Local and Systemic Immunity Against RSV Induced by a Novel Intranasal Vaccine: A Randomised, Double- Blind, Placebo-Controlled Trial

Authors:

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Online Data Supplement

Supplementary Methods

Study design

Healthy volunteers were recruited for the randomised double-blind placebo-controlled study, according to the inclusion and exclusion criteria in Table E1 (clinicaltrials.gov identifier NCT02958540). Enrolment took place between October 2016 and January 2017 with final follow-up visits in July 2017. Volunteers received either placebo (phosphate buffered saline (PBS) + 2.5% glycerol) or SynGEM vaccine administered intranasally. The vaccine was given at 2 dose levels; a low dose (140 µg F protein and 2mg BLP) and a high dose (350 µg F protein and 5mg BLP) at day 0 and day 28. Blood and nasal lavage samples were collected 0, 7, 28, 35 and 56 days post-prime vaccination. Intention to treat analysis was performed for primary safety analyses.

Ethics statement

The phase I study was approved by the UK National Research Ethics Service (reference 16/SC/0441), overseen by a Data Safety Monitoring Committee and carried out at the Imperial Clinical Research Facility (ICRF) at the Hammersmith Hospital. Adenotonsillar tissues were obtained from children and adults undergoing tonsillectomy; ethics approval was obtained (reference 14/SS/1058) and written informed consent was obtained in all cases.

Outcomes

The primary endpoint was the safety and tolerability assessed by solicited (local and systemic) and unsolicited adverse events (Table E3). Events were graded mild, moderate or severe and association with vaccination was recorded. Blood test abnormalities were determined using the

FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. Secondary endpoints assessed humoral systemic and mucosal immune responses to the vaccine. Exploratory endpoints were defined as the measurement of cellular immune responses.

F and G protein-specific enzyme linked immunoassay (ELISA)

Anti-RSV IgG and IgA antibodies were measured using stabilized pre-fusion or unstabilized F protein or Ga (from RSV A) or Gb protein (from RSV B) in ELISA assays as previously described (E1). Serum IgG titer was calculated as a midpoint EC_{50} and s-IgA titers were calculated as endpoint titers, defined as the highest titer exhibiting an optical density of ≥10x the background. Endpoint titers for the IgA ELISAs were normalized using the ratio of urea in serum and nasal lavage measured using the Abcam Urea Assay Kit, the method adapted from the manufacturer's protocol. The dilution factor for normalization was calculated as follows:

Dilution Factor = (Serum urea concentration) / (Nasal Lavage urea concentration). Normalized IgA titer=Dilution factors x Nasal s-IgA Titer.

RSV microneutralization assay

The titer of RSV-neutralizing antibodies was determined in serum by plaque reduction neutralization titer (PRNT) assays performed at Viroclinics Biosciences, Rotterdam, The Netherlands, as previously described (E2).

Palivizumab and D25 competing ELISA

Palivizumab- and D25-competing antibodies were quantified in serum by competition ELISA, with furin cleavage site-mutated F protein ectodomain extended with GCN4 trimerization motifs (FlysGCN4) as antigen. Briefly, 100 µL/well of solid phase FlysGCN4 was adsorbed to 96-well EIA plates (Greiner, UK). Dilutions of serum were prepared in duplicate and mixed with biotin-labeled epitope-specific antibodies (D25, Mucosis BV and Palivizumab, MedImmune). Biotinylation was achieved using an EZ-Link Sulfo-NHS-LC-Biotinylation Kit (Thermo Scientific). Plates were then incubated with streptavidin-HRP (Jackson ImmunoResearch), TMB (KPL) was added and the colorimetric reaction was stopped by addition of HCl. Absorbance was read at 450 nm on a microplate reader. Human RSV antiserum (NR-4021, NR-4022 and NR-4023, BEI Resources) and IgG-depleted human serum (SF142-7 and SF505-2, BBI Solutions) were used as controls.

Antibody-secreting cell ELISpots

Human antibody-secreting cells (ASCs) were quantified using enzyme-linked immunospot (ELISpot) assays as previously described (E3). Spots were counted using an automated ELISpot reader (AID), and results expressed as spot forming cells per million PBMCs.

Flow cytometry

Flow cytometry analysis was performed using heparinized whole blood. Cells were stained with anti-CD19 FITC, anti-CD27 APC, anti-CD38 PE and anti-CD3/anti-CD20 both on PE-CF594 (BD Biosciences). Fixed cells were run on a Fortessa flow cytometer (BD Biosciences) and analyzed with FlowJo software.

