Supplementary Material

Safety and immunogenicity of the ID93 + GLA-SE tuberculosis vaccine in BCG-vaccinated healthy adults: A randomized, double-blind, placebo-controlled phase 2 trial

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Table of Contents

Supplementary Material Appendix

1. Methods and materials

1.1 Enrollment

Screening numbers were sequentially assigned to the subjects who signed the informed consent to participate in the study. Eligible subjects who met the inclusion criteria were randomized according to the study randomization plan. The subjects were sequentially assigned to unique randomization numbers in the order of study entry as determined by the investigator. The randomization ratio is 1:1:1 for each cohort. The randomization list was generated by the randomization manager using a SAS program (SAS v9.4) based on the units determined prior to the study initiation. The Investigational Product was dispensed by an unblinding pharmacist according to the subject's randomization number in the randomization list.

1.2 Inclusion criteria and exclusion criteria 1.2.1

Inclusion criteria

Subjects had to meet all the following inclusion criteria to be eligible for participation in the study:

1) Male or female who were ≥ 19 and < 65 years of age.
2) Healthcare workers who were QuantiFERON-TB Gold Plus negative (not latently infected with *Mycobacterium tuberculosis*) at screening.
3) Were able to comply with the scheduled visits and expected to continue working in the current medical institution and be available for a continuous follow-up by the investigator via provided contact information.
4) Only for female subjects of childbearing potential:

① Must be human chorionic gonadotropin (hCG)-negative from serum or urine pregnancy test, at screening.

② Agreed to use one of the following acceptable birth control methods to avoid pregnancy until the end of study (Visit 9): hormonal contraceptives, intrauterine device (IUD) or intrauterine system (IUS), tubal

ligation, or combination of barrier methods (combined use of barrier methods such as male condoms, female condoms, cervical cap, diaphragm, sponge, or implant).

5) History of BCG vaccination that was confirmed through medical examination (i.e., asking a subject about his/her condition) or presence of a scar.
6) Body mass index (BMI) ≥ 19 and ≤ 33 (kg/㎡) at screening. (Body mass index (BMI) results are rounded off to the first decimal place)
7) Understood the study procedures, voluntarily decide to participate in the study and signed the informed consent form.

1.2.2 Exclusion criteria

Subjects must have met none of the following exclusion criteria to be eligible for participation in the study: 1) History of positive tuberculin skin test or positive QuantiFERON-TB results.

2) History of severe chronic disease that may compromise the safety of the subject during the study (e.g., impairment of pulmonary function from tuberculosis infection or other pulmonary disease; chronic illness with signs of cardiac or renal failure; suspected progressive neurological disease or uncontrolled epilepsy).
3) Had acute fever (body temperature ≥ 38 ℃ at the time of randomization or within 24 hours before randomization), acute respiratory diseases, or active infection.
4) Malignant tumors or a history of malignant tumors.
5) Planned surgery during the study period.
6) Impaired immune functions including autoimmune disease or immunodeficiency disease.
7) History of Guillain-Barre syndrome.
8) History of anaphylaxis or severe allergic reaction to vaccines, eggs, or other allergens.
9) Was living with a household member with active TB or infectious TB.
10) Clinically significant abnormal laboratory values for any of the following tests conducted in the study center, prior to randomization (however, even if clinical laboratory test result was abnormal, the subject could be randomly assigned if the result was Not Clinically Significant under the investigator's judgment)

- Hemoglobin, hematocrit, absolute neutrophil count, absolute lymphocyte count, or platelet count: < Lower limit of normal (LLN)
- White blood cell count: > Upper limit of normal (ULN) or < LLN (i.e., had to be within normal limits)

- Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, alkaline phosphatase, creatinine, or blood urea nitrogen (BUN): > ULN

11) Received immunosuppressant, immunity-modifying drug, or other treatment that might affect the immune system including cytotoxic anti-cancer agents or radiotherapy, within 3 months prior to the randomization.
12) Use of systemic steroids (equivalent to daily prednisone ≥ 15 mg/day for more than 14 days), inhaled or intranasal steroids, within 3 months prior to randomization; however, use of topical corticosteroids were acceptable, regardless of dose.
13) Use of immunoglobulin or blood products within 3 months before randomization or planned to use them during the study period.
14) Human Immunodeficiency Virus (HIV) positive at screening.
15) Had chronic hepatitis (e.g., hepatitis B core antibody or hepatitis C antibody positive) at screening. 'Chronic hepatitis' meant current hepatitis results with virus test at screening.
16) Unable to discontinue current chronic drug therapy such as thyroxin, insulin, or other medications with hepatotoxicity or myelotoxicity; however, estrogen and progesterone replacement therapy or contraceptives, and topical medications were acceptable.
17) Pregnant or lactating.
18) Use of other vaccines within 4 weeks before screening or planned to use them from the day of screening to 4 weeks after the last vaccination with the Investigational Product or within 4 weeks before the End Visit.
19) Use of other investigational drugs within 4 weeks before screening.
20) Deemed ineligible by investigator based on other reasons.

