Science Advances
AAAS

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# Supplementary Materials for

## The adjuvant GLA-AF enhances human intradermal vaccine responses

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Published 12 September 2018, *Sci. Adv.* **4**, eaas9930 (2018)
DOI: 10.1126/sciadv.aas9930

**This PDF file includes:**

**SUPPLEMENTARY MATERIALS**

The clinical summary of inclusion and exclusion criteria are from our clinical submissions and are also found at: https://www.clinicaltrials.gov/ct2/show/NCT01657929.

**Section S1. Inclusion Criteria**

Subjects were required to meet ALL of the following criteria to be eligible for inclusion in the study: Males and females ≥18 years and ≤49 years of age; Must be in good general health as confirmed by a medical history and physical exam, vital signs, and screening laboratories conducted no more than 30 days prior to study injection administration; All female subjects, regardless of birth control history, must have a negative serum pregnancy test at screening, a negative urine or serum pregnancy test on the day of each study injection, must not be breast-feeding or lactating, and are required to consistently use one of the following methods of contraception through the first three months of the study: hormonal (e.g. oral, transdermal, intravaginal, implant, or injection); double barrier (i.e., condom, diaphragm with spermicide); intrauterine device (IUD) or system (IUS); vasectomized partner (6 months minimum); abstinence (or agrees to use the approved birth control methods if subject becomes sexually active); or bilateral tubal ligation (if no conception post-procedure). These precautions are necessary due to unknown effects that H5-VLP + GLA-AF or H5-VLP alone might have in a fetus or newborn infant. The subject must have no plan to become pregnant during the first three months of the study period.

The following screening laboratory blood tests must have values within the normal ranges (as provided by each clinical site) or not clinically significant as determined by the Principal Investigator (or designated sub-investigator) and Medical Monitor (all test results must be within

30 days prior to first study injection): sodium, potassium, BUN, ALT, AST, total bilirubin, alkaline phosphatase, creatinine, fasting glucose, fasting lipid panel, total WBC count, hemoglobin, and platelet count.

The following serology tests must be negative: HIV 1/2 antibody, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) antibody; Negative urine test for recreational drugs and alcohol per Clinical Research Unit standards; Urinalysis not clinically significant as determined by the study clinician.

In the opinion of the Investigator, must show comprehension of the study requirements, competence and willingness to provide written, informed consent for participation after reading the informed consent form. The subject must have adequate opportunity to discuss the study with an Investigator or qualified designee. Must be capable of completing a study memory aid in English or Spanish. Must be able and willing to attend all evaluation visits, be reachable by telephone on a consistent basis by the study site personnel, and have a permanent address.

**Section S2. Exclusion Criteria**

Subjects who met ANY of the following criteria were ineligible and excluded from the study: Previous exposure to H5N1 vaccines or experimental products containing GLA-AF; History of allergy to any of the constituents of H5-VLP (H5N1) + Alhydrogel (aluminum hydroxide) vaccine. Participation in another experimental protocol or receipt of any investigational or non-registered products within the past 3 months or planned use during the study period. Subjects may not participate in any other drug study while participating in this study. Treatment with immunosuppressive drugs (e.g., oral or injected steroids, such as prednisone; high dose inhaled steroids) or cytotoxic therapies (e.g., chemotherapy drugs or radiation) within the past 6 months.

Received a blood transfusion or immunoglobulin within the past 3 months. Donated blood products (platelets, whole blood, plasma, etc.) within past 1 month. Poor venous access. Administration of any vaccine (including any other influenza vaccine) within a 30-day period prior to study enrollment, or planned administration of any vaccines within the period from the first study injection up to blood sampling at Day 42 or within 30 days prior to blood sampling at Day 189. Immunization on an emergency basis of a tetanus and diphtheria toxoids adsorbed for adult use (Td) will be allowed provided the vaccine is not administered within two weeks prior to study injection administration. Receipt of any other emergency immunizations (e.g. rabies) will result in a case-by-case review of continued participation. History of autoimmune disease or other causes of immunosuppressive states. Any confirmed or suspected immunosuppressive condition or immunodeficiency including history of human immunodeficiency virus (HIV) infection or presence of lymphoproliferative disease. History or evidence of any acute or chronic illness (including cardiovascular, pulmonary, neurological, hepatic, rheumatic, hematological, metabolic, or renal disorders, controlled hypertension), or use of medication that, in the opinion of the Principal Investigator (or designated sub-investigator), may interfere with the evaluation of the safety or immunogenicity of the vaccine. Cancer or treatment for cancer within 3 years of study injection administration. Persons with a history of cancer who are disease-free without treatment for 3 years or more are eligible. Persons with treated and uncomplicated basal cell carcinoma of the skin are eligible. History of significant psychiatric illness with current use of medication. Rash, tattoos or any other dermatological condition that could adversely affect the vaccine injection site or interfere with its evaluation. BMI <18 or >30 $kg/m^2$. Hypertension (systolic >150 mmHg or diastolic >90 mmHg). Resting pulse rate <40 bpm or >100 bpm. Any medical or neuropsychiatric condition which, in the Investigator's opinion, would render the

