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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☆

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ABSTRACT

Background: Respiratory syncytial virus (RSV) causes significant illness in older adults resulting in substantial health and economic impact. A successful vaccine would reduce morbidity in this growing segment of the population.
Methods: In this double-blind phase 1 study, subjects 60 years of age and older were enrolled by cohort and randomized to receive vaccines containing escalating doses (20, 50, or 80 μg) of soluble RSV fusion protein (sF) alone or adjuvanted with 2.5 μg of glucopyranosyl lipid A, a toll-like receptor-4 agonist, in 2% stable emulsion (GLA-SE). Each cohort included 20 vaccine and 4 placebo recipients. Immune responses were evaluated using assays for RSV microneutralizing, anti-F IgG, and palivizumab competitive antibodies and for F-specific interferon (IFN)-γ enzyme-linked immunospot (ELISPOT) responses.
Results: The inclusion of adjuvant increased local reactogenicity, with the majority of subjects who received sF and adjuvant reporting low-grade injection site pain or tenderness. At all doses, the safety profile was acceptable for further development. Immune responses were antigen dose-dependent, and the inclusion of adjuvant increased both humoral and cellular immune responses, with responses statistically higher than for placebo recipients in all 4 assays. At the highest dosage level with adjuvant, half of the subjects had a ≥3-fold rise from day 0 in RSV neutralizing antibody titers, and all had a ≥3-fold rise in antibody levels by anti-F IgG and palivizumab competitive antibody assays on day 29. For the day 8 IFNγ ELISPOT assay, 74% of subjects in the highest dosing cohort had a ≥3-fold rise from baseline.
Conclusions: The safety and immunogenicity results from this study support inclusion of the GLA-SE adjuvant in this RSV vaccine for older adults and also support assessment of the efficacy of the vaccine in a larger clinical trial. Clinicaltrials.gov NCT02115815.
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1. Introduction

Respiratory syncytial virus (RSV) is gaining recognition as an important cause of disease in older adults [1–3]. RSV-induced illness is clinically indistinguishable from influenza and has a peak incidence in winter in temperate zones [4,5]. Physicians do not routinely test for RSV in adults, as there is no specific treatment; however, RSV is a recognized cause of adult illness worldwide [6–8]. Estimates suggest that the current burden of RSV in older adults is comparable to, or greater than, that of influenza [9]. RSV reinfection is common, presumably because of a short duration of immunity after natural infection [10,11]. An adult vaccine would most likely require annual administration, which could occur at

Abbreviations: ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot; GLA, glucopyranosyl lipid A; GLA-SE, glucopyranosyl lipid A in stable emulsion; IFN-γ, interferon gamma; MN, microneutralizing; PCA, palivizumab competitive antibodies; SE, stable emulsion; sF, soluble RSV fusion protein; SFC, spot forming cells; TLR-4, toll-like receptor-4.

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the time of influenza vaccination. An effective RSV vaccine for older adults would decrease RSV-related morbidity and be cost-effective [12,13].

We report the results of a phase 1 study of investigational RSV vaccines as the initial step in developing a vaccine for the prevention of RSV disease in adults ≥60 years of age. This population was selected because RSV disease severity increases with age, likely due to the increase in comorbid conditions as well as to immunosenescence [14–16]. Given the ubiquity of RSV infection, all adults are seropositive. Older adults have antibody levels similar to those of younger adults, and higher antibody titers are associated with decreased risk of RSV disease in older adults [16,17]. However, cell-mediated immune responses to RSV are deficient in older adults compared with younger adults [17]. Thus, the use of an adjuvant selected to boost cellular as well as humoral immunity was assessed in this study.

The RSV vaccines tested in this study contained soluble RSV fusion protein (sF) in the postfusion form. F protein is essential for productive infection and is the only highly conserved RSV protein capable of eliciting substantial neutralizing antibody titers [18]. RSV F is a clinically validated target, as demonstrated by the efficacy of palivizumab, a monoclonal antibody that binds to both the pre- and postfusion forms of F and decreases severe RSV disease in high-risk infants [19]. Unlike influenza vaccines, which require annual reformulation based upon worldwide circulating influenza strains, an RSV F vaccine is expected to maintain annual efficacy without requiring reformulation.