1.3 Definition of Adverse Events

Adverse event was defined as any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease experienced by a subject who was administered with IP, and which does not necessarily have a causal relationship with the IP. An adverse drug reaction (ADR) was defined as any noxious and unintended responses to an IP, related to any dose for which causality cannot be excluded. An unexpected ADR was defined as any ADR of which the nature or severity is not consistent with the applicable product information such as IB or package insert. For further definitions of AEs, refer to the study protocol.

Solicited AEs, including local and systemic AEs are listed in the study protocol. AESIs were pre-defined AEs listed of the study protocol. Solicited local AEs included tenderness, pain, erythema/flare, and induration/edema; solicited systemic AEs included fatigue, myalgia, headache, diarrhea, nausea/vomiting, chills, arthralgia, fever, and hives. All unsolicited AEs were classified by the MedDRA System Organ Class (SOC) and Preferred Term
(PT). Each subject was counted only once within an SOC or a PT.

1.3.1 Severity Assessment

For solicited AEs, severity was assessed according to the Korea Ministry of Food and Drug Safety "Guideline on Grading Scale for Adverse Events in Vaccine Clinical Trial" (Dec 2011) and Food and Drug Administration guidance "Toxicity Grading scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (Sep 2007).

For unsolicited AEs, severity was assessed based on the following criteria:

- Grade 1 (Mild): Transient or mild discomfort (< 48 hours); no medical intervention or therapy required; daily activity was not affected.
- Grade 2 (Moderate): Mild to moderate limitation in activity (able to conduct ≥ 50% of daily activity); some assistance might be needed (no or minimal medical intervention or therapy required).
- Grade 3 (Severe): Marked limitation in activity (able to conduct <50% of daily activity); some assistance usually required (medical intervention or therapy required, hospitalization possible).
- Grade 4 (Potentially Life Threatening): Life-threatening, extreme limitation in activity; significant assistance required (significant medical intervention or therapy required, hospitalization or hospice probable).

1.3.2 Causality Assessment

Causality of AEs was assessed based on the following criteria:

1.3.2.1 Related to IP:

Definitely related:

- There was evidence that the IP was administered; temporal relationship of the onset of the event, relative to IP administration, was reasonable.

- The occurrence of the event was most plausibly explained by administration of the IP than any other cause.
    - The AE disappeared upon withdrawal of the IP and reappeared following IP reintroduction.
- An event, which exhibited the pattern consistent with the information already known to the IP or other drug within the same class.

Probably related:

- There was evidence that the IP was administered; temporal relationship of the onset of the event, relative to administration of the IP, was reasonable.
- The occurrence of the event was more plausibly explained by IP administration than other causes.
- The AE disappeared upon withdrawal of the IP.

Possibly related:

- There was evidence that the IP was administered; temporal relationship of the onset of the event, relative to administration of the IP, was reasonable.
- The event could have been due to another, equally likely, cause.
- The AE disappeared upon withdrawal of the IP.

Probably not related:

- There was evidence that the IP was administered.
- There was another more likely cause of the event.
- The AE disappeared or became vague upon withdrawal of the IP.
- The AE did not reappear or became vague following the reintroduction of the IP.

Unknown (Not assessable/Unclassifiable):

- An AE, which could not be judged because information was insufficient or contradictory, and which could not be supplemented or verified.

1.3.2.2 Not related to IP:

Temporal relationship of the onset of the event, relative to administration of the IP, was not reasonable or the occurrence of the event was plausibly explained by causes other than administration of the IP.