subject incompetent to provide informed consent or unable to provide valid safety observations and reporting. Known or suspected alcohol or drug abuse within the past 6 months. Chronic smoker (>20 pack years). History of allergy to tobacco or eggs. Subjects with a history of previous anaphylaxis or severe allergic reaction to vaccines, or unknown allergens. Subjects who are unlikely to cooperate with the requirements of the study protocol.

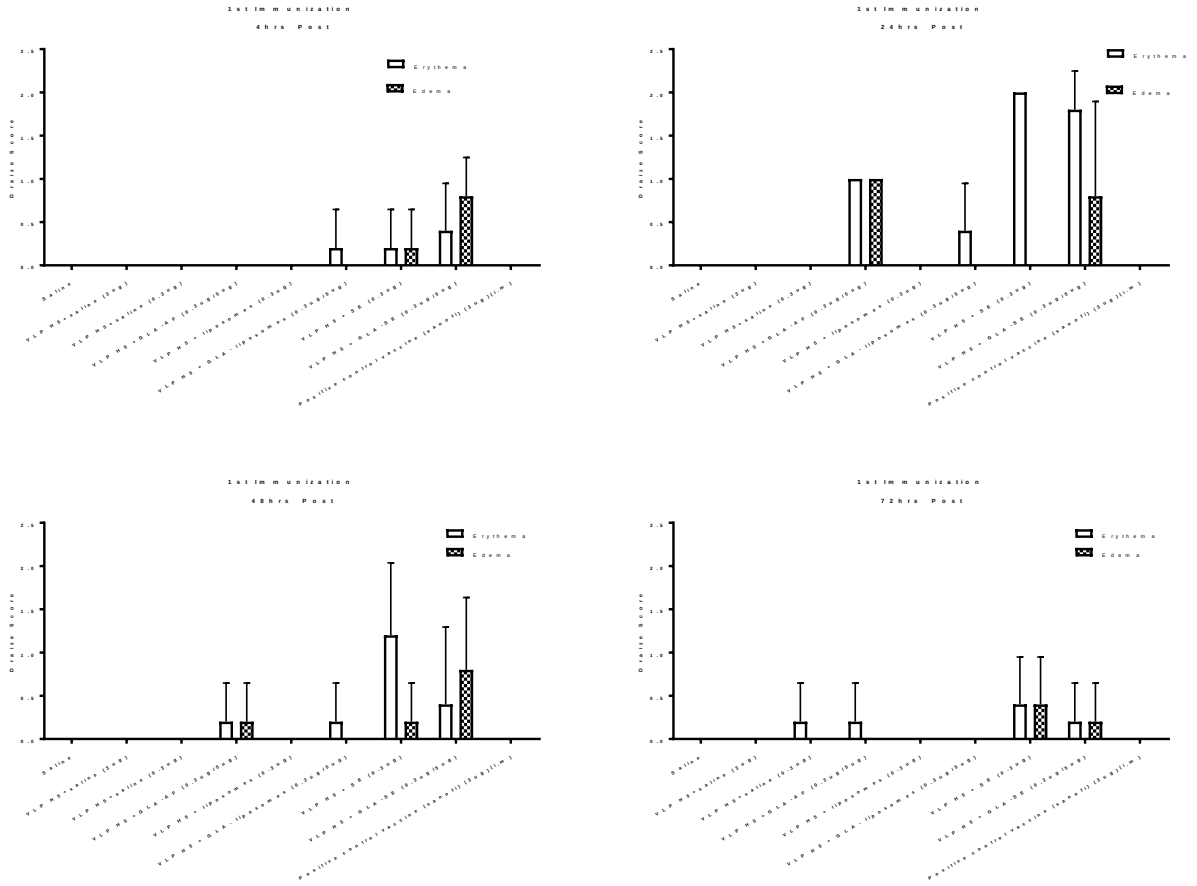


**Fig. S1. Safety assessments in the guinea pig.** A guinea pig experiment using the vaccine candidate was started to evaluate the safety of the vaccine in an accepted intradermal model. The various formulations were given ID to the guinea pigs as labeled in the graph and were evaluated using the Draize scale for erythema and edema at 4, 24, 48, and 72 hours post injection. Averages and standard deviations for 5 animals per group are shown. Peak reactogenicity was around 24 hours and mostly resolved by 72 hours after injection.

