assess immunogenicity of a single vaccine dose. The Department of Health and Human Services Guidelines for the protection of human research

Research in context

Evidence before this study

Respiratory syncytial virus (RSV) remains a significant cause of global morbidity and mortality, and there is currently no licensed vaccine. Several vaccines based on the concept of stabilising the F protein in the prefusion conformation (pre-F) to retain the most neutralisation-sensitive antigenic sites are in clinical evaluation. The pre-F subunit vaccine DS-Cav1 strongly boosted neutralising activity in healthy adults at doses of 50 µg and 150 µg with and without aluminium hydroxide (AlOH) adjuvant, yet the benefits of further increasing antigen dose and inclusion of AlOH adjuvant are unknown. More critically, the durability of DS-Cav1-boosted immunity and the effect of the dose and adjuvant on longitudinal RSV neutralising activity have not been determined. We searched PubMed and Google Scholar on Jan 2, 2021, with no language restrictions, for clinical trial reports addressing the durability of immune responses to stabilised pre-F subunit vaccines using the following search strategy: "RSV" AND "clinical trial" AND "pre-F subunit" AND "durability". We found no primary data reporting the durability of neutralising activity elicited by a structurally defined, stabilised pre-F subunit vaccine in humans.

Added value of this study

In this study, we delineate the immunogenicity of the DS-Cav1 subunit vaccine at escalating doses of 50 µg, 150 µg, and 500 µg, as well as the acute and longitudinal effect of altering dose, inclusion of the AlOH adjuvant, and administration of a second immunisation at week 12. We show sustained neutralising activity following DS-Cav1 vaccination and determine that, although increases in dose or a second immunisation have a modest transient effect, they do not further improve the maintenance of serum neutralising activity. Finally, we show increased levels of F-specific antibody in the mucosa of the upper respiratory tract after DS-Cav1 immunisation, indicating more RSV-specific immunity at the site of infection.

Implications of all the available evidence

We provide timely results anticipating the outcome of ongoing efficacy trials in the rapidly evolving field of RSV vaccines. Our results might be of particular relevance for the vaccination of healthy pregnant women, as our findings suggest that the use of higher doses of antigen and adjuvant are not necessary to provide sustained neutralising activity conferred by optimised, pre-F stabilised immunogens.

participants were followed and institutional review board approval was obtained. All participants provided written informed consent before enrolment. VRC investigators developed the vaccine, and the VRC Clinical Trials Program ran the study.

Randomisation and masking

Study procedures were followed as previously described.[10] Under the VRC 317 study protocol, participants were assigned to receive escalating doses of either 50 µg, 150 µg, or 500 µg DS-Cav1 and within each dose were randomly allocated 1:1 to receive the vaccine with or without AlOH adjuvant, using permuted block randomisation with block sizes of 4 or 6, chosen at random. Participants were not randomly allocated between doses, due to the dose-escalation design. The protocol statistician generated the randomisation sequence which was programmed into the database. Participants were assigned to a group upon electronic enrolment by a study clinician. Participants and investigators were not masked to group assignment.

Procedures

The prefusion subunit vaccine VRC-RSVRGP084–00-VP (DS-Cav1) was manufactured under cGMP conditions at the VRC Pilot Plant, in contract with the Vaccine Clinical Material Program, Leidos Biomedical Research (Frederick, MD, USA). DS-Cav1 is a sterile, aqueous, buffered solution that contains the RSV A2 fusion glycoprotein ectodomain assembled as a prefusion-stabilised trimer as described previously.[10] The vaccine is supplied in single dose vials. The adjuvant, Alhydrogel 2% (CRODA, Frederikssund, Denmark), is a GMP-grade AlOH suspension. To prepare the adjuvanted dose, DS-Cav1 was contemporaneously mixed with 500 µg of Alhydrogel before administration.

Following randomisation, a single unmasked injection of the assigned DS-Cav1 vaccine was administered in the deltoid muscle by needle and syringe at weeks 0 and 12, except in the participants who declined the optional second dose. Only those participants who had not received the second dose after enrolment of 71 participants (when amendment was approved) had the option to forgo the second dose. All second immunisations given at week 12 were administered at the same dose as the first. Participants were observed for at least 60 min following vaccination. Before discharge from the clinic, vital signs were recorded, and the injection site was inspected for evidence of local reaction.

Safety endpoints were obtained by patient reports, targeted physical examination, and medical history review, and monitoring of haematological and chemical parameters. Participants recorded solicited reactogenicity for 7 days after each vaccination. The solicited parameters included local events at the injection site of pain or tenderness, redness, and swelling, as well as systemic events of malaise, myalgia, headache, chills, nausea, and temperature. Adverse events were recorded for 28 days after each vaccination and were graded according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (modified from US Food and Drug Administration Guidance, September 2007). Severe adverse events and new chronic medical conditions were recorded for the duration of the study (weeks 0–44). A nasopharyngeal PCR swab was collected if participants experienced any symptoms of RSV infection while in the study. All participants were followed up to week 44.

Outcomes

The primary endpoints were vaccine safety and tolerability at each dose, assessed by solicited local and systemic reactogenicity and participant reports of adverse events up to 28 days after vaccine administration. The secondary endpoints include the evaluation of neutralising activity 4 weeks after each immunisation. All other endpoints were exploratory.