1.3.3 Seriousness Assessment

An AE was considered serious if it resulted in any of the following outcomes:

- Death or was life-threatening
- Inpatient hospitalization or prolongation of existing hospitalization

- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Other medically important conditions that might not have resulted in outcomes listed above (e.g., development of drug dependency, drug abuse, or blood dyscrasias)

However, AE was not considered serious if a subject was hospitalized for any of the following reasons:

- Hospitalization planned prior to the subject's inclusion in the study
- Hospitalization as part of the medical examination, cosmetic procedures, beauty, and nursing care
- Emergency room visits for fewer than 24 hours. These events could be considered and reported as an SAE in the judgment of the investigator

Other medical events that might have a significant impact on the safety and health conditions of the subject, could be considered serious based upon medical judgment of physician or relevant experts.

1.3.4 Collection and recording of AE

Data on AEs were obtained based on voluntary reporting from the subjects, monitoring and interviews at site visits, phone-call follow-ups, and subject diaries. The injection site was monitored for at least 30 minutes for acute reactions, and any observed symptoms were recorded after each IP administration. Subjects were instructed to record any AEs (both solicited and unsolicited) in their subject diaries after each IP administration. Solicited AEs were collected up to 7 days from each IP administration (revised from 28 days after the final IP administration to 7 days from each IP administration in protocol), and unsolicited AEs were collected up to 28 days. A site visit or phone-call monitoring was conducted for a safety follow-up 7 days after each IP administration.

Clinically significant abnormalities from safety assessments including clinical laboratory tests, vital signs, and physical examination that met the definition of an AE after IP administration were reported as AEs. Any preexisting conditions prior to IP administration were recorded as medical history. For long-term safety assessment, SAEs and AESIs were monitored up to 12 months (Visit 9) after the final IP administration. AEs that occurred after Visit 9 were reported only when serious and related to IP.

All AEs were followed until the event was resolved or became stable, or the subject was lost to follow-up. AE documentation included AE term, onset and resolution date, severity, causal relationship to the IP, actions taken

to the IP, AE outcome, treatment of the AE, and AE seriousness. When recording an AE, the investigator used the overall diagnosis or syndrome using standard medical terminology, rather than recording individual symptoms or signs. Unsolicited AEs were recorded by the MedDRA Version 21.0 terminology.

1.4 Measurements

1.4.1 IgG enzyme-linked immunosorbent assay (ELISA)

Ninety-six-well High Binding Plates (Corning, NY) were coated with 100 µl ID93 at a concentration of 1 µg/ml in coating buffer (eBiosciences, CA, cat# 00-0044-59) overnight at 4°C. Wells were washed three times with Wash Buffer A (Teknova, CAP2192) (PBS/0·1% Tween-20), and then blocked overnight at 4°C with blocking buffer (1% BSA/PBS/0·05% Tween). Plates were washed 3 times with Wash Buffer A. Serum samples were serially diluted 4-fold in duplicate with sample diluent (0·1% BSA/PBS/0·05%) for a 12-point dilution series starting at 1:100. Plates were incubated overnight at 4°C. Plates were washed five times, followed by the addition of 100 µl/well of anti-human rec Protein-G-HRP (Invitrogen, CA 10-1223) for 1 hour at room temperature. Plates were washed six times and developed with 100 µl SureBlue™ TMB Peroxidase Substrate (SeraCare, MD 5120-0077) followed by 50 µl Stop Solution (1N H_2SO_4; RICCA, 8300-5). Optical Density (OD) readings at 450 nm and 570 nm were taken within 1 hour of Stop Solution addition using a SpectraMax i3x plate reader and SoftMax Pro 6.4.2 Software. Endpoint titers were calculated by subtracting OD_{570} from OD_{450} for interpolating unknowns from the last value greater than the threshold given by naïve serum using a 4parameter logistic model (Parameter 208) XL-Fit software as a Microsoft Excel add-in.

1.4.2 Peripheral blood mononuclear cells (PBMCs)-based enzyme-linked immunospot (ELISpot)

PBMCs were cryopreserved prior to ELISpot assays. To thaw, vials were defrosted in a 37°C water bath and the contents transferred to a 50 ml centrifuge tube (Greiner) containing 25 ml of R10 medium containing RPMI1640, 10% FBS, 2 mM L-Glutamine and 100 U/ml PCN-Streptomycin, and tubes were centrifuged at 400 × *g* for 10 minutes. After discarding supernatants, pellets were carefully resuspended in 10 ml R10 medium, and cells were counted using Guava®easyCyte™. Vials containing 2 x 10^5 cells/ml were incubated at 37°C, 5% CO_2 for 18–24 hours. Recovered PBMC were enumerated as described above. Cells were not used when cell viability was < 66%.