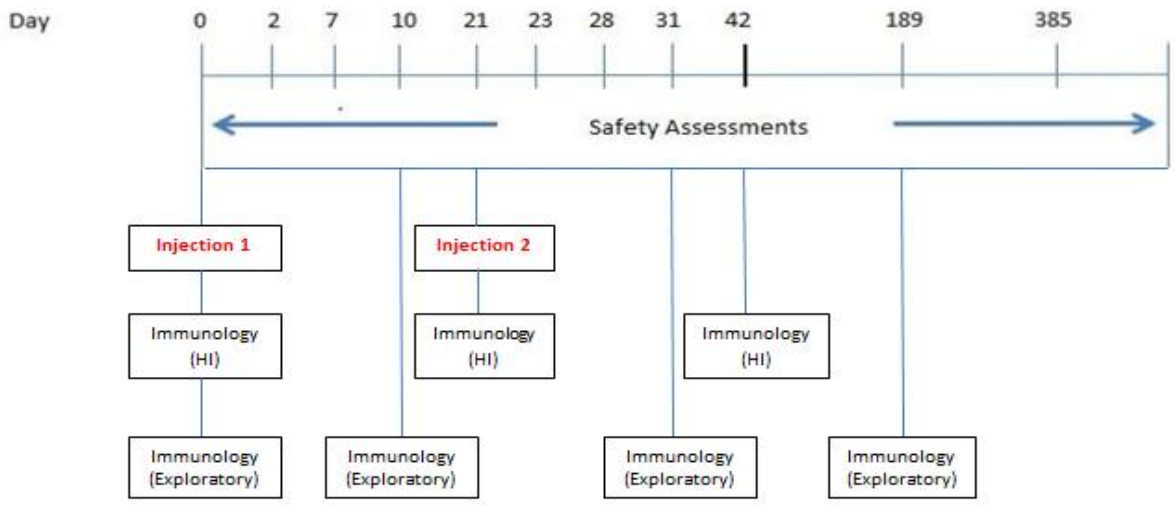


**Fig. S2. Assessment schedule.** Safety assessments were performed on Days 0, 2, 7, 10, 21, 23, 28, 31, 42, 189, and 385 and the study comprised three periods: Period 1: The treatment period commencing on Day 0 (after the first study injection) through the Day 42 visit. Period 2: The follow-up period commencing after the Day 42 visit through the Day 189 visit. Period 3: The follow-up period commencing after the Day 189 visit through the Day 385 visit. Screening evaluations included: medical history; physical examination; serology for hepatitis B, C, and HIV-1; serum chemistry; CBC with differential; lipid profile; urinalysis; and concomitant medications. Safety assessments included focused physical examinations (including targeted neuro-muscular examination), vital signs, clinical chemistry, hematology, and local and systemic reactogenicity assessments.

**Table S1. Schedule of study visits and procedures.**

| Study Period | Screen | Schedule for Study Injection Phase | | | | | | | | | Follow-up | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day -30 to Day 0 | Day 0 | Day 2 (+1) | Day 7 (±1) | Day 10 (+2) | Day 21 (+2) | Day 23 (+1) | Day 28 (±1) | Day 31 (+2) | Day 42 (±2) | Day 189 (±14) | Day 385 (±14)[6] |
| Informed Consent(s) | X | | | | | | | | | | | |
| Inclusion/Exclusion Criteria | X | X | | | | | | | | | | |
| Medical History | X | | | | | | | | | | | |
| Record Concomitant Med. | X | X | X | X | | X | X | X | | X | X | X |
| Physical Exam | X | X[1] | | X[1] | | X[1] | | X[1] | | X[1] | X[1] | X[7] |
| Vital Signs, Oral Temp | X | X[2] | X | X | X | X[2] | X | X | X | X | X | X[7] |
| Height & Weight | X | | | | | | | | | | | |
| Hematology/Chemistries[3] | X | | | X | | | | X | | X | | |
| Serology (HIV, HCV, HbsAg) | X | | | | | | | | | | | |
| Lipid Panel | X | | | | | | | | | | | |
| Pregnancy Testing | X | X | | | | X | | | | X | | |
| UA + drug/alcohol test | X | | | | | | | | | | | |
| Visual acuity (Snellen chart) | X | | | | | | | | | | | |
| Immunology Blood | | X | | | X | X | | | X | X | X | |
| Study Injection[4] | | **X** | | | | **X** | | | | | | |
| Injection Site Evaluation | | X | X | X | | X | X | X | | | | |
| Dispense memory aid | | X | | | | X | | | | | | |
| Review memory aid | | | X | X[5] | | | X | X[5] | | | | |
| Adverse Events (AE) | X | X | X | X | X | X | X | X | X | X | | |
| SAE/AESI | X | X | X | X | X | X | X | X | X | X | X | X |