The effect of the adjuvant glucopyranosyl lipid A (GLA), a synthetic analog of monophosphoryl lipid A (a toll-like receptor-4 [TLR-4] agonist) formulated in a squalene-based oil-in-water stable emulsion (SE), on the immunogenicity of the investigational vaccines was evaluated in this study. GLA stimulates immune responses conducive to a T-helper type 1 vaccine response [20]. In animal models, a vaccine containing RSV sF and adjuvant GLA-SE elicited high neutralizing antibody titers and a T-helper type 1-biased immune response with strong F-specific interferon (IFN)-γ T-cell responses, a response profile that we hypothesize will be optimal in older adults [21,22].

We present data from our first-time-in-human study of an RSV sF vaccine assessed in the presence and absence of adjuvant: 2.5 μg of GLA in 2% SE (GLA-SE). The primary objective was to assess the safety and tolerability of a single ascending dose of RSV sF alone and in the presence of GLA-SE in adults ≥60 years of age who were healthy or who had stable, chronic underlying medical conditions. RSV-specific humoral and cellular immune responses pre- and postvaccination were assessed.

2. Materials and methods

2.1. Ethics statement

The study (clinicaltrials.gov NCT02115815) was carried out in accordance with the Declaration of Helsinki and good clinical practice guidelines. The study protocol and amendments, along with the subject informed consent document, were approved by an institutional review board. All subjects provided written informed consent prior to any study procedures.

2.2. Study design

This was a double-blind, randomized, placebo-controlled study. Subjects were randomized at three sites in the United States between April and June 2014. Cohorts of 24 subjects were enrolled consecutively; allocation to cohort was unblinded. The 6 cohorts consisted of 3 dosage levels of RSV sF (20, 50, and 80 μg) and those same doses of RSV sF with GLA-SE. Within a cohort, subjects were randomly allocated by an internet-based interactive response system in a 5:1 ratio (20 active, 4 saline placebo). In a preplanned interim analysis, the study was partially unblinded after subjects had completed 90 days of safety follow-up. Site staff and subjects remained blinded through the end of the study (day 361).

2.3. Subject selection

Subjects were required to be ≥ 60 years of age and not institutionalized or homebound, to weigh ≥110 lbs (50 kg), and to have normal hemoglobin concentrations. Major exclusions included an unstable medical condition or recent change in therapy, receipt of products that could include exogenous antibodies, clinically significant abnormalities in screening tests, viral hepatitis, history of or current autoimmune disorder other than hypothyroidism, immunosuppression, body mass index ≥ 40 kg/m^2, or medications or conditions that could cause injection site bleeding.

2.4. Vaccine preparation and administration

RSV sF (in the post-fusion conformation [18]), which differs from the pre-fusion conformation described by McLellan et al. [23] was expressed in a Chinese hamster ovary cell line. GLA-SE was provided by and licensed from Immune Design Corporation pursuant to an existing agreement (Seattle, WA). Dose preparation occurred on-site with administration within 3 h of mixing and 30 min of syringe filling. The barrel was covered to blind administration. A single 0.5 mL intramuscular dose was administered into the deltoid muscle with needle gauge and length selected for gender and weight [24].

2.5. Safety assessments

Study visits occurred on dosing day 1 and (approximately) post-dose days 8, 29, 61, 91, 181, 271, and 361. Safety data assessed included specified solicited symptoms collected by diary card during days 1–7; adverse events (AEs) collected days 1–28; and serious AEs, new onset chronic diseases, and AEs of special interest including autoimmune events, collected days 1–361. An independent, external safety monitoring committee reviewed 7 days of safety data for cohort escalation. Interim follow-up by telephone occurred at least monthly. Toxicity grading was in general accordance with a standard toxicity table [25].

2.6. Immunogenicity assessments

Immunogenicity data collected included RSV microneutralizing (MN), anti-F IgG (F IgG), and palivizumab competitive antibodies (PCA) and F-specific IFN-γ enzyme-linked immunospot (ELISPOT) responses. RSV-neutralizing antibody titers in heat-inactivated sera were measured using a green fluorescent protein-tagged RSV A2 MN assay (without complement) as previously described [26]. Anti-F IgG antibodies were determined using a multiplex F, Ga, Gb (RSV A and B subtypes of the G protein), and N-specific IgG assay [27] developed on the Meso Scale Discovery platform (Gaithersburg MD). Subjects with a ≥4-fold rise in Ga, Gb, or N-specific antibodies were considered to be exposed to wildtype RSV, and their immunogenicity data were excluded from that timepoint onward; however, no subject was excluded from day 29 analyses. PCAs were assessed using an enzyme-linked immunosorbant assay (ELISA) format where biotin-labeled palivizumab was mixed with serum samples and added to RSV F antigen-coated plates, and bound palivizumab was detected with horseradish peroxidase-conjugated streptavidin. PCA values were reported relative to a reference standard. Values below the lower limit of quantification

Table 1
Solicited symptoms reported during days 1–7 by treatment group.