ELISpot assays were performed using Human IFN-γ ELISpot PRO kits (Mabtech AB, Sweden, cat# 3420-2APT-10), according to the manufacturer's instructions. Briefly, plates were washed four times with sterile PBS and blocked with R10 medium; 2×10^5 PBMCs were stimulated in triplicate with ID93 antigen at a concentration of 1 µg/ml in a 37°C (5% CO_2) for 20 and 24 hours. Negative controls, medium only and positive controls [phytohemagglutinin (PHA)] were also included. All assays were run in triplicate. Following incubation, the plates were washed five times with PBS, probed with detection alkaline phosphatase conjugated 7-B6-1 monoclonal antibody for 2 hours and developed using BCIP/NBT-plus (Mabtech substrate solution).

The number of spots was counted using an Immunospot analyzer (Cellular Technology Limited, Cleveland, OH). Mean spot counts for negative control wells were subtracted from the mean of test wells to generate normalized readings; these are presented as the number of spot forming cells (SFC) per million PBMCs.

1.4.3 Intracellular cytokine staining (ICS) and flow cytometry

Sample Processing:

Peripheral blood mononuclear cells (PBMCs) were isolated from blood collected from participants using CPT 8 ml BD Vacutainer (cat# 362780) within 8 hours of venipuncture according to the manufacturer's recommendation and were cryopreserved and shipped to Center for Global Infectious Disease Research (CGIDR) of Seattle Children's Research Institute (SCRI), Seattle, WA, USA. PBMC were thawed in the presence of benzonase (Millipore, Burlington, MA), assessed for viability using Trypan Blue (Life Technologies), then rested overnight at 37°C/5% CO_2 in R10 prior to stimulation. Viability was again determined after resting. A minimum cell viability of 66% measured on the day following thaw was required for use in ICS assays. This threshold is arbitrary and has been used within the Clinical Immunology Group for many previous clinical trials.

***In Vitro* Stimulations:**

A million viable PBMCs were dispensed per well into 96-well round bottom plates (Corning, Corning, NY) to assess *ex vivo* cytokine responses. Cells were stimulated with 10 µg/ml ID93 whole protein in R10 medium or 0·25 µg/ml staphylococcal enterotoxin B (SEB; Toxin Technologies, Sarasota, FL) as a positive control or left unstimulated. The co-stimulatory antibodies CD28 and CD49d (eBioscience) were included in the culture medium. After 2 h of incubation at 37°C and 5% CO_2, 1 µg/ml Brefeldin A (eBioscience) was added. Plates

were cultured an additional 10 hours in an EchoTherm programmable incubator (Torrey Pines Scientific, Carlsbad, CA), then maintained at 4°C until staining.

Intracellular Cytokine Staining:

Cells were first incubated at room temperature with LIVE/DEAD fixable aqua viability dye (Invitrogen) diluted in PBS (Life Technologies) for 20 minutes. PBMC were then washed with 150 µl FACS wash buffer (FACS wash, 1% FBS in PBS). Cells were stained with surface stain antibody cocktail containing: CD14 BV510 (clone M5E2 BioLegend), CD19 BV510 (clone HIB19, BD Biosciences), CCR7 BV605 (clone G043H7, BioLegend), and CD45RA PerCP-Cy5.5 (clone HI10, BioLegend) in Brilliant Stain buffer (BD Biosciences) for 30 min. After washing with FACS wash, PBMCs were fixed with BD Cytofix/Cytopem for 10 min at room temperature and washed with BD Perm/Wash Buffer. Subsequently, PBMCs were incubated for 30 min with an intracellular antibody cocktail containing CD3 ECD (clone UTCHI, Beckman Coulter), CD4 APC-eFluor780 (clone SK3, Invitrogen), CD8 AlexaFluor700 (clone HIT8a, BioLegend), TNFα FITC (clone Mab11, eBioscience), IL-2 PE (clone MQ1-17H12, BioLegend), CD154 PE-Cy5 (clone 24-31, BioLegend), and IFN-γ v450 (clone B27, BD Biosciences) in Perm/Wash. Cells were washed with Perm/Wash, then resuspended in 200 µl 1% paraformaldehyde (stock 10% paraformaldehyde, EMS) diluted in distilled water (Life Technologies) prior to acquisition. All samples were acquired the day of staining on an LSR II cytometer with High Throughput Sampler (BD) capable of measuring 18 colors, collecting on average a range of 100,000–200,000 lymphocyte-gated events.