1 Directed physical exam. 2 Vital signs and oral temp were performed prior to and at 30 and 60 minutes post study injection. 3 Specific items reported in the hematology and chemistry panels performed by different local laboratories may vary slightly, with required blood volume ranging between 12-16 mL per test. Only the test parameters required by the protocol were documented in the case report forms. 4 Must not inject if subject has temperature of >38.5ºC/101.2ºF, has other acute illness, or if discontinuation rules have been met. 5 Retain memory aid in source document. 6 Telephone follow-up. 7 Only if an in-clinic visit was required.

**Table S2. Subjects with adverse events by study period, injection interval, severity, and relatedness to study injection (safety population).**

| | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | |
|---|---|---|---|---|---|---|
| | H5-VLP + GLA-AF | H5-VLP + GLA-AF | H5-VLP | H5-VLP + Alhydrogel | Influenza Virus Vaccine, H5N1 | |
| | ID | IM | ID | IM | IM | |
| | n=20 | n=23 | n=22 | n=20 | n=20 | $p$[1] |
| **Any Adverse Event** | 20 (100%) | 22 ( 95.7%) | 21 ( 95.5%) | 19 ( 95.0%) | 19 ( 95.0%) | 1.000 |
| **Study Period** [2] | | | | | | |
| Period I | 20 (100%) | 22 ( 95.7%) | 21 ( 95.5%) | 19 ( 95.0%) | 19 ( 95.0%) | 1.000 |
| Period II and III | 0 | 0 | 0 | 0 | 0 | N/A |
| **Injection Interval** [3] | | | | | | |
| Injection Interval 1 | 20 (100%) | 21 ( 91.3%) | 21 ( 95.5%) | 18 ( 90.0%) | 15 ( 75.0%) | 0.099 |
| Injection Interval 2 | 17 ( 85.0%) | 14 ( 60.9%) | 19 ( 86.4%) | 17 ( 85.0%) | 13 ( 65.0%) | 0.137 |
| **Severity** | | | | | | |
| Grade 1 (Period I) | 20 (100%) | 22 ( 95.7%) | 21 ( 95.5%) | 19 ( 95.0%) | 19 ( 95.0%) | 1.000 |
| Grade 2 (Period I) | 9 ( 45.0%) | 10 ( 43.5%) | 8 ( 36.4%) | 12 ( 60.0%) | 3 ( 15.0%) | 0.055 |
| Grade 3 (Period I) | 0 | 0 | 0 | 0 | 0 | N/A |
| Grade 4 (Period I) | 0 | 0 | 0 | 0 | 0 | N/A |
| SAE (Period I to III) | 0 | 0 | 0 | 0 | 0 | N/A |
| **Relatedness (Period I)** [4] | | | | | | |
| Any | 20 (100%) | 22 ( 95.7%) | 18 ( 81.8%) | 19 ( 95.0%) | 16 ( 80.0%) | 0.096 |
| Possibly | 12 ( 60.0%) | 12 ( 52.2%) | 10 ( 45.5%) | 14 ( 70.0%) | 10 ( 50.0%) | 0.538 |
| Probably | 9 ( 45.0%) | 7 ( 30.4%) | 5 ( 22.7%) | 5 ( 25.0%) | 8 ( 40.0%) | 0.501 |
| Definitely | 19 ( 95.0%) | 19 ( 82.6%) | 18 ( 81.8%) | 18 ( 90.0%) | 12 ( 60.0%) | 0.066 |

1 $p$-value for comparison across all five treatment groups obtained by Fisher's exact test; if significant, pairwise comparisons were performed with Fisher's exact test with Bonferroni adjustments; $p<0.05$ marked with *. 2 Period I: Day 0-42; Period II: Day 43-189, Period III: Day 190-385. Only SAE and AESI reported for Periods II and III. 3 Injection Interval 1: Day 0-20; Injection Interval 2: Day 21-42. 4 No study-injection related adverse events in Study Periods II or III.