Solicited symptom	Placebo *N* = 24 *n* (%)	RSV sF 20 μg *N* = 20 *n* (%)	RSV sF 20 μg + GLA-SE[a] *N* = 20 *n* (%)	RSV sF 50 μg *N* = 20 *n* (%)	RSV sF 50 μg + GLA-SE[a] *N* = 20 *n* (%)	RSV sF 80 μg *N* = 20 *n* (%)	RSV sF 80 μg + GLA-SE[a] *N* = 20 *n* (%)
Any symptom	6 (25.0)	4 (20.0)	15 (75.0)	7 (35.0)	14 (70.0)	9 (45.0)	17 (85.0)
Fatigue or tiredness	4 (16.7)	3 (15.0)	4 (20.0)	4 (20.0)	2 (10.0)	4 (20.0)	6 (30.0)
Fever ≥100.4 °F	0	1 (5.0)	0	0	0	0	0
Generalized muscle aches	1 (4.2)	1 (5.0)	2 (10.0)	1 (5.0)	3 (15.0)	1 (5.0)	2 (10.0)
Headache	2 (8.3)	2 (10.0)	3 (15.0)	3 (15.0)	1 (5.0)	4 (20.0)	2 (10.0)
Pain at the site of injection	1 (4.2)	0	8 (40.0)	1 (5.0)	13 (65.0)	1 (5.0)	12 (60.0)
Redness at the site of injection	0	0	1 (5.0)	0	1 (5.0)	0	0
Swelling at the site of the injection	0	0	0	0	2 (10.0)	0	1 (5.0)
Tenderness or soreness at the site of the injection	1 (4.2)	1 (5.0)	13 (65.0)	4 (20.0)	11 (55.0)	2 (10.0)	13 (65.0)

GLA = glucopyranosyl lipid A; RSV = respiratory syncytial virus; SE = stable emulsion; sF = RSV soluble fusion protein.
[a] 2.5 μg of GLA in 2% SE.

(LLOQ) were assigned a value of 2.28 μg/mL (1/2 of the LLOQ) for analyses. The ELISPOT assay was performed using RSV F peptides as previously described [28]. For results <LLOQ, a value equal to the LLOQ (33.3 spot-forming cells [SFC] per 10^6 peripheral blood mononuclear cells) was imputed. Seroresponse data are presented as ≥3-fold rise from baseline. Based on the precision of the assays, there was a <5% chance that a 3-fold rise from baseline would be due to chance.

2.7. Statistical analysis

The sample size was selected to provide initial assessment of safety and immunogenicity; cohort size did not support multiple pairwise comparisons between treatment groups. Placebo data from 6 cohorts were grouped for presentation. There was no imputation of missing data. Formal statistical comparisons among groups were not performed; for data presented with 95% confidence intervals (CIs), nonoverlapping CIs determined statistical significance. Correlation among assays was assessed with Pearson correlation for pairwise responses.

3. Results

3.1. Subjects

As planned, 144 subjects (24 per cohort) received vaccine or placebo as allocated (Fig. S1). Two placebo recipients withdrew consent; all others were followed through day 361. The median age at enrollment was 68 (range 60–87) years, and 42% were >69 years of age. Half (53%) were male, 89% were White race, and 34% were Hispanic/Latino ethnicity (Table S1). Pre-existing illness was common: for example, hypertension 51%, lipid disorder 43%, diabetes mellitus 18%, hypothyroidism 12%, ongoing pulmonary disease 7%, coronary artery disease 7%.

3.2. Tolerability and safety

Subjects receiving vaccines containing adjuvant were more likely to report solicited symptoms, most commonly pain or tenderness at the injection site (Table 1). All solicited symptoms were mild-to-moderate in severity and generally of short duration; for pain or tenderness, maximum duration was 2 days. The single fever observed was associated with gastroenteritis. The pattern of events was not antigen dose-dependent.

All AEs during days 1–28 were grade 1 or 2 except for a grade 3 event of viral gastroenteritis in a subject who received RSV sF 20 μg, and two grade 3 events in two subjects who received 50 μg of RSV sF: bladder cancer detected after hematuria was observed on day 7, and day 1 worsening of staphylococcal abscesses on the neck present at dosing. AEs occurred in only one subject per cohort, except for back pain, which occurred in two subjects who received 50 μg of RSV sF. Serious AEs through day 361 are presented in Table S2 and new onset chronic diseases in Table S3. None were considered related to study dosing. No AE of special interest was reported. Two subjects developed hypothyroidism, but both had received only RSV sF; no other potentially autoimmune events were reported.