Flow cytometry analysis:

Data analysis was performed using FlowJo software (v.9.9.6, BD). Data files were uploaded to a pre-designed analysis template. Individual gates were adjusted to only include cell populations that were predefined to yield outcomes of interest. The following inclusion/exclusion criteria were applied to determine which data to include in the final analysis:

- The negative (unstimulated) control had to be present and interpretable for each set of samples from a study day.
- CD4+ T cells in the SEB-stimulated sample (positive control) had to include at least one of the following cytokines: IFN-γ, IL-2, or TNF-α production ≥ 3%.

Supplementary Material Tables

Supplementary Material Table S1. Overall frequencies of participants (%) in the study groups with solicited local AEs.

Time Point	Cohort 1 (N = 35)		Cohort 2 (N = 36)		Cohort 3 (N = 36)		Total (N = 107)	
Symptom	(2 μg ID93 + 5 μg GLA-SE)		(10 μg ID93 + 5 μg GLA-SE)		(0·9% normal saline placebo)			
Severity	n	%	n	%	n	%	n	%
First Dose of IP, total (# of Subjects with any local symptoms)	27	77.14	33	91.67	12	33.33	72	67.29
Second Dose of IP, total (# of Subjects with any local symptoms)	29	85.29	34	94.44	6	17.65	69	66.35
Third Dose of IP, total (# of Subjects with any local symptoms)	25	75.76	27	77.14	6	18.18	58	57.43

First Dose of IP	Cohort 1 (N = 35)		Cohort 2 (N = 36)		Cohort 3 (N = 36)		Total (N = 107)	
(# of 1st dose completed subjects)	n	%	n	%	n	%	n	%
Pain, total	25	71.43	27	75.00	7	19.44	59	55.14
Mild	20	57.14	23	63.89	7	19.44	50	46.73
Moderate	4	11.43	3	8.33	0	0·00	7	6.54
Severe	1	2.86	1	2.78	0	0·00	2	1.87
Tenderness, total	25	71.43	31	86.11	9	25.00	65	60·75
Mild	11	31.43	18	50·00	8	22.22	37	34.58
Moderate	10	28.57	10	27.78	1	2.78	21	19.63

Severe	4	11.43	3	8.33	0	0·00	7	6.54
Erythema/flare, total	4	11.43	2	5.56	0	0·00	6	5.61
Mild	4	11.43	2	5.56	0	0·00	6	5.61
Induration/edema, total	3	8.57	3	8.33	0	0·00	6	5.61
Mild	3	8.57	3	8.33	0	0·00	6	5.61

Second Dose of IP	Cohort 1 (N = 34)		Cohort 2 (N = 36)		Cohort 3 (N = 34)		Total (N = 104)	
(# of 2nd dose completed subjects)	n	%	n	%	n	%	n	%
Pain, total	28	82.35	32	88.89	3	8.82	63	60·58
Mild	23	67.65	28	77.78	2	5.88	53	50·96
Moderate	3	8.82	4	11.11	1	2.94	8	7.69
Severe	2	5.88	0	0·00	0	0·00	2	1.92
Tenderness, total	28	82.35	33	91.67	4	11.76	65	62.50
Mild	20	58.82	17	47.22	3	8.82	40	38.46
Moderate	2	5.88	12	33.33	0	0·00	14	13.46
Severe	6	17.65	4	11.11	1	2.94	11	10·58
Erythema/flare, total	5	14.71	4	11.11	0	0·00	9	8.65
Mild	3	8.82	1	2.78	0	0·00	4	3.85
Moderate	0	0·00	3	8.33	0	0·00	3	2.88
Severe	2	5.88	0	0·00	0	0·00	2	1.92

Induration/edema, total	2	5.88	4	11.11	0	0·00	6	5.77
Mild	1	2.94	3	8.33	0	0·00	4	3.85
Moderate	1	2.94	1	2.78	0	0·00	2	1.92