**Table S3. Subjects with AEs occurring in ≥5 subjects in study period I (safety population).**

| | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | |
|---|---|---|---|---|---|---|
| | H5-VLP + GLA-AF | H5-VLP + GLA-AF | H5-VLP | H5-VLP + Alhydrogel | Influenza Virus Vaccine, H5N1 | |
| | ID | IM | ID | IM | IM | |
| | n=20 | n=23 | n=22 | n=20 | n=20 | $p^1$ |
| **Injection related reactions**[2] | | | | | | |
| Anorexia | 3 ( 15.0%) | 2 ( 8.7%) | 1 ( 4.5%) | 3 ( 15.0%) | 3 ( 15.0%) | 0.735 |
| Chills | 0 | 0 | 2 ( 9.1%) | 1 ( 5.0%) | 2 ( 10.0%) | 0.397 |
| Fatigue | 9 ( 45.0%) | 9 ( 39.1%) | 6 ( 27.3%) | 8 ( 40.0%) | 7 ( 35.0%) | 0.829 |
| Injection site erythema/redness | 15 ( 75.0%) | 0 | 13 ( 59.1%) | 0 | 1 ( 5.0%) | <0.001* |
| Injection site pain | 4 ( 20.0%) | 14 ( 60.9%) | 4 ( 18.2%) | 15 ( 75.0%) | 8 ( 40.0%) | <0.001* |
| Injection site swelling/induration | 2 ( 10.0%) | 1 ( 4.3%) | 2 ( 9.1%) | 2 ( 10.0%) | 2 ( 10.0%) | 0.945 |
| Injection site tenderness | 11 ( 55.0%) | 14 ( 60.9%) | 11 ( 50.0%) | 17 ( 85.0%) | 11 ( 55.0%) | 0.142 |
| Headache | 9 ( 45.0%) | 4 ( 17.4%) | 6 ( 27.3%) | 5 ( 25.0%) | 6 ( 30.0%) | 0.396 |
| Myalgia | 1 ( 5.0%) | 3 ( 13.0%) | 5 ( 22.7%) | 5 ( 25.0%) | 0 | 0.062 |
| **Laboratory Investigations** | | | | | | |
| Blood bilirubin increased | 0 | 1 ( 4.3%) | 3 ( 13.6%) | 1 ( 5.0%) | 0 | 0.245 |
| Blood sodium decreased | 0 | 2 ( 8.7%) | 4 ( 18.2%) | 2 ( 10.0%) | 0 | 0.119 |
| Hemoglobin decreased | 4 ( 20.0%) | 5 ( 21.7%) | 2 ( 9.1%) | 4 ( 20.0%) | 3 ( 15.0%) | 0.795 |
| Platelet count decreased | 1 ( 5.0%) | 0 | 0 | 1 ( 5.0%) | 3 ( 15.0%) | 0.100 |
| White blood cell count decreased | 2 ( 10.0%) | 2 ( 8.7%) | 7 ( 31.8%) | 2 ( 10.0%) | 2 ( 10.0%) | 0.225 |
| **General AEs** | | | | | | |
| Dizziness | 1 ( 5.0%) | 1 ( 4.3%) | 0 | 3 ( 15.0%) | 0 | 0.164 |
| Headache | 2 ( 10.0%) | 2 ( 8.7%) | 1 ( 4.5%) | 2 ( 10.0%) | 2 ( 10.0%) | 0.958 |
| Myalgia | 0 | 0 | 1 ( 4.5%) | 3 ( 15.0%) | 2 ( 10.0%) | 0.117 |
| Oropharyngeal pain | 1 ( 5.0%) | 1 ( 4.3%) | 3 ( 13.6%) | 2 ( 10.0%) | 0 | 0.515 |
| Upper respiratory tract infection | 3 ( 15.0%) | 4 ( 17.4%) | 3 ( 13.6%) | 4 ( 20.0%) | 3 ( 15.0%) | 0.994 |

**Pairwise statistical comparison *p*-values**

| | Group 1 | Group 2 | Group 3 | Group 4 | | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|---|---|---|---|---|
| Injection site erythema/redness | | | | | Injection site pain | | | | |
| Group 2 | <0.001* | | | | Group 2 | 0.087 | | | |
| Group 3 | 1.000 | <0.001* | | | Group 3 | 1.000 | 0.046* | | |
| Group 4 | <0.001* | NA | <0.001* | | Group 4 | 0.011* | 0.908 | 0.005* | |
| Group 5 | <0.001* | 1.000 | <0.001* | 1.000 | Group 5 | 0.908 | 0.908 | 0.873 | 0.321 |

Note: Study Period I: Day 0-42.

1 *p*-value for comparison across all five treatment groups obtained by Fisher's exact test; if significant, pairwise comparisons were performed with Fisher's exact test with Bonferroni adjustments; $p<0.05$ marked with *. 2 Protocol-specified solicited local and systemic adverse events deemed related to treatment.