3.3. Immunogenicity

All subjects had pre-existing antibodies as measured in the MN and F-specific IgG assays at baseline; however 47.2% had baseline values <LLOQ in the PCA assay, and 63.9% did not have measurable F-specific IFN-γ T cells at baseline. Day 29 immune responses were RSV sF dose-dependent and were enhanced by GLA-SE (Figs. 1 and 2). At all dosage levels, humoral and cellular responses in all immunogenicity assays were significantly higher than those in placebo recipients (Fig. 2). At the highest dosage level, 80 μg of RSV sF + GLA-SE, the mean $\log_2$ MN titer on day 29 was 10.46 (95% CI: 10.08, 10.84) compared with 8.75 (95% CI: 8.16, 9.34) in placebo recipients. Among subjects in the highest dose group, 50% (95% CI: 27.2, 72.8), 100% (95% CI: 83.16, 100), and 100% (95% CI: 83.16, 100) had a geometric mean fold rise from baseline (GMFR) in MN, F IgG, and PCA values, respectively, that was at least ≥3-fold higher than baseline compared with 0 (95% CI: 0, 14.25) for each assay in placebo recipients (Fig. 2). For the IFN-γ ELISPOT assay, 73.7% (95% CI: 48.80, 90.85) of subjects at this highest dose had a ≥3-fold rise from baseline compared with 0 (95% CI: 0, 17.65) placebo recipients.

Humoral assay results were significantly correlated with one another (Fig. 3) and, to a lesser extent, with IFN-γ ELISPOT results: Pearson correlations were 0.763–0.852 for relationship between humoral assay results and 0.231–0.281 between humoral and ELISPOT responses. There was a trend among humoral responses for an effect of baseline value on the magnitude of antibody rise (data not shown), with those subjects entering the study with levels below the median having a greater increase than those with higher baseline values.