Measurement of antibodies and cytokines in adenotonsillar cell culture supernatants

Mononuclear cells (MNC) were isolated from adenotonsillar tissues and cultured, as described previously (E4). Tissues were obtained from children (age 2-10 years) and adults (16-30 years) undergoing adenoidectomy and/or tonsillectomy at Liverpool Alder Hey Children's Hospital and Royal Liverpool and Broadgreen University Hospitals. Patients with known immunodeficiency and tissue samples with signs of gross inflammation were excluded. Adenotonsillar MNC were co-cultured with SynGEM BLP-F with F-protein concentration at 1µg/mL or 5µg/mL, BLP alone (25µg/mL) and F-Protein alone (1µg/mL) or medium. Cell culture supernatants were harvested at day 12 and F protein-specific antibodies were measured by ELISA as described previously (E5).

Following stimulation of adenotonsillar MNC for 3 days with the SynGEM BLP-F (5µg/mL), culture supernatants were analysed using cytometric bead array for cytokines (LEGENDplexTM, Biolegend, UK) following manufacturer's instructions. T cell responses in adenotonsillar MNC were analyzed by Carboxyfluorescein succinimidyl ester (CFSE) (Molecular Probes, UK) labelling as previously described (E4, E6).

Statistical analysis

Data analyses and graphs were produced using the software R and Graphpad Prism. Non-parametric data was compared using Mann-Whitney-Wilcoxon tests with Holm's correction for multiple comparisons. Binary response variables were related to continuous explanatory variables using logistic regression. Odds ratios (OR) and 95% confidence intervals (CI) of the OR for the explanatory variables were calculated. For estimation of serum neutralizing antibody titers,

weighted (1/y) four-parameter logistic models were fitted to the plaque counts and the 50% neutralizing titer (EC50) was derived from the midpoint of the curve using package ‘drc’.

Supplementary References

E1. Habibi MS, Jozwik A, Makris S, Dunning J, Paras A, DeVincenzo JP, de Haan CAM, Wrammert J, Openshaw PJM, Chiu C. 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;191:1040–1049.

E2. Sande CJ, Mutunga MN, Medley GF, Cane PA, Nokes DJ. Group and genotype specific neutralising antibody responses against respiratory syncytial virus (RSV) in infants and young children with severe pneumonia. J Infect Dis. 2012 Feb 1; 207(3): 489–492.

E3. Saletti G, Çuburu N, Yang JS, Dey A, Czerkinsky C. Enzyme-linked immunospot assays for direct ex vivo measurement of vaccine-induced human humoral immune responses in blood. Nat Protocols 2013;8:1073–1087.

E4. Aljurayyan A, Puksuriwong S, Ahmed M, Sharma R, Krishnan M, Sood S, Davies K, Rajashekar D, Leong S, McNamara PS, Gordon S, Zhang Q. Activation and Induction of Antigen-Specific T Follicular Helper Cells Play a Critical Role in Live-Attenuated Influenza Vaccine-Induced Human Mucosal Anti-influenza Antibody Response. J Virol 2018;92:e00114-18.

E5. Mullin J, Ahmed MS, Sharma R, Upile N, Beer H, Achar P, Puksuriwong S, Ferrara F, Temperton N, McNamara P, Lambe T, Gilbert SC, Zhang Q. Activation of cross-reactive mucosal T and B cell responses in human nasopharynx-associated lymphoid tissue in vitro by Modified Vaccinia Ankara-vectored influenza vaccines. Vaccine 2016;34:1688–1695.

E6. Gray C, Ahmed MS, Mubarak A, Kasbekar AV, Derbyshire S, McCormick MS, Mughal MK, McNamara PS, Mitchell T, Zhang Q. Activation of memory Th17 cells by domain 4 pneumolysin in human nasopharynx-associated lymphoid tissue and its association with pneumococcal carriage. Mucosal Immunology 2014;7:705–717.

Supplementary Legends

Figure E1 Flowchart summarizing recruitment and vaccination of 48 participants.

Healthy adult volunteers were enrolled and randomized to receive SynGEM intranasally at low-dose (n=18), high-dose (n=18) or placebo (n=12).

Figure E2. RSV infection in the study cohort revealed by antibodies against G protein.

(A) RSV A and (B) RSV B G protein-specific serum antibodies were measured by ELISA at time-points up to 180 days post-prime. Fold-changes compared with pre-vaccination are shown. A cut-off of 2-fold increase was defined as sero-conversion.

Figure E3. Intranasal SynGEM induces pre-fusion F protein-specific serum antibodies. Volunteers were given SynGEM or placebo and serum IgG was measured by ELISA using pre-fusion F protein as coating antigen at time-points up to 180 days post-"prime". Titers and fold-changes compared to baseline are shown following (A and B) placebo, (C and D) low-dose and (E and F) high-dose. Geometric means are shown in red. Wilcoxon ranked sign test was used to test statistically significant rises compared to pre-vaccination. * $p<0{\cdot}05$; ** $p<0{\cdot}01$; *** $p<0{\cdot}001$· Vaccinations are indicated by red triangles.