Statistical analysis

The sample size for this phase 1 dose escalation study was limited to a total of 95 participants receiving a DS-Cav1 vaccine, with 20 participants in the unadjuvanted 150 µg group and 15 participants in all other groups. In the group allocated to 150 µg without AlOH, five additional participants were enrolled who agreed to undergo apheresis under protocol VRC 200 (A Multicenter Specimen Collection Protocol to Obtain Human Biological Samples for Research Studies, NCT00067054). Therefore, a total of 35 participants were in the 150 µg dose group, and the 50 µg dose group and 500 µg dose group both included 30 participants each. This sample size was selected as adequate and reasonable for an initial review of the safety profile, with approximately 80% chance of seeing one or more adverse events in a dose group size of n=30 if the true rate is at last 5%. Immunogenicity analyses were secondary, but we estimated from previous data that the standard deviation of ELISA and neutralisation results might be approximately 0·5 on a $\log_{10}$ scale, and if that held, then we would have at least 90% power to detect a difference between groups of 0·45 $\log_{10}$ with n=15 in each group.

Methods for assessment of RSV neutralising activity and F-specific antibody readouts are included in the appendix (p 1). Lognormal immunology assay results were $\log_2$ transformed for analysis, and all analyses were done using R or GraphPad Prism version 7.0. For the primary endpoints, adverse event rates were summarised using proportions and Clopper-Pearson CIs. Secondary analysis focused on the antigen-specific antibody response using paired *t* tests to compare concentrations before and after doses, and linear regression to determine the significance of dose, adjuvant, and number of vaccinations in predicting the antibody concentration at week 4. Exploratory analysis repeated

See Online for appendix

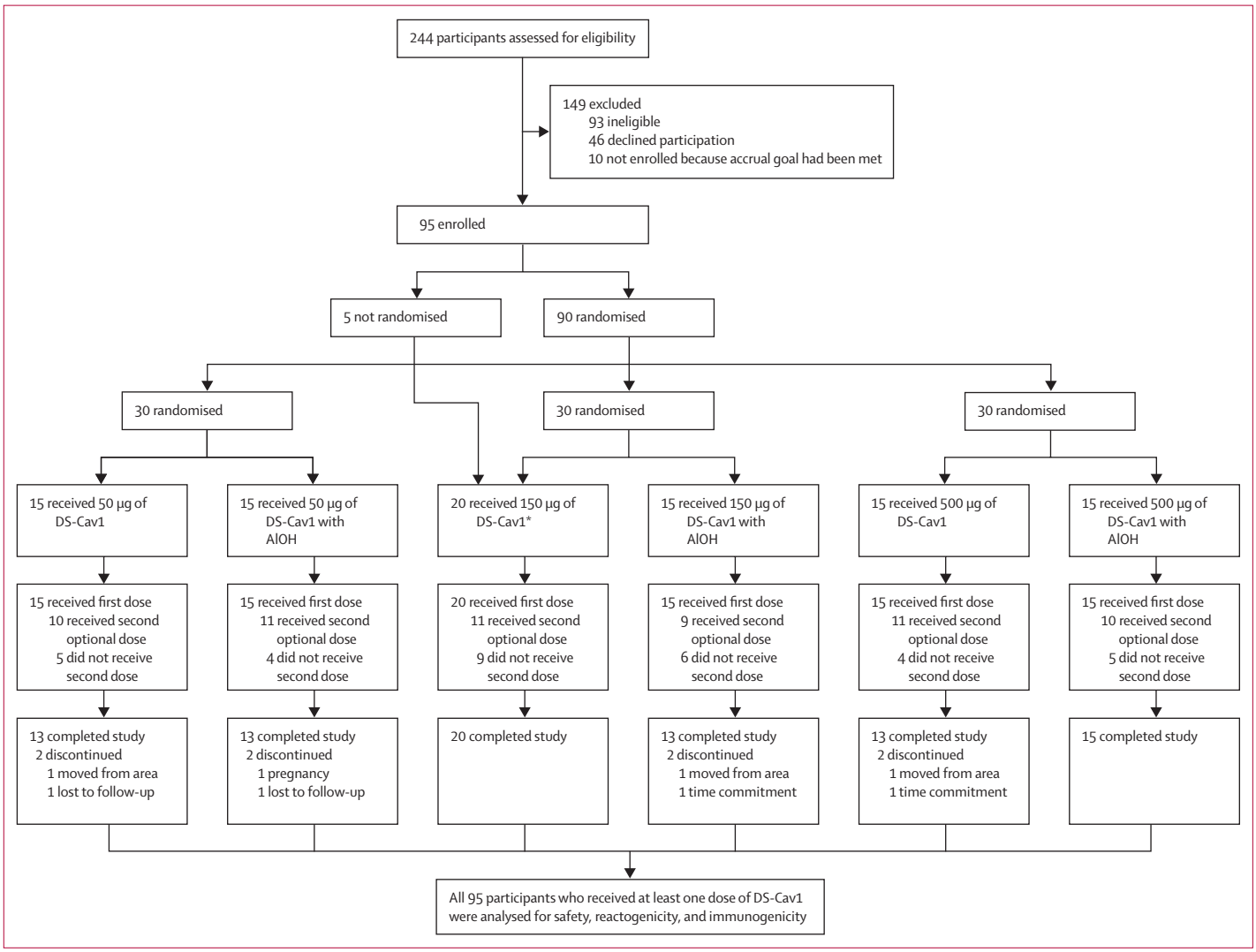

Figure 1: **Trial profile**
AlOH=aluminium hydroxide. *Five additional participants were enrolled who agreed to undergo apheresis under protocol VRC 200 (A Multicenter Specimen Collection Protocol to Obtain Human Biological Samples for Research Studies, NCT00067054).

these investigations at weeks 16 and week 44, supplemented by a longitudinal mixed model assuming random effects for participants, with fixed effects for dose, boosting, adjuvant, and baseline covariates as appropriate. Exploratory analyses were not prespecified and do not include adjustments for multiple comparisons, so we consider these results to be hypothesis generating, with those yielding p values of less than 0·01 taken as the most promising. All pairwise correlations are based on Spearman's correlation coefficient. Analyses were done by intention to treat, with assessment of safety in all participants that received at least one dose of DS-Cav1; secondary and exploratory immunogenicity analyses include all participants with available data at a given visit.