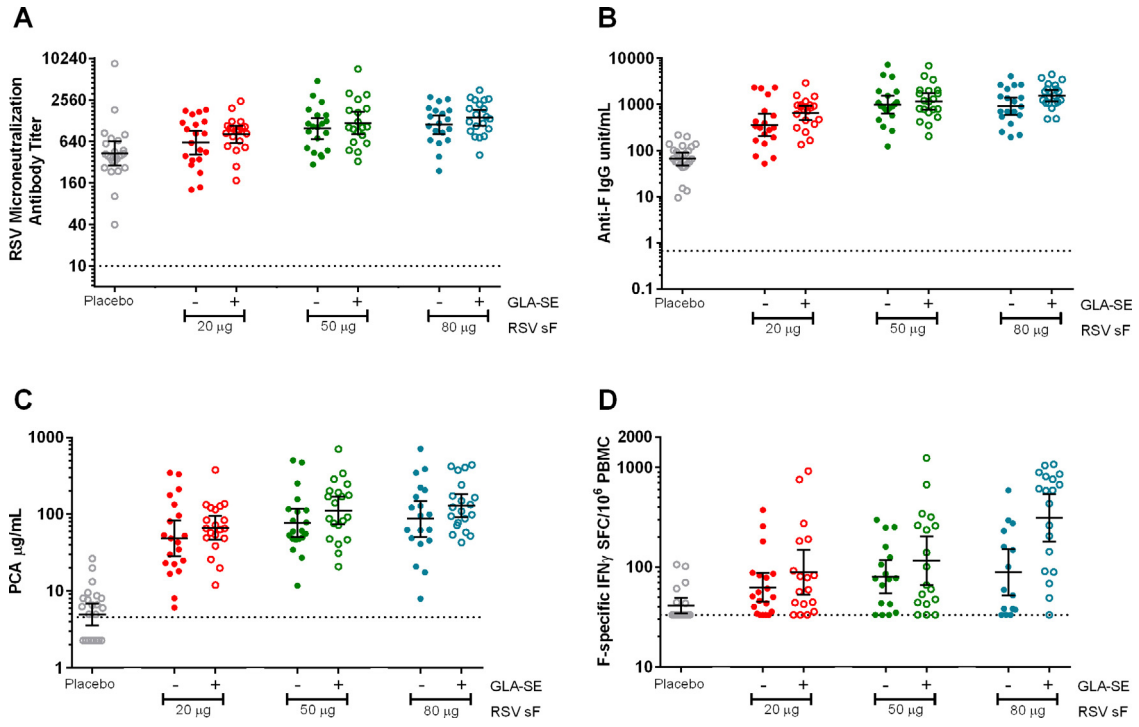

Fig. 1. Immunogenicity of an RSV sF vaccine dosed at 20, 50, and 80 µg, unadjuvanted and adjuvanted with 2.5 µg of GLA in 2% SE (GLA-SE). Panel A: RSV microneutralization titers, postdose day 29; Panel B: anti-F IgG antibodies, postdose day 29; Panel C: palivizumab competitive antibodies, postdose day 29; Panel D: RSV F-specific interferon-γ ELISPOT SFCs/10^6 PBMCs, postdose day 8. Bars represent 95% confidence intervals. For microneutralization responses, there was overlap of 95% CIs between placebo and RSV sF 20 µg alone and with GLA-SE only. Dotted line = lower limit of quantitation. Ab = antibody; ELISPOT = enzyme-linked immunospot; GLA = glucopyranosyl lipid A; PBMC = peripheral blood mononuclear cell; RSV = respiratory syncytial virus; SE = stable emulsion; sF = soluble fusion protein; SFC = spot-forming cells.

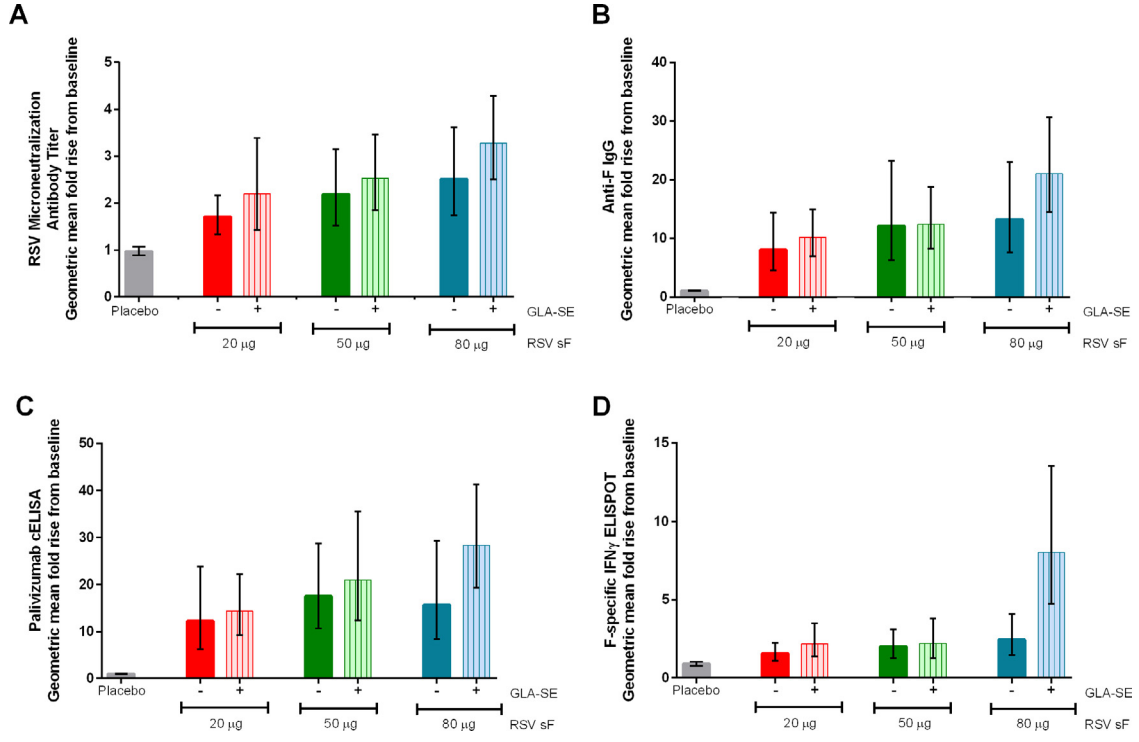

Fig. 2. Geometric mean fold rises from baseline in antibody (day 29) and interferon-γ ELISPOT values (day 8) after receipt of an RSV sF vaccine dosed at 20, 50, and 80 µg, unadjuvanted and adjuvanted with 2.5 µg of GLA in 2% SE (GLA-SE). Panel A: RSV microneutralization antibody titers; Panel B: anti-F IgG antibodies; Panel C: palivizumab competitive antibodies; Panel D: RSV F-specific interferon-γ ELISPOT responses. Bars represent 95% confidence intervals (CI). No vaccine CI overlapped with that of placebo. ELISPOT = enzyme-linked immunospot; GLA = glucopyranosyl lipid A; RSV = respiratory syncytial virus; SE = stable emulsion; sF = soluble fusion protein.