Third Dose of IP	Cohort 1 (N = 33)		Cohort 2 (N = 35)		Cohort 3 (N = 33)		Total (N = 101)	
(# of 3rd dose completed subjects)	n	%	n	%	n	%	n	%
Pain, total	20	60·61	25	71.43	5	15.15	50	49.50
Mild	17	51.52	23	65.71	4	12.12	44	43.56
Moderate	3	9.09	2	5.71	1	3.03	6	5.94
Tenderness, total	25	75.76	26	74.29	6	18.18	57	56.44
Mild	20	60·61	15	42.86	5	15.15	40	39.60
Moderate	4	12.12	10	28.57	0	0·00	14	13.86
Severe	1	3.03	1	2.86	1	3.03	3	2.97
Erythema/flare, total	4	12.12	3	8.57	0	0·00	7	6.93
Mild	3	9.09	0	0·00	0	0·00	3	2.97
Moderate	1	3.03	3	8.57	0	0·00	4	3.96
Induration/edema, total	2	6.06	2	5.71	0	0·00	4	3.96
Mild	2	6.06	1	2.86	0	0·00	3	2.97
Moderate	0	0·00	1	2.86	0	0·00	1	0·99

Supplementary Material Table S2. Overall frequencies of participants (%) in the study groups with solicited systemic AEs.

Time Point Symptom Severity	Cohort 1 (N = 35) (2 μg ID93 + 5 μg GLA-SE)		Cohort 2 (N = 36) (10 μg ID93 + 5 μg GLA-SE)		Cohort 3 (N = 36) (0·9% normal saline placebo)		Total (N = 107)	
	n	%	n	%	n	%	n	%
First Dose of IP, total (# of Subjects with any systemic symptoms)	23	65.71	19	52.78	11	30·56	53	49.53
Second Dose of IP, total (# of Subjects with any systemic symptoms)	18	52.94	24	66.67	6	17.65	48	46.15
Third Dose of IP, total (# of Subjects with any systemic symptoms)	17	51.52	18	51.43	3	9.09	38	37.62

First Dose of IP (# of 1st dose completed subjects)	Cohort 1 (N = 35)		Cohort 2 (N = 36)		Cohort 3 (N = 36)		Total (N = 107)	
	n	%	n	%	n	%	n	%
Fever, total	0	0·00	0	0·00	0	0·00	0	0·00
Mild	0	0·00	0	0·00	0	0·00	0	0·00
Moderate	0	0·00	0	0·00	0	0·00	0	0·00
Severe	0	0·00	0	0·00	0	0·00	0	0·00
Nausea / Vomiting, total	3	8.57	1	2.78	2	5.56	6	5.61
Mild	2	5.71	1	2.78	2	5.56	5	4.67
Moderate	1	2.86	0	0·00	0	0·00	1	0·93

Diarrhea, total	5	14.29	1	2.78	1	2.78	7	6.54
Mild	4	11.43	1	2.78	1	2.78	6	5.61
Moderate	1	2.86	0	0·00	0	0·00	1	0·93
Headache, total	12	34.29	7	19.44	2	5.56	21	19.63
Mild	8	22.86	4	11.11	2	5.56	14	13.08
Moderate	3	8.57	3	8.33	0	0·00	6	5.61
Severe	1	2.86	0	0·00	0	0·00	1	0·93
Fatigue, total	14	40·00	17	47.22	9	25.00	40	37.38
Mild	11	31.43	16	44.44	8	22.22	35	32.71
Moderate	2	5.71	1	2.78	1	2.78	4	3.74
Severe	1	2.86	0	0·00	0	0·00	1	0·93
Myalgia, total	20	57.14	12	33.33	3	8.33	35	32.71
Mild	13	37.14	10	27.78	3	8.33	26	24.30
Moderate	4	11.43	2	5.56	0	0·00	6	5.61
Severe	3	8.57	0	0·00	0	0·00	3	2.80
Chills, total	4	11.43	2	5.56	0	0·00	6	5.61
Mild	2	5.71	2	5.56	0	0·00	4	3.74
Moderate	1	2.86	0	0·00	0	0·00	1	0·93
Severe	1	2.86	0	0·00	0	0·00	1	0·93
Arthralgia, total	5	14.29	0	0·00	0	0·00	5	4.67

Mild	4	11.43	0	0·00	0	0·00	4	3.74
Moderate	0	0·00	0	0·00	0	0·00	0	0·00
Severe	1	2.86	0	0·00	0	0·00	1	0·93
Hives, total	2	5.71	0	0·00	0	0·00	2	1.87
Mild	2	5.71	0	0·00	0	0·00	2	1.87