This study is registered with ClinicalTrials.gov, NCT03049488.

Role of the funding source
This work was supported with intramural funding from the NIAID. The funding source had no involvement with the study design, execution, or preparation of the manuscript.

Results
In total, 95 participants were enrolled and vaccinated from Feb 21, 2017, to Nov 29, 2018, and the final study visit occurred on Oct 3, 2019, (figure 1). The study population was composed of 49 women and 46 men, aged 18–50 years (mean age 31 years; table). Of the 95 participants included in the study, 62 were vaccinated with DS-Cav1 at both week 0 and week 12, and 33 did not have a second dose at week 12 and only received a single vaccination at week 0. 87 of the 95 participants completed the study. Of the eight participants who did not complete

	Group 1; 50 µg (n=15)	Group 2; 50 µg with AlOH (n=15)	Group 3; 150 µg (n=20)	Group 4; 150 µg with AlOH (n=15)	Group 5; 500 µg (n=15)	Group 6; 500 µg with AlOH (n=15)	Overall (n=95)
Sex							
Male	6 (40%)	9 (60%)	11 (55%)	7 (47%)	6 (40%)	7 (47%)	46 (48%)
Female	9 (60%)	6 (40%)	9 (45%)	8 (53%)	9 (60%)	8 (53%)	49 (52%)
Age, years	32·4 (5·8)	32·9 (9·3)	32·2 (9·6)	31·8 (9·3)	31·5 (8·5)	27·6 (7·6)	31·4 (8·5)
Race							
Asian	4 (27%)	1 (7%)	4 (20%)	0	3 (20%)	3 (20%)	15 (16%)
Black or African American	1 (7%)	3 (20%)	1 (5%)	4 (27%)	0	0	9 (9%)
White	10 (67%)	11 (73%)	13 (65%)	9 (60%)	12 (80%)	10 (67%)	65 (68%)
Multiracial	0	0	2 (10%)	2 (13%)	0	2 (13%)	6 (6%)
Ethnicity							
Hispanic or Latino	0	1 (7%)	2 (10%)	0	4 (27%)	1 (7%)	8 (8%)
Non-Hispanic or Non-Latino	15 (100%)	14 (93%)	18 (90%)	15 (100%)	11 (73%	14 (93%)	87 (92%)
Body-mass index	26·0 (3·5)	28·1 (6)	26·7 (4·1)	27 (4·2)	25·8 (3·3)	26·2 (3·5)	26·6 (4·1)
College or higher educational level	13 (87%)	13 (87%)	19 (95%)	14 (93%)	15 (100%)	15 (100%)	89 (94%)

Data are n (%) or mean (SD). AlOH=aluminium hydroxide.

Table: **Characteristics of the participants at enrolment**

the protocol visits as scheduled, three moved from the area, two were lost to follow-up, two withdrew due to the required time commitment, and one became pregnant.

In analysis of the primary endpoint of safety, there was no effect of antigen dose, number of immunisations, or adjuvant with respect to local reactogenicity. When present, reactogenicity was usually mild to moderate and most commonly consisted of local injection site pain or swelling, or systemic headache, malaise, or myalgias. Although not statistically significant, there was a trend toward increased symptoms of mild malaise, myalgia, and headache after the first vaccine dose and in participants who received AlOH adjuvant (appendix pp 7–9).

There were no severe adverse events reported (appendix pp 8–9). 18 adverse events (three in the 50 µg without adjuvant group, three in the 50 µg with adjuvant group, two in the 150 µg without adjuvant group, six in the 500 µg without adjuvant group, and four in the 500 µg with adjuvant group) were deemed as possibly or probably related to the study product, and all resolved without residual effects. Mild related adverse events included one episode each of headache, vivid dreams, leukocytosis, lymph node enlargement, elevated creatinine, lymphopenia, leukopenia, chest wall pain, and muscle spasm, three episodes of neutropenia, and two episodes of dizziness. Moderate related adverse events included three episodes of neutropenia and one episode of presyncope. There was no evidence of a dose, number of immunisations, or adjuvant effect with respect to adverse events.

One case of RSV was diagnosed in the 50 µg without adjuvant group during the trial. The participant displayed symptoms shortly after receiving the first dose of vaccine and tested positive for RSV from a nasal swab on day 5.