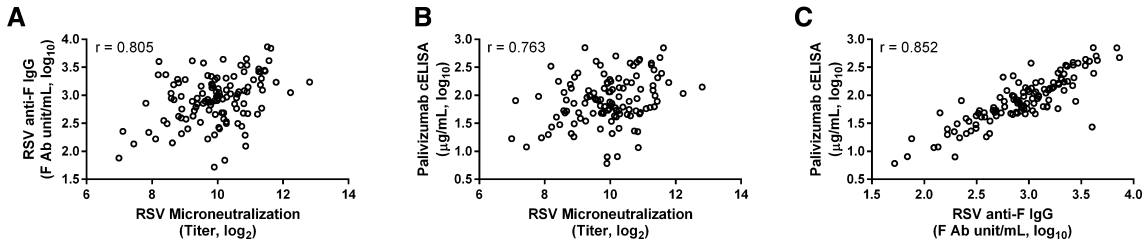

Fig. 3. Correlation between day 29 postdose antibody responses to an RSV sF vaccine, unadjuvanted and adjuvanted with 2.5 μg GLA in 2% SE. Panel A: RSV microneutralization antibody titers vs anti-F IgG antibodies; Panel B: palivizumab competitive antibodies vs RSV microneutralization antibody titers; Panel C: palivizumab competitive antibodies vs anti-F IgG antibodies. $P < 0.01$ for Pearson correlation of all pairwise comparisons. GLA = glucopyranosyl lipid A; r = Pearson's correlation; RSV = respiratory syncytial virus; SE = stable emulsion; sF = soluble fusion protein.

At the highest dosage level, 95.0% (95% CI: 75.13, 99.87) of subjects had a day 29 MN titer ≥67% of the baseline titer in the evaluable study population (compared with 29.2% [95% CI: 12.62, 51.09] in the placebo group); 100% (95% CI: 83.16, 100) achieved a titer ≥67% of the baseline in RSV F IgG and PCA assays, without overlap of 95% CIs with those of the placebo group. For day 8 F-specific IFN-γ responses, 94.7% (95% CI: 73.97, 99.87) of subjects in the highest dosing group had responses ≥67% of the baseline compared with only 30.0% (95% CI: 11.89, 54.28) of subjects who received placebo. The effect of dosage level on the responses at the population level can be seen in the reverse cumulative distribution curves (Figs. 4 and S2).

By RSV F IgG assay, GMFRs in F IgG antibodies in subjects who received RSV sF 80 μg + GLA-SE declined slowly over time. GMFR values were 15.39 at day 61; 12.58 at day 91; 8.19 at day 181; 5.12 at day 271; and 5.27 at day 361. At each of these timepoints, 95% CIs of F IgG geometric mean titers did not overlap with those of the placebo group. The IFN-γ ELISPOT responses diminished by day 29 (data not shown) and were not evaluated at later timepoints. Two of 24 placebo recipients and five subjects who received 80 μg of RSV sF seroconverted to a nonvaccine antigen (Ga, Gb, or N) between days 29 and 361; no subject who received an adjuvanted vaccine seroconverted.

4. Discussion

In this first-time-in-human study of a vaccine comprising RSV sF with and without GLA, a TLR-4 agonist, in a stable emulsion, an acceptable safety profile and significant immunogenicity were demonstrated. Reactogenicity was not RSV sF dose-dependent; however, GLA-SE increased local reactogenicity compared with the study vaccines containing RSV sF alone. Solicited symptoms were mild-to-moderate in severity and of short duration even in the presence of adjuvant. The most common solicited symptoms were tenderness and pain at injection sites. Other local symptoms, such as swelling or redness at injection site, were uncommon, as were other systemic symptoms. There was no pattern of AEs of concern. The overall safety profile of RSV sF with GLA-SE was acceptable at all doses studied.