Second Dose of IP	Cohort 1 (N = 34)		Cohort 2 (N = 36)		Cohort 3 (N = 34)		Total (N = 104)	
(# of 2nd dose completed subjects)	n	%	n	%	n	%	n	%
Fever, total	1	2.94	2	5.56	0	0·00	3	2.88
Mild	0	0·00	2	5.56	0	0·00	2	1.92
Moderate	1	2.94	0	0·00	0	0·00	1	0·96
Nausea / Vomiting, total	2	5.88	2	5.56	1	2.94	5	4.81
Mild	1	2.94	1	2.78	1	2.94	3	2.88
Moderate	1	2.94	1	2.78	0	0·00	2	1.92
Diarrhea, total	4	11.76	1	2.78	0	0·00	5	4.81
Mild	3	8.82	1	2.78	0	0·00	4	3.85
Moderate	1	2.94	0	0·00	0	0·00	1	0·96
Headache, total	9	26.47	8	22.22	3	8.82	20	19.23
Mild	5	14.71	6	16.67	3	8.82	14	13.46
Moderate	4	11.76	2	5.56	0	0·00	6	5.77

Fatigue, total	8	23.53	17	47.22	4	11.76	29	27.88
Mild	7	20·59	12	33.33	4	11.76	23	22.12
Moderate	1	2.94	5	13.89	0	0·00	6	5.77
Myalgia, total	15	44.12	18	50·00	2	5.88	35	33.65
Mild	11	32.35	12	33.33	2	5.88	25	24.04
Moderate	1	2.94	6	16.67	0	0·00	7	6.73
Severe	3	8.82	0	0·00	0	0·00	3	2.88
Chills, total	2	5.88	7	19.44	0	0·00	9	8.65
Mild	2	5.88	4	11.11	0	0·00	6	5.77
Moderate	0	0·00	3	8.33	0	0·00	3	2.88
Arthralgia, total	6	17.65	3	8.33	0	0·00	9	8.65
Mild	6	17.65	2	5.56	0	0·00	8	7.69
Moderate	0	0·00	1	2.78	0	0·00	1	0·96
Hives, total	0	0·00	0	0·00	0	0·00	0	0·00

Third Dose of IP	Cohort 1 (N = 33)		Cohort 2 (N = 35)		Cohort 3 (N = 33)		Total (N = 101)	
(# of 3rd dose completed subjects)	n	%	n	%	n	%	n	%
Fever, total	2	6.06	2	5.71	0	0·00	4	3.96
Mild	2	6.06	2	5.71	0	0·00	4	3.96

Nausea / Vomiting, total	0	0·00	2	5.71	0	0·00	2	1.98
Mild	0	0·00	1	2.86	0	0·00	1	0·99
Moderate	0	0·00	1	2.86	0	0·00	1	0·99
Diarrhea, total	1	3.03	0	0·00	0	0·00	1	0·99
Mild	1	3.03	0	0·00	0	0·00	1	0·99
Headache, total	6	18.18	7	20·00	2	6.06	15	14.85
Mild	3	9.09	6	17.14	1	3.03	10	9.90
Moderate	3	9.09	1	2.86	1	3.03	5	4.95
Fatigue, total	5	15.15	15	42.86	3	9.09	23	22.77
Mild	4	12.12	13	37.14	2	6.06	19	18.81
Moderate	1	3.03	2	5.71	1	3.03	4	3.96
Myalgia, total	14	42.42	14	40·00	2	6.06	30	29.70
Mild	9	27.27	10	28.57	1	3.03	20	19.80
Moderate	5	15.15	3	8.57	1	3.03	9	8.91
Severe	0	0·00	1	2.86	0	0·00	1	0·99
Chills, total	4	12.12	4	11.43	1	3.03	9	8.91
Mild	3	9.09	2	5.71	0	0·00	5	4.95
Moderate	1	3.03	1	2.86	1	3.03	3	2.97
Severe	0	0·00	1	2.86	0	0·00	1	0·99
Arthralgia, total	6	18.18	2	5.71	1	3.03	9	8.91

Mild	6	18.18	2	5.71	0	0·00	8	7.92
Moderate	0	0·00	0	0·00	1	3.03	1	0·99
Hives, total	0	0·00	0	0·00	0	0·00	0	0·00