The secondary objective of the study was to measure the increase in neutralising activity elicited by DS-Cav1 vaccination. At week 4 after the first vaccination, there was a robust increase in neutralising activity to both RSV subtypes A and B in all dose groups ($p<0·0001$ compared with baseline; figure 2A, B, appendix p 9–10), with no effect of AlOH adjuvant within each dose (appendix p 2). RSV A neutralising activity waned between weeks 4 and 44, but remained 3·1-fold above baseline activity in the 50 µg group, 3·8-fold in the 150 µg group, and 4·5 fold in the 500 µg group ($p<0·0001$); our modelling (data not shown) suggested that it would take more than a year after the second immunisation for neutralising activity to return to the level measured at baseline (figure 2A, appendix p 9). A similar pattern was evident for RSV B neutralising activity which remained 2·8 fold (50 µg), 3·4 fold (150 µg), and 3·7 fold (500 µg) higher at week 44 than neutralising activity at baseline ($p<0·0001$; figure 2B, appendix p 10). At week 4, neutralising activity against RSV A was marginally higher in the 150 µg dose group compared with the 50 µg dose group ($p=0·016$, fold-change 10·5 in the 150 µg dose group and 7·5 in the 50 µg dose group) and highest in participants immunised with 500 µg of DS-Cav1 (fold-change 12·6; $p=0·002$ compared with the 50 µg dose group; figure 2C). By week 44, there was no significant difference in RSV A neutralisation between groups immunised with 50 µg and 150 µg, and only a marginal increase in RSV A neutralisation for the 500 µg dose compared with the 50 µg dose group ($p=0·016$, figure 2C; appendix p 9). At week 4, RSV B neutralising activity was significantly higher in 500 µg ($p=0·0002$) and 150 µg dose recipients ($p=0·0029$) compared with the 50 µg dose recipients (fold changes of 9·8, 8·4, and 6·4, respectively; figure 2D, appendix p 10). Similar to RSV A neutralising activity, only the 500 µg dose group had marginally higher RSV B neutralising activity than the 50 µg dose group at week 44

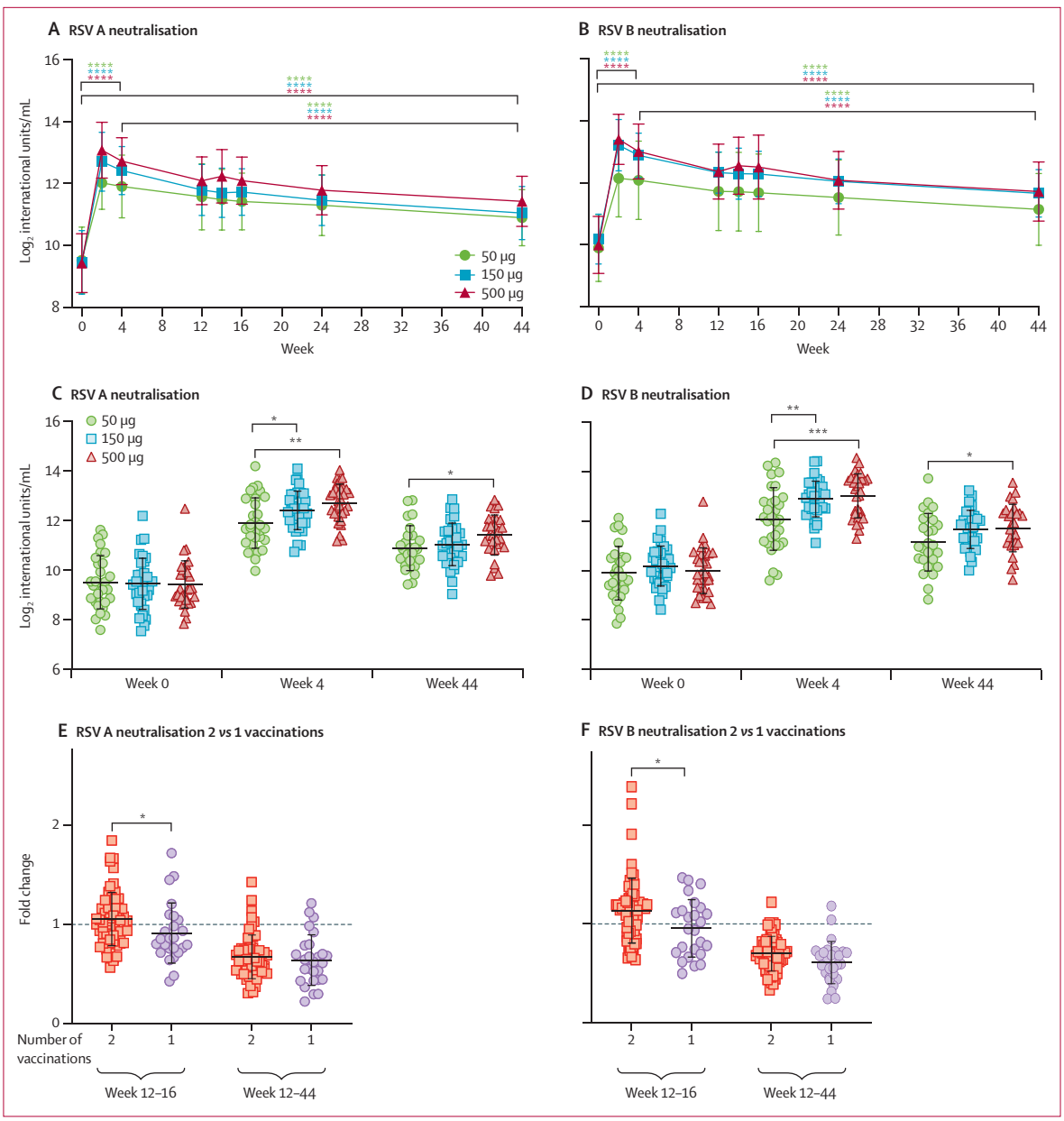

Figure 2: **RSV neutralising activity post DS-Cav1 vaccination**
A and B show neutralising activity in the serum against a reporter RSV A2 virus (RSV A) and RSV B18537 virus (RSV B), respectively at weeks 0, 2, 4, 12, 14, 16, 24, and 44 for participants immunised with 50 µg, 150 µg, or 500 µg of DS-Cav1 (adjuvanted and unadjuvanted groups combined for each dose). Significance determined using Student's *t* test to compare log fold-change (at specific timepoints) without adjustment for multiple comparisons. C and D show neutralising activity in the serum against RSV A and RSV B, respectively, for each participant vaccinated with 50 µg, 150 µg, or 500 µg of DS-Cav1 at baseline (week 0) and week 4, and week 44. For the number of samples obtained at each timepoint, see appendix (p 11). Significance between dose groups determined by linear regression. E and F show the fold-change in neutralising activity against RSV A and B, respectively, between week 12 and week 16 in participants who received two (n=59) or one (n=28) vaccinations and between week 12 and week 44 in these groups. Symbols (A, B) and horizontal lines (C–F) represent the mean, and error bars represent the SD. Dotted line represents a fold-change of 1. Significance determined by linear regression without adjustment for multiple comparisons. Significance indicated as ****p<0·0001, ***p<0·001, **p<0·01, and *p<0·05.