RSV sF, particularly in the presence of GLA-SE, resulted in substantial and statistically significant (vs placebo) humoral and cellular immune responses as assessed by RSV A MN, F IgG, PCA, and RSV F-specific IFN-γ ELISPOT assays. The clinical significance of the induction of high levels of antibodies that compete with palivizumab is unknown, because the level of palivizumab antibody used for the protection of infants may not be relevant to adults, and antibodies that sterically hinder binding of palivizumab, but do not themselves bind to the palivizumab epitope, could affect assay results. Overall, immune responses were RSV sF dose-dependent and increased in the presence of adjuvant. In an ad hoc analysis of immunity in subjects who did and did not receive adjuvant that used a general linear model, the ratios of results in the adjuvant group divided by the unadjuvanted group were all significantly >1 (data not shown, P values 0.005–0.040 for all assays). The greatest immunogenicity was observed at the highest dose tested—80 μg of RSV sF + 2.5 μg of GLA in 2% SE. Fold rises in MN antibody titers were relatively low, which could be due to subjects' high baseline titers. We have explored the effect of adding complement to the MN assay and found that MN titers were enhanced in the presence of guinea pig complement, especially in samples from subjects who received RSV sF and GLA-SE [29]. Importantly, 95% of subjects in the high dose group achieved a MN titer ≥67% of baseline, and this population shift can also be seen in the reverse cumulative distribution curves. This suggests that vaccination moved those least likely to be protected at baseline toward lower risk.

In the highest dose group, cellular immune responses measured by IFN-γ ELISPOT showed a ≥3-fold rise in 74% of subjects. This study is the first successful demonstration of enhanced cellular immunity induced by an RSV vaccine for older adults. We believe a robust T-helper type 1 vaccine response is an important contributor to protection from disease, perhaps through clearance of virally-infected cells [30,31]. In an ongoing study, the cellular immune response to the vaccine is being characterized in greater detail. In animal models, both the GLA and SE portions of the adjuvant were important contributors to humoral and cellular immunogenicity [22]. Data from this phase 1a study confirm that inclusion of GLA-SE in an sF-containing vaccine induces greater humoral and cellular immune responses. The immune response did not plateau with escalating antigen doses, suggesting that even greater immunogenicity might be achieved with higher doses of RSV sF. For this reason, the follow-up phase 1b study (NCT02289820) assesses antigen doses of 120 μg of RSV sF as well as 3 dosage levels of GLA (administered with 2% SE).

There are several limitations to this study. Although cohort sizes were appropriate for a first-in-human study, they were too small to distinguish statistically significant differences between dosing groups; however, the dose–response data were useful for dose selection. The use of a single dose level of adjuvant is a limitation being addressed in an ongoing phase 1b study. In addition, although subjects were permitted to enroll with stable chronic illnesses and medication usage expected in this age group, exclusion criteria may have skewed the population toward one that is less representative of the overall population ≥60 years of age. Broader inclusion criteria are used in subsequent studies. Finally, although duration of immunity at higher doses is still being explored, data from this study suggest that annual vaccination is likely to be required. Given that RSV and influenza illnesses occur with similar seasonality, it would be convenient to administer an adult RSV vaccine at the same time as an influenza vaccine (administered in the contralateral arm to avoid inadvertent adjuvant effect on the influenza vaccine). No interference between a subunit RSV vaccine and influenza vaccine is expected based on previous studies of RSV vaccines and other