Figure E4. The magnitude of sIgA anti-F fold-change at any time-point from both low and high-dose groups correlates with lower pre-existing antibody titers.

Linear regression and Spearman correlation of pre-vaccination antibody titers and maximal antibody fold-change compared with baseline from placebo, low- and high-dose groups are shown (A-C). Fold-changes compared to baseline are shown following administration of placebo (D),

low-dose (E) and high-dose (F). Wilcoxon ranked sign test was used to test statistically significant rises compared to pre-vaccination.

Figure E5. Plasmablasts are not significantly increased following SynGEM.

Whole blood was stained with anti-CD3, CD20, CD19, CD38 and CD27 for analysis by flow cytometry. (A) Representative plots are shown from time-points up to 28 days post-boost. Plots are gated on CD3-CD19+CD20+/- lymphocytes. (B, C and D) Box and whisker plots show frequencies of plasmablasts at each time-point. No significant differences are seen by Wilcoxon ranked sign test.

Figure E6. Tonsil cells cultured with SynGEM do not express Th2 cytokines.

Tonsil cells from healthy donors were cultured with SynGEM. Cytokines were measured in culture supernatant by cytometric bead array. Mann-Whitney U test, and Wilcoxon Signed Rank Test was used to test significant differences; no significant differences are seen.

Table E1. Eligibility criteria

Table E2. Study endpoints

Table E3. Hematology and biochemistry abnormalities by maximum severity (any visit)

Table E4. Solicited adverse events within 1 hour of dosing

Table E5. Solicited adverse events

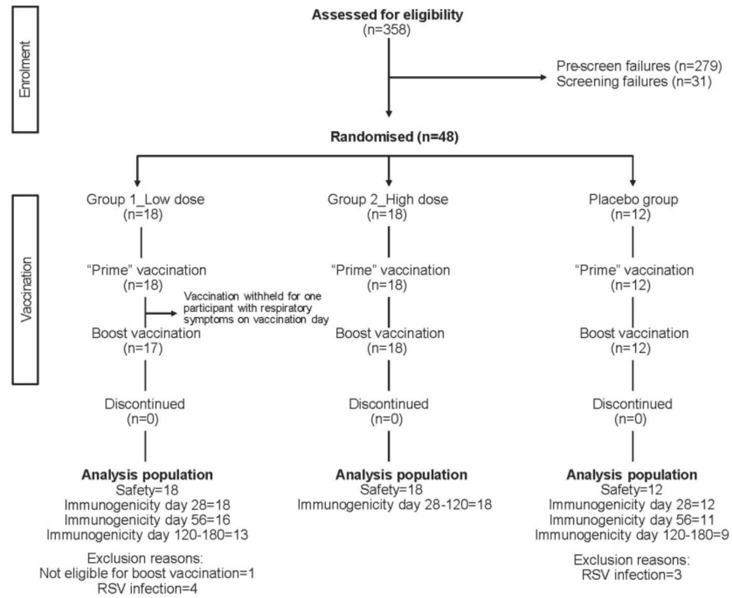

Figure E1 Flowchart summarizing recruitment and vaccination of 48 participants.
Healthy adult volunteers were enrolled and randomized to receive SynGEM intranasally at low-dose (n=18), high-dose (n=18) or placebo (n=12).

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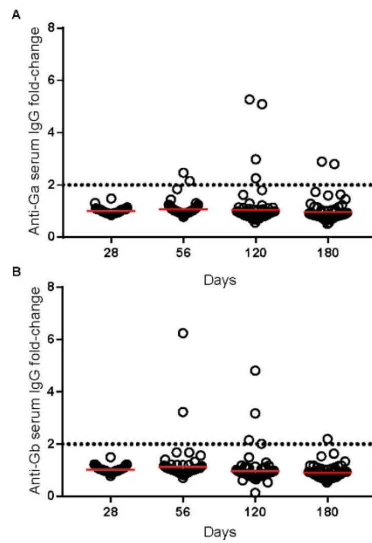