(p=0·028, figure 2D). Neutralising activity against RSV A was directed towards pre-F-exclusive sites as well sites shared by the pre-F and post-F protein (appendix p 3).

To address whether the second immunisation with DS-Cav1 at week 12 significantly affected neutralising activity, we compared the fold change in neutralising activity between week 12 to week 16 in participants who received both immunisations with DS-Cav1 (n=59; 59 of 62 participants had samples available at both time points) to those who only received a single immunisation (n=28; 28 of 33 participants receiving a single immunisation had samples available at both time points).

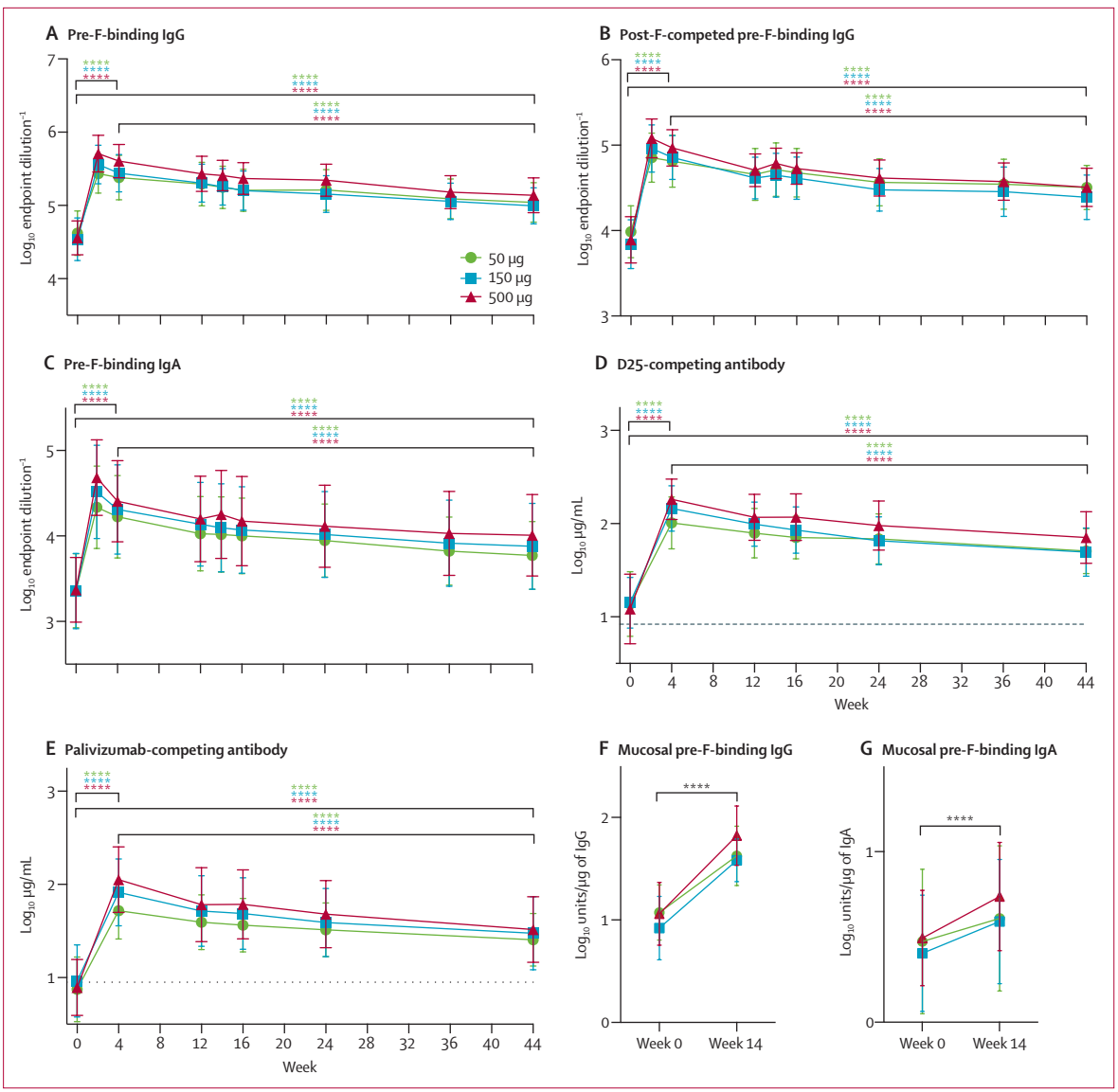

Figure 3: **Serum and mucosal antibody binding profile after DS-Cav1 immunisation**

Pre-F-binding IgG (A) and IgA (C) measured by ELISA and pre-F binding IgG in the presence of excess post-F (B) that competes for post-F exclusive and dual-binding antibodies, showing that binding antibodies are directed to the pre-F-exclusive antibodies and shared surfaces of pre-F and post-F antibodies. D shows the level of pre-F-binding antibodies that compete with binding of the D25 antibody (apex-binding) and panel E shows the level post-F-binding antibodies that competed with palivizumab antibody (side-binding). Statistical differences comparing week 0, week 4, and week 44 are determined by linear regression with no adjustment for multiple comparisons. For the number of samples obtained at each time point, see appendix (p 11). Adjuvanted and unadjuvanted groups combined for each dose. F and G show levels of pre-F-binding IgG (F; n=79 at week 0, n=77 at week 14) and IgA (G; n=78 at week 0, n=71 at week 14) in mucosal samples. Symbols represent the mean, and error bars represent the SD. Statistical significance between week 0 and week 14 was determined using a paired *t* test across all dose groups. Significance indicated as ****p<0·0001.