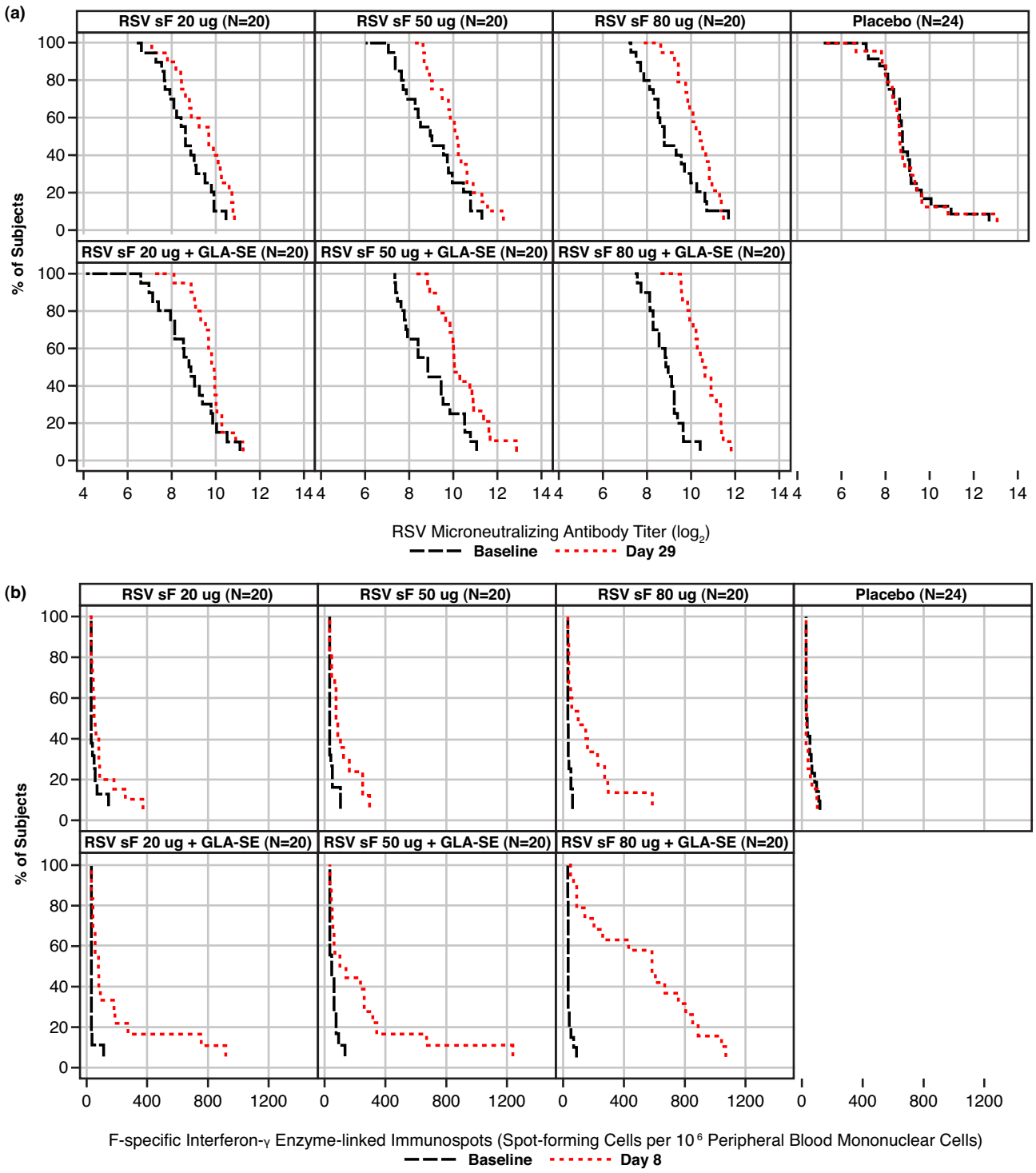

Fig. 4. Reverse cumulative distribution curves of antibody titers and IFN-γ spot-forming cells per 10^6 PBMCs before and after receipt of an RSV sF vaccine unadjuvanted and adjuvanted with 2.5 μg of GLA in 2% SE. Panel A: RSV microneutralization titers; Panel B: RSV F-specific interferon-γ ELISPOT SFCs/10^6 PBMCs. Baseline values are in black barred line; day 29 (antibody) and day 8 (ELISPOT) postdose values are in red dotted line. ELISPOT = enzyme-linked immunospot; GLA = glucopyranosyl lipid A; RSV = respiratory syncytial virus; SE = stable emulsion; sF = soluble fusion protein.

subunit vaccines; however, potential interference will be assessed in future studies [32].

In conclusion, safety and immunogenicity data from this phase 1a study of an RSV sF vaccine in adults ≥60 years of age support continued development of the vaccine and confirm the immunogenic benefit of including GLA-SE in the vaccine. Although GLA-SE contributed to local reactogenicity, the safety profile was acceptable at all doses tested. The absence of a clear plateau of immune response led to inclusion of a higher dosage level of RSV sF in subsequent studies. A phase 1b study assessing escalating doses of GLA (with 2% SE) (NCT02289820) and a phase 2b efficacy study (NCT02508194) are ongoing.

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Conflict of interest: The following authors are or were employees of MedImmune and may own AstraZeneca stock or stock options: J. Falloon, F. Ji, F. Dubovsky, S. Lambert, T. Takas, T. Villafana, M. Esser. The following authors received support from MedImmune for participation as principal investigators in the study: C. Curtis, S. Bart, E. Sheldon, D. Krieger.

Authors contributions: Judith Falloon, Fei Ji, Filip Dubovsky, Mark Esser, Therese Takas, and Tonya Villafana conceived of, designed, and wrote the study protocol. Drs. Curtis, Sheldon, and Bart reviewed the protocol, and Drs. Curtis, Bart, Sheldon, and Krieger enrolled subjects and acquired their safety data and blood for immunogenicity analyses. Fei Ji performed statistical analysis. Stacie Lambert and Mark Esser were responsible for development and implementation of assays. All authors had access to all study data for review, and all participated in interpretation of the data and in drafting and revising the article for important intellectual content and provided final approval of the submitted manuscript. All assume responsibility for the integrity and completeness of the reported data.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.04.002.

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