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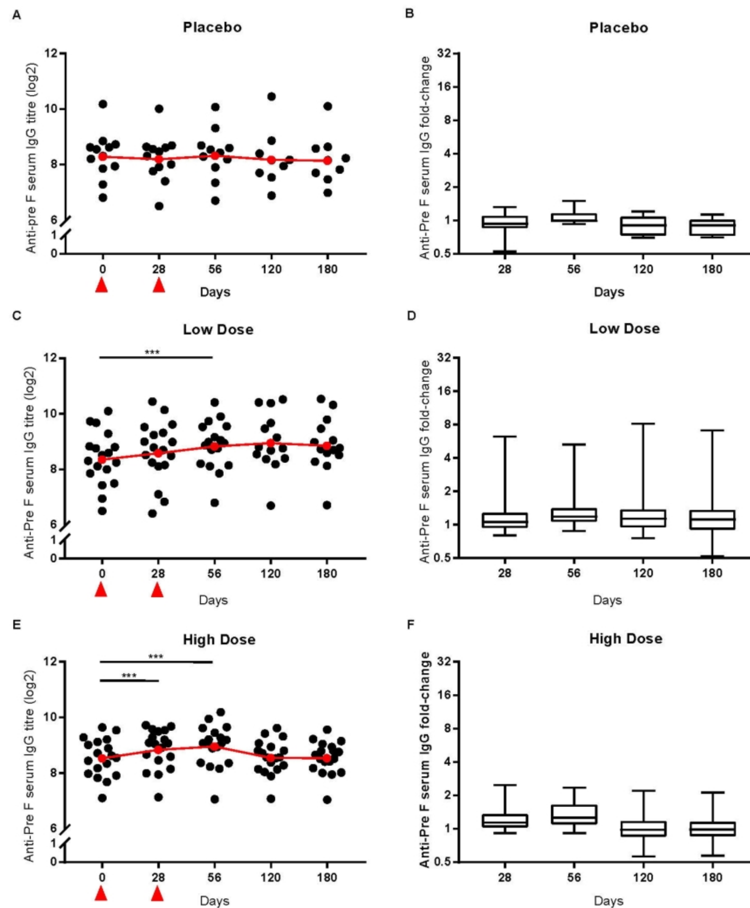

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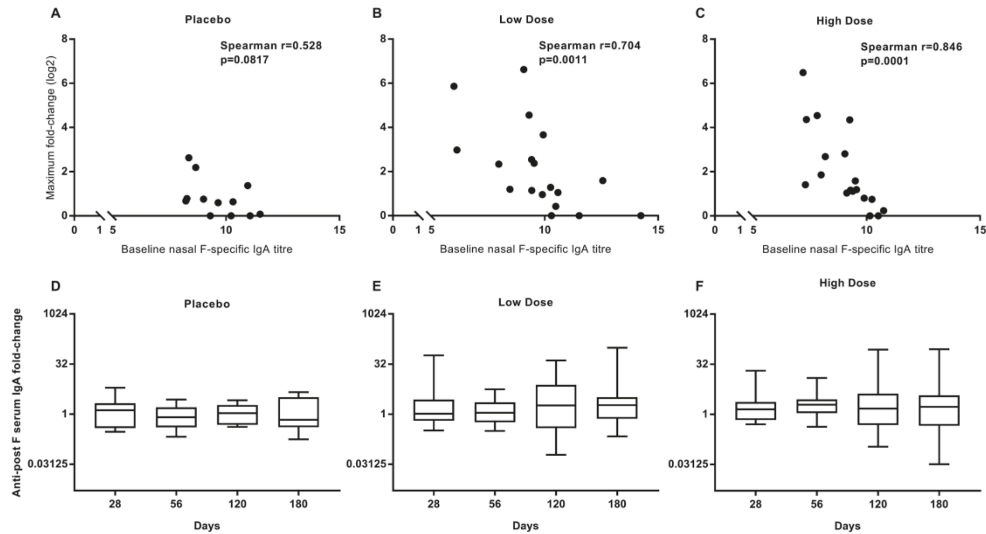

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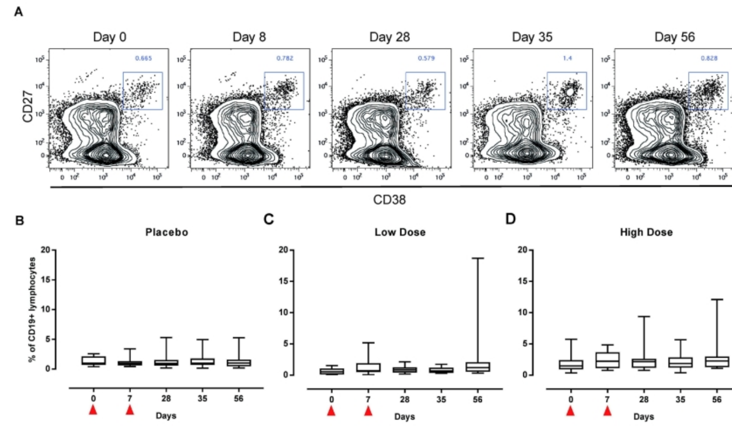