Neutralising activity against RSV A was marginally greater at week 16 in the participants that received a second immunisation compared with those who received only one immunisation (fold change 1·0 *vs* 0·91; p=0·031) and that against RSV B showed similar effect (0·82 *vs* 0·71; p=0·015; figure 2E, F). By week 44, there was no significant difference in neutralising activity between participants receiving two or one vaccinations, suggesting that durable neutralising activity is not affected by a second immunisation with DS-Cav1 (figure 2E, F).

Mirroring neutralising activity, the first immunisation with DS-Cav1 elicited a robust boost in serum IgG and IgA binding to subtype A pre-F at week 4 compared with baseline (p<0·0001, figure 3A, C). A significant increase in antibodies targeting pre-F-exclusive epitopes was confirmed by measurement of post-F-competed pre-F-binding IgG (figure 3B). Pre-F-binding IgG waned between week 4 and week 44 but remained significantly above baseline at week 44 (p<0·0001, 3·2 fold at 50 µg, 3·4 fold at 150 µg, and 4·0 fold at 500 µg). The 500 µg

dose group had significantly higher pre-F-binding IgG at week 4 than the 50 µg group (p=0·0003), but there was no significant difference by week 44 suggesting only a transient dose effect (appendix p 3). Compared with baseline, pre-F-binding serum IgA remained 4·1 fold (50 µg), 4·3 fold (150 µg), and 4·8 fold (500 µg) higher at week 44 (p<0·0001). Vaccine dose had no effect on pre-F-binding IgA at either week 4 or week 44 (appendix p 3). To further characterise serum antibody, we quantified antibodies that compete with D25 for binding to the apex of pre-F, and antibodies targeting surfaces shared by both conformations of F (palivizumab-competing, side-binding).[11] Apex-binding and side-binding antibodies were strongly induced by week 4 after vaccination (p<0·0001) and remained significantly above baseline at week 44 (p<0·0001; figure 3 D, E), showing that DS-Cav1 vaccination elicited robust and durable antibody responses targeting both the apex and the side of the F protein.

A second DS-Cav1 immunisation mitigated antibody waning between week 12 and week 16 as measured by pre-F binding IgG (p=0·0003) and IgA (p=0·0045) and concentrations of D25-competing or palivizumab-competing antibody (p=0·0002 and p=0·0005, respectively; appendix p 4). However, the difference in these measures was abrogated between week 12 and week 44 in participants receiving one or two immunisations, suggesting that a second immunisation of DS-Cav1 did not have an effect on long-term antibody concentrations (appendix p 4).

We did a correlates analysis to identify assays that could serve as a surrogate for neutralisation in large, advanced stage clinical trials. At week 4 and week 44, RSV A neutralisation was highly correlated with post-F-competed RSV A neutralisation (r=0·81 at week 4, r=0·85 at week 44) and RSV B neutralisation (r=0·79 at week 4, r=0·73 at week 44; appendix p 5). Pre-F-binding IgG and D25-competing antibody measures were highly correlated with RSV A neutralisation at week 4 (r>0·7; appendix p 5). At week 44, D25-competing antibody remained highly correlated with neutralising activity (r=0·76; appendix p 5). Post-F-binding IgG and palivizumab-competing antibody were less correlated with RSV A neutralisation (r=0·54 at week 4 and r=0·51 at week 44; appendix p 5), suggesting that these readouts might not serve as reliable surrogates for neutralisation.

As mucosal surfaces are the first line of defence against RSV, and mucosal F-specific antibodies have been correlated with protection from infection,[12–16] we quantified pre-F-specific IgG and IgA in nasopharyngeal fluid obtained at week 0 and week 14 (2 weeks after the second immunisation). At week 14, DS-Cav1 vaccination significantly boosted mucosal pre-F-specific IgG (p<0·0001), with a more modest increase in mucosal pre-F-specific IgA (p<0·0001; figure 3F, G; appendix p 6). The fold change in mucosal pre-F-specific IgG was similar to the fold change in pre-F-specific IgG in the serum. However, the fold-change in pre-F-specific IgA was greater in the serum than in the mucosa (appendix p 6). In summary, intramuscular immunisation with DS-Cav1 not only increased serum F-specific antibody and neutralising activity, but also significantly increased pre-F-binding IgG and IgA at the site of RSV infection.

Discussion

Analysis of all 95 participants in the VRC 317 phase 1 study to 44 weeks post-vaccination showed that the DS-Cav1 subunit vaccine was safe and well tolerated up to doses as high as 500 µg. One vaccination with subtype A protein was capable of eliciting a robust increase in neutralising activity against both RSV A and B subtypes that exceeds the increase seen after RSV infection in people in a similar age category.[12] A modest, transient advantage of increasing antigen dose or providing a second vaccination at 12 weeks was observed, but the effect of dose or number of vaccinations on long-term neutralising activity was marginal and unlikely to be clinically or biologically meaningful in an efficacy trial. Of note, the effect of a single dose on neutralising activity proved durable until completion of the trial at 44 weeks, suggesting the possibility that protective immunity could be improved for more than one RSV season.