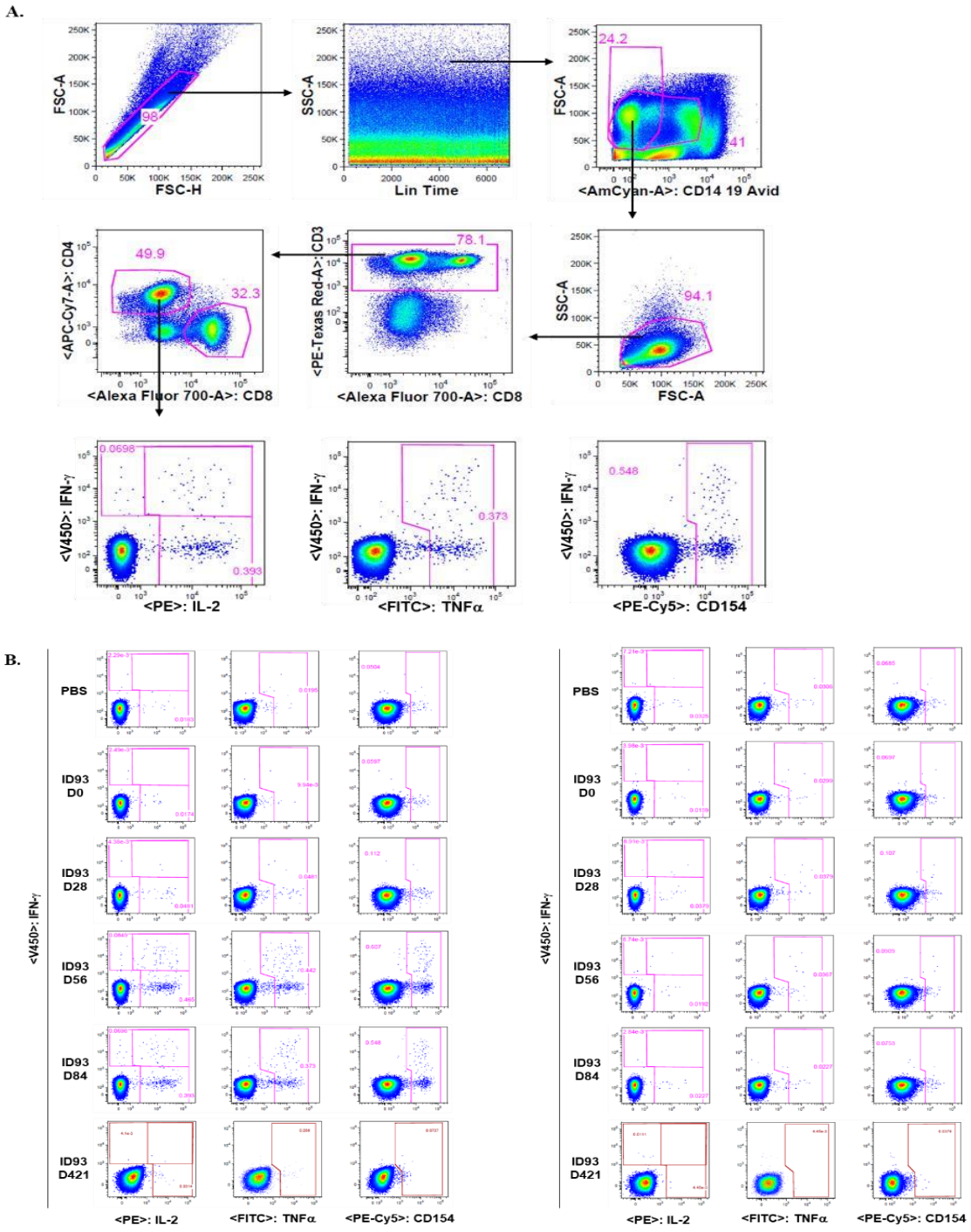
Supplementary Material Table S3. The most frequent unsolicited adverse events via SOC and PT.

AEs by System Organ Class Preferred Term	Cohort 1 (N = 35) (2 μg ID93 + 5 μg GLA-SE)			Cohort 2 (N = 36) (10 μg ID93 + 5 μg GLA-SE)			Cohort 3 (N = 36) (0·9% normal saline placebo)			Total (N = 107)		
	E	N	%	E	N	%	E	N	%	E	N	%
Subjects with any AEs, Total	25	14	40·00	38	23	63.89	27	14	38.89	90	51	47.66
Mild	18	11	31.43	27	15	41.67	16	9	25.00	61	35	32.71
Moderate	6	4	11.43	10	10	27.78	10	8	22.22	26	22	20·56
Severe	1