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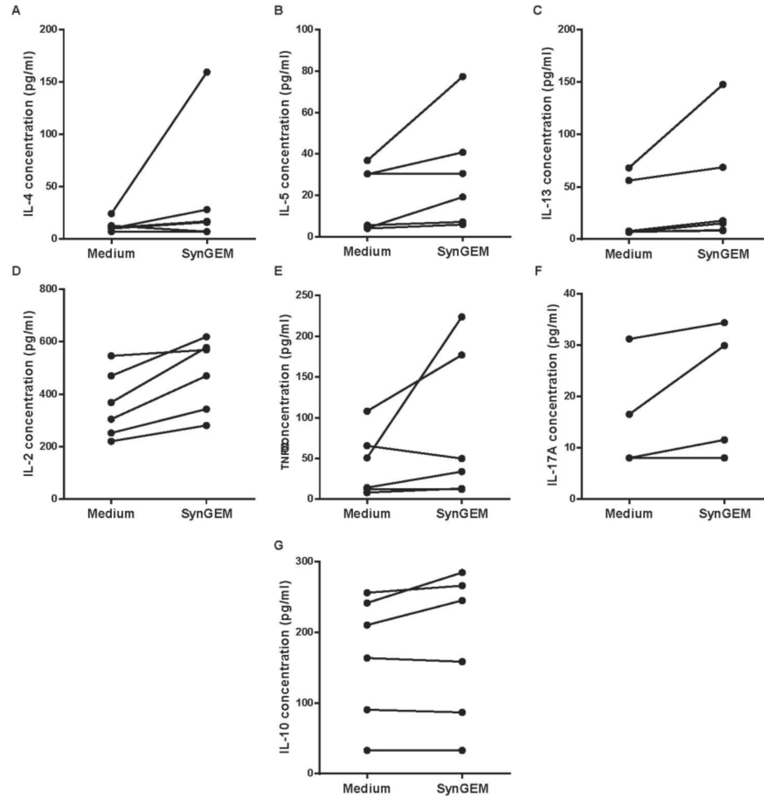

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	Inclusion criteria
1.	Male or female aged 18-49 years inclusive
2.	Able to give written informed consent to participate
3.	Comprehension of the study requirements, expressed availability for the required study period and ability to attend scheduled visits
4.	Healthy, as determined by medical history, physical examination, vital signs and clinical judgement
5.	Having acceptable laboratory parameters within 28 days before study day, defined as: haemoglobin, Red Blood Cell (RBC) count and haematocrit, White Blood Cell (WBC) count, sodium, potassium and total bilirubin within normal laboratory range and alanine aminotransferase (ALT)/ aspartate aminotransferase (AST) and serum creatinine ≤1·1x institutional upper limit normal (ULN)
6.	Body Mass Index (BMI) between 18 and 32, inclusive
7.	Women of childbearing potential are to have a negative serum β-human chorionic gonadotropin (β-hCG) pregnancy test at screening and a negative urine β-hCG pregnancy test within 24 hours preceding receipt of each dose and agree to practice, if not already practicing, highly effective birth control measures from 28 days before the prime vaccination until at least 90 days after the boost vaccination. For women already practicing highly effective birth control measurements for at least 28 days at screening start, recruitment could occur as soon as all screening procedures were completed. The following birth control measures were considered highly effective: a. Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable or implantable), intrauterine device, intrauterine hormone releasing system, bilateral tubal ligation, vasectomized partner (if the partner was the sole sexual partner and had received medical assessment of the surgical success) b. True abstinence: when this was in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial, and withdrawal are not acceptable methods of contraception c. If not heterosexually active at screening, must agree to practice highly effective birth control measures described above if they became heterosexually active from that moment onwards until at least 90 days after the boost vaccination d. Agree not to donate eggs (ova, oocytes) for the purposes of assisted reproduction from the start of screening onwards until at least 90 days after the boost vaccination
8.	Women of non-childbearing potential, defined as postmenopausal (>45 years of age with amenorrhea for ≥2 years; for female of >45 years of age with amenorrhea for more than 6 months but less than 2 years confirmation of a serum follicle-stimulating hormone (FSH) >40 mIU/mL are required to consider them of non-childbearing potential) or surgically sterile (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy), were not required to use the birth control methods as described in Inclusion Criterion #7
9.	A man who has not had a vasectomy with medical assessment of the surgical success and is sexually active with a woman of childbearing potential must agree to consistently use a barrier method of birth control, such as condom with spermicidal foam/gel/film/cream/suppository. Men also gave to agree not to donate sperm from the first study vaccine administration (Day 1) until 90 days after the boost vaccination
10.	Subjects have to be willing to provide verifiable identification and their National Insurance/Passport number for the purpose of The Over-volunteering Prevention System (TOPS) registration
11.	Subject has to have a means to be contacted
	Exclusion criteria
1.	History of acute respiratory disease in the 30 days preceding start of screening or documented infection with RSV in the previous 3 months
2.	Any chronic disease of the nasal cavity such as chronic hypertrophic or atrophic rhinitis, chronic sinusitis, ozena, Wegener's granulomatosis or granulomatosis with polyangiitis.
3.	History of asthma or chronic obstructive pulmonary disease
4.	Presence of significant uncontrolled medical or psychiatric illness (acute or chronic). This includes institution of a new medical or surgical treatment, or a significant dose alteration for uncontrolled symptoms or drug toxicity within 3 months of screening
5.	Subjects who are positive for hepatitis B surface antigen, hepatitis C antibodies or HIV
6.	Pregnant or breastfeeding women or planning to become pregnant while enrolled in the study or within 90 days after the boost vaccination
7.	Cancer, or treatment for cancer, within 3 years, excluding basal cell carcinoma or squamous cell carcinoma of the skin, which is allowed
8.	Presence of any medical condition that may be associated with impaired immune responsiveness, including diabetes mellitus
9.	Receiving at study start or history of receiving, during the preceding 3-month period, any medications or other treatments that may adversely affect the immune system such as allergy injections, immune globulins, interferon, immunomodulators, cytotoxic drugs or other drugs known to be frequently associated with significant major organ toxicity, or systemic corticosteroids (oral or injectable)
10.	Receipt of any intranasal administration of drug or vaccine within the 30 days prior to the first administration of study vaccine or plans to receive any intranasal administration of drug or vaccine until the end of study visit
11.	Receipt of live attenuated vaccine within 30 days of first SynGEM® administration or plans to receive within 30 days after the last study vaccine administration, and receipt of any other vaccine within 15 days of first SynGEM® administration or plans to receive within 15 days after the last study vaccine administration

12. Positive history of illicit drug use, of drug or alcohol abuse within the previous 6 months
13. History of anaphylactic type reaction to injected vaccines
14. History of allergic rhinitis or of allergy to food
15. History of allergy to insect bites, latex, pollens, house dust mites that were considered significant by the Investigator
16. Treatment with another investigational medicinal product (IMP) within 3 months prior to screening or with more than 2 IMPs in the past year
17. Receipt of blood or blood products 8 weeks prior to vaccination or planned administration during the study period
18. Loss of > 500 mL blood within 3 months prior to screening
19. Any major neurological disease, including migraine
20. Any condition that, in the Investigator's opinion, might interfere with the primary study objectives
21. Acute disease within 72 hours prior to vaccination, defined as the presence of a moderate or severe illness (as determined by the Investigator through medical history and physical examination) with or without fever, or a fever >38ºC did not represent an absolute exclusion criterion, but an exclusion criterion at that moment in time. Prime vaccination could be re-scheduled as deemed necessary by the Investigator

Table E1.

Primary objective	
1.	To assess the safety and tolerability of two different doses of SynGEM® (140 µg F- protein-FP/2mg BLPs or 350 µg F-protein-FP/5mg BLPs) administered 28 days apart (Day 1 and Day 29) in healthy adult subjects.
Secondary objectives	
1.	To assess humoral systemic and mucosal immune responses to F-protein-BLP of the two doses measured by
a.	RSV A virus neutralization by plaque reduction neutralization titers (PRNT) assay
b.	F specific serum IgG and nasal Secretory IgA (S-IgA) antibody titers by enzyme-linked immunosorbent assay (ELISA).

Table E2.

Parameter	Low dose		High dose		Placebo	
	Decrease	Increase	Decrease	Increase	Decrease	Increase
	Any grade (%) [grade 3 or more (%)]	Any grade (%) [grade 3 or more (%)]	Any grade (%) [grade 3 or more (%)]	Any grade (%) [grade 3 or more (%)]	Any grade (%) [grade 3 or more (%)]	Any grade (%) [grade 3 or more (%)]
Haematology						
Haemoglobin	5 (27·8)	0	2 (11·1)	0	2 (16·7) [2 (16·7)]	0
White Blood Cells (total)	6 (33·3)	6 (33·3)	4 (22·2)	4 (22·2)	6 (50)	6 (50)
Absolute lymphocytes	2 (11·1)	0	1 (5·6)	0	2 (16·7)	0
Absolute neutrophils	3 (16·7)	0	5 (27·8)	0	3 (25·0)	0
Absolute eosinophils	1 (5·6)	0	0	0	0	0
Biochemistry						
Sodium	0	0	0	0	0	0
Potassium	0	0	0	0	0	0
Creatinine	0	0	0	0	0	0
Urea	0	0	0	0	0	0
Alanine aminotransferase	0	1 (5·6)	0	0	0	2 (16·7)
Aspartate aminotransferase	0	2 (11·1)	0	1 (5·6)	0	1 (8·3)

Table E3.

	Low dose		High dose		Placebo		Test of significance
	Post prime (n=18)	Post boost (n=17)	Post prime (n=18)	Post boost (n=18)	Post prime (n=12)	Post boost (n=18)	
	Any grade (%) [grade 3 (%)]	Any grade (%) [grade 3 (%)]	Any grade (%) [grade 3 (%)]	Any grade (%) [grade 3 (%)]	Any grade (%) [grade 3 (%)]	Any grade (%) [grade 3 (%)]	
Local							
Epistaxis	0	0	0	0	0	0	ns
Facial discomfort	0	0	0	0	2 (16·7)	0	ns
Facial numbness	0	0	0	1 (5·6)	0	0	ns
Facial swelling	0	0	0	0	0	0	ns
Lacrimation	0	0	0	0	0	0	ns
Loss of smell	0	0	0	0	2 (16·7)	0	ns
Nasal discomfort	1 (5·6)	0	0	0	1 (8·3)	0	ns
Nasal pain	0	0	0	0	0	0	ns
Red eyes	0	0	0	0	0	0	ns
Rhinorrhea	3 (16·7)	2 (11·8)	1 (5·6)	0	0	1 (8·3)	ns
Sneezing	1 (5·6)	0	0	0	0	0	ns
Sore throat	1 (5·6)	0	0	0	0	0	ns
Stuffy nose	3 (16·7)	1 (5·9)	0	0	1 (8·3)	1 (8·3)	ns
Systemic							
Arthralgia	0	0	0	0	0	0	ns
Chills	0	1 (5·9)	0	0	0	0	ns
Fatigue	2 (11·1)	2 (11·8)	0	0	3 (25)	1 (8·3)	ns
Feeling feverish	2 (11·1)	0	0	0	0	0	ns
Itching	0	0	0	0	0	0	ns
Headache	0	1 (5·9)	0	0	1 (8·3)	1 (8·3)	ns
Malaise	1 (5·6)	0	0	0	0	0	ns
Myalgia	0	0	0	0	0	0	ns
Nausea	0	0	0	0	0	1 (8·3)	ns
Rash	0	0	0	0	0	0	ns
Vomiting	0	0	0	0	0	0	ns

Table E4.

	Low dose						High dose						Placebo					
	Post prime			Post boost			Post prime			Post boost			Post prime			Post boost		
	(n=18)			(n=17)			(n=18)			(n=18)			(n=12)			(n=12)		
	Any grade (%) [grade 3 (%)]	Median TTO (IQR) (days)	Median duration (IQR) (days)	Any grade (%) [grade 3 (%)]	Median TTO (IQR) (days)	Median duration (IQR) (days)	Any grade (%) [grade 3 (%)]	Median TTO (IQR) (days)	Median duration (IQR) (days)	Any grade (%) [grade 3 (%)]	Median TTO (IQR) (days)	Median duration (IQR) (days)	Any grade (%) [grade 3 (%)]	Median TTO (IQR) (days)	Median duration (IQR) (days)	Any grade (%) [grade 3 (%)]	Median TTO (IQR) (days)	Median duration (IQR) (days)
Local																		
Epistaxis	0 (0)			0			0			2 (11·1)	2·5 (1)	1 (0)	0			2 (16·7)	2 (2)	1 (0)
Facial discomfort	0			0			0			1 (5·6)	1 (0)	6 (0)	2 (16·7)	1 (0)	1 (0)	1 (8·3)	1 (0)	1 (0)
Facial numbness	0			0			1 (5·6)	1 (0)	1 (0)	1 (5·6)	1 (0)	5 (0)	0			0		
Facial swelling	1 (5·6)	2 (0)	1 (0)	0			1 (5·6)	3 (0)	1 (0)	1 (5·6)	1 (0)	1 (0)	0			0		
Lacrimation	1 (5·6)	1 (0)	1 (0)	1 (5·9)	7 (0)	1 (0)	1 (5·6)	2 (0)	3 (0)	1			1 (8·3)	1 (0)	1 (0)	0		
Loss of smell	3 (16·7)	2 (1.0)	3 (2)	4 (23·5)	4·5 (4·5)	1·5 (1·5)	1 (5·6)	1 (0)	7 (0)	0			3 (25)	1 (0)	1 (4)	0		
Nasal discomfort	3 (16·7)	1 (2)	1 (2)	3 (17·6)	5 (6)	1 (2)	3 (16·7)	1 (1)	2 (1)	2 (11·1)	1 (0)	3 (2)	3 (25)	1 (0)	2 (2)	1 (8·3)	1 (0)	1 (0)
Nasal pain	1 (5·6)	4 (0)	1 (0)	0			1 (5·6)	1 (0)	2 (0)	2 (11·1)	4·5 (1)	1 (0)	1 (8·3)	1 (0)	1 (0)	0		
Red eyes	0			1 (5·9)	7 (0)	1 (0)	0			1 (5·6)	3 (0)	1 (0)	0			0		
Rhinorrhea	8 (44)	1 (1)	2·5 (2)	5 (29·4)	5 (4)	1 (2)	6 (33·3)	1 (3)	1·5 (1)	4 (22·2)	1 (1)	3 (3)	3 (25)	1 (0)	2 (5)	3 (25)	3 (3)	4 (4)
Sneezing	6 (33·3)	1·5 (4)	2 (1)	2 (11·8)	3 (4)	1 (0)	1 (5·6)	2 (0)	1 (0)	3 (16·7)	1 (2)	3 (2)	2 (16·7)	1·5 (1)	1 (0)	2 (16·7)	3·5 (1)	1·5 (1)
Sore throat	2 (11·1)	1 (0)	1 (0)	1 (5·9) [1(5·9)]	6 (0)	2 (0)	5 (27·8)	1 (1)	3 (1)	3 (16·7)	6 (3)	2 (1)	3 (25)	1 (3)	2 (2)	5 (41·7)	3 (1)	4 (3)
Stuffy nose	8 (44·4)	1 (0·5)	2·5 (2·5)	5 (29·4)	1 (4)	1 (1)	6 (33·3)	2 (1)	1·5 (3)	4 (22·2)	2 (2)	2 (2)	4 (33·3)	1 (1)	3 (4·5)	7 (58·3)	1 (2)	1 (3)
Systemic																		
Arthralgia	1 (5·6)	1 (0)	2 (0)	2 (11·8)	5 (2)	1 (0)	1 (5·6)	2 (0)	1 (0)	0			0			0		
Chills	0			2 (11·8)	4 (6)	1 (0)	1 (5·6)	4 (0)	1 (0)	1 (5·6)	4 (0)	1 (0)	0			1 (8·3)	7 (0)	1 (0)
Fatigue	9 (50) [1 (5·6)]	1 (0)	2 (2)	7 (41·2)	1 (2)	1 (1)	6 (33·3) [1(5·6)]	1 (1)	1·5 (1)	6 (33·3)	1·5 (2)	2 (3)	7 (58·3)	1 (2)	2 (2)	5 (41·7)	1 (0)	1 (1)
Feeling feverish	3 (16·7)	1 (0)	2 (3)	1 (5·9)	6 (0)	2 (0)	1 (5·6)	1 (0)	1 (0)	1 (5·6)	3 (0)	1 (0)	1 (8·3)	1 (0)	1 (0)	1 (8·3)	5 (0)	1 (0)
Itching	1 (5·6)	4 (0)	2 (0)	0			1 (5·6)	4 (0)	1 (0)	1 (5·6)	3 (0)	1 (0)	0			0		
Headache	8 (44·4)	1·5 (2)	1·5 (1)	4 (23·5)	2 (3·5)	1 (0·5)	8 (44·4)	1·5 (4·5)	1 (1)	8 (44·4)	2·5 (3·5)	1 (1)	4 (33·3)	1 (0·5)	1·5 (1·5)	3 (25)	1 (6)	1 (3)
Malaise	2 (11·1) [1 (5·6)]	2 (2)	2 (2)	2 (11·8) [1(5·9)]	4·5 (3)	1·5 (1)	2 (11·1)	1 (0)	3·5 (5)	0			1 (8·3)	1 (0)	2 (0)	1 (8·3)	6 (0)	2 (0)
Myalgia	5 (27·8)	1 (1)	2 (1)	3 (17·6)	5 (2)	2 (2)	0			2 (11·1)	1·5 (1)	1 (0)	2 (16·7)	2 (2)	1·5 (1)	1 (8·3)	1 (0)	1 (0)
Nausea	1 (5·6)	3 (0)	1 (0)	1 (5·9)	6 (0)	2 (0)	1 (5·6)	2 (0)	1 (0)	1 (5·6)	1 (0)	1 (0)	0			1 (8·3)	1 (0)	1 (0)
Rash	1 (5·6)	4·0 (4·0-4·0)	3·0 (3·0-3·0)	0			1 (5·6)	4 (0)	1 (0)	1 (5·6)	3 (0)	1 (0)	0			0		
Vomiting	0			0			0			0			0			0		

Table E5.