In contrast to other trials comparing adjuvanted with unadjuvanted RSV F subunit protein in the post-F conformation, we observed no significant effect of AlOH adjuvant on immunogenicity.[17–19] This could be the result of age differences of vaccinated participants (60 years and older in previous studies *vs* 18–50 years old in this study) or the small number of participants in each dose group, but it is more likely due to the overall higher immunogenicity of pre-F,[9] which preserves and elicits potent antibody to pre-F exclusive sites not present on post-F. A different adjuvant might affect neutralising activity.[20] As evidenced by the similar induction and maintenance of antibodies to both the apex and side of the pre-F, antibodies are generated to pre-F exclusive and shared antigenic sites, and we did not observe a preferential elicitation or decay of antibodies of either specificity. The boost in polyclonal antibody to all neutralisation-sensitive epitopes offers an advantage over post-F, which presents fewer epitopes, and approaches such as prophylactic monoclonal antibody, for which efficacy might be susceptible to minor antigenic drift in selected RSV F epitopes.[8,21–23]

The greatest limitation of our study is that it is a small phase 1 study, where responses to vaccine were tested in 15–20 healthy adults per study group. The major target populations for a pre-F subunit protein vaccine are pregnant women and older adults. For maternal immunisation, the goal of vaccination is to extend the window in which placentally transferred antibody confers protection. Together with studies showing a near 1:1 mother to child transfer ratio for RSV-specific IgG, these data suggest that a single vaccination during the second or third trimester could result in neutralising

activity in infants at birth that exceeds typical levels by ten fold.[24] Maternal immunisation with an unstabilised F vaccine that boosted neutralising activity two to three fold afforded partial protection of infants up to 90 days of life.[25] Although our study did not involve pregnant women, a ten-fold rise in neutralising activity in infants could extend protection through the first 6 months of life, when an increase in airway size and maturation of the immune response improve clinical outcomes of RSV infection.[25–29] The robust antibody response in the absence of adjuvant is promising for maternal populations where adjuvant use might be contraindicated. Although there was no advantage for AlOH adjuvant in the young, healthy VRC 317 cohort, our study did not involve any older adults, and does not offer insight into whether adjuvant effects could counteract the waning immunity and comorbidities that increase risk of severe disease in adults older than 65 years. Vectored vaccine platforms are also being explored to deliver pre-F for this high-risk group.[30]

Mucosal IgA and IgG have been associated with protection from RSV infection and severe disease.[12–16] Intramuscular immunisation with a pre-F vaccine elicited a similar fold-increase in mucosal IgG in the nasopharynx and upper respiratory tract as in the sera. We would anticipate similar concentrations in the lower respiratory tract, where IgG could prevent the manifestation of severe disease.[24,26,31,32] There was also a modest increase of IgA in the mucosa which was not proportional to the more dramatic increase in serum IgA. This is not surprising, since intramuscular immunisation is not expected to induce anti-RSV secretory IgA compared with intranasal antigen delivery.[12]

In summary, DS-Cav1 offers a marked improvement over previous RSV vaccines based on post-F or structurally undefined F antigens. A single dose of unadjuvanted pre-F was able to induce robust and durable neutralising activity, which exceeded that of RSV infection. Although we cannot evaluate efficacy in this small phase 1 trial in a low-risk population, a sustained increase in neutralising activity in high-risk cohorts following DS-Cav1 immunisation or perinatal antibody transfer from vaccinated mothers might protect against severe lower respiratory tract disease caused by RSV infection, and consequently, possible long-term sequelae on lung development and function. Various vaccine approaches based on stabilised RSV pre-F antigens have advanced into clinical evaluation, and efficacy studies in both maternal and older adult populations are ongoing. After decades of RSV vaccine development efforts, a structure-guided approach to precision antigen design has yielded an effective immunogen and engendered hope that an RSV vaccine is on the near horizon. Furthermore, these findings have implications for vaccines against other enveloped viruses, such as the SARS-CoV-2 spike protein as a COVID-19 vaccine, showing the value of structure-based vaccine design and advantages of stabilising immunogens based on trimeric class I fusion proteins in the prefusion conformation.

Contributors

TJR, JRM, and BSG conceptualised the study. MCC, PJC, LAH, SPH, NMB, IJG, GVY, MRG, LAK, and JAS curated the data. TJR, KMM, EP, LAC, AMO, and MCN did the formal analysis. JRM and BSG acquired the funding. EP, LAC, BL, and RB did the investigations. TJR, EP, LAC, BL, RB, and MC overviewed the methodology. KMM, MCC, MRG, KC, and GC administered the project. AK overviewed the resources. MCC, RMS, JAS, KC, JGG, JRM, GC, and BSG supervised the project. TJR, EP, MCC, LAC, NMB, and LAK validated the data. KMM, EP, MCC, SPH, and TN visualised the data. TJR, KMM, EP, and BSG wrote the original draft. TJR, KMM, EP, MCC, PJC, LAH, LAC, SPH, NMB, IJG, GVY, MRG, BL, RB, MC, AMO, TN, RMS, LAK, JAS, KC, JGG, MCN, JRM, GC, and BSG edited and reviewed the manuscript. TJR and EP verified the final data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

MC and BSG are inventors on patents for the stabilisation of the RSV F protein. The other authors declared no competing interests.

Data sharing

Qualified researchers can request access to the raw data and clinical study protocol with amendments. Study documents will be redacted to protect the privacy of trial participants. For access, please contact the corresponding authors. Approvals are at the discretion of the authors.

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References

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

2 Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. *N Engl J Med* 2005; **352:** 1749–59.

3 Shi T, Denouel A, Tietjen AK, et al. Global disease burden estimates of respiratory syncytial virus-associated acute respiratory infection in older adults in 2015: a systematic review and meta-analysis. *J Infect Dis* 2020; **222** (suppl 7): 577–83.

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

5 Chin J, Magoffin RL, Shearer LA, Schieble JH, Lennette EH. Field evaluation of a respiratory syncytial virus vaccine and a trivalent parainfluenza virus vaccine in a pediatric population. *Am J Epidemiol* 1969; **89:** 449–63.

6 Fulginiti VA, Eller JJ, Sieber OF, Joyner JW, Minamitani M, Meiklejohn G. Respiratory virus immunization: a field trial of two inactivated respiratory virus vaccines; an aqueous trivalent parainfluenza virus vaccine and an alum-precipitated respiratory syncytial virus vaccine. *Am J Epidemiol* 1969; **89:** 435–48.

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

8 Mazur NI, Higgins D, Nunes MC, et al. The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates. *Lancet Infect Dis* 2018; **18:** e295–311.

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

10 Crank MC, Ruckwardt TJ, Chen M, et al. A proof of concept for structure-based vaccine design targeting RSV in humans. *Science* 2019; **365:** 505–09.

11 Phung E, Chang LA, Morabito KM, et al. Epitope-specific serological assays for RSV: conformation matters. *Vaccines (Basel)* 2019; **7:** E23.

12 Habibi MS, Jozwik A, Makris S, et al. Impaired antibody-mediated protection and defective IgA B-cell memory in experimental infection of adults with respiratory syncytial virus. *Am J Respir Crit Care Med* 2015; **191:** 1040–49.

13 Vissers M, Ahout IML, de Jonge MI, Ferwerda G. Mucosal IgG levels correlate better with respiratory syncytial virus load and inflammation than plasma IgG levels. *Clin Vaccine Immunol* 2015; **23:** 243–45.

14 Bagga B, Cehelsky JE, Vaishnaw A, et al. Effect of preexisting serum and mucosal antibody on experimental respiratory syncytial virus (RSV) challenge and infection of adults. *J Infect Dis* 2015; **212:** 1719–25.

15 Mills J 5th, Van Kirk JE, Wright PF, Chanock RM. Experimental respiratory syncytial virus infection of adults. Possible mechanisms of resistance to infection and illness. *J Immunol* 1971; **107:** 123–30.

16 Walsh EE, Falsey AR. Humoral and mucosal immunity in protection from natural respiratory syncytial virus infection in adults. *J Infect Dis* 2004; **190:** 373–78.

17 Falloon J, Yu J, Esser MT, et al. An adjuvanted, postfusion F protein-based vaccine did not prevent respiratory syncytial virus illness in older adults. *J Infect Dis* 2017; **216:** 1362–70.

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

19 Leroux-Roels G, De Boever F, Maes C, Nguyen TL, Baker S, Gonzalez Lopez A. Safety and immunogenicity of a respiratory syncytial virus fusion glycoprotein F subunit vaccine in healthy adults: results of a phase 1, randomized, observer-blind, controlled, dosage-escalation study. *Vaccine* 2019; **37:** 2694–703.

20 Cunningham AL, Lal H, Kovac M, et al. Efficacy of the herpes zoster subunit vaccine in adults 70 years of age or older. *N Engl J Med* 2016; **375:** 1019–32.

21 Ruckwardt TJ, Morabito KM, Graham BS. Immunological Lessons from respiratory syncytial virus vaccine development. *Immunity* 2019; **51:** 429–42.

22 Ruzin A, Pastula ST, Levin-Sparenberg E, et al. Characterization of circulating RSV strains among subjects in the OUTSMART-RSV surveillance program during the 2016–17 winter viral season in the United States. *PLoS One* 2018; **13:** e0200319.

23 Griffin MP, Yuan Y, Takas T, et al. Single-Dose Nirsevimab for prevention of RSV in preterm infants. *N Engl J Med* 2020; **383:** 415–25.

24 Chu HY, Steinhoff MC, Magaret A, et al. Respiratory syncytial virus transplacental antibody transfer and kinetics in mother-infant pairs in Bangladesh. *J Infect Dis* 2014; **210:** 1582–89.

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

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

27 Ruckwardt TJ, Morabito KM, Graham BS. Determinants of early life immune responses to RSV infection. *Curr Opin Virol* 2016; **16:** 151–57.

28 Lambert L, Sagfors AM, Openshaw PJM, Culley FJ. Immunity to RSV in early-life. *Front Immunol* 2014; **5:** 466.

29 Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet* 2010; **375:** 1545–55.

30 Sadoff J, De Paepe E, Haazen W, et al. Safety and immunogenicity of the Ad26.RSV.preF investigational vaccine coadministered with an influenza vaccine in older adults. *J Infect Dis* 2021; **223:** 699–708.

31 Lamprecht CL, Krause HE, Mufson MA. Role of maternal antibody in pneumonia and bronchiolitis due to respiratory syncytial virus. *J Infect Dis* 1976; **134:** 211–17.

32 Ogilvie MM, Vathenen AS, Radford M, Codd J, Key S. Maternal antibody and respiratory syncytial virus infection in infancy. *J Med Virol* 1981; **7:** 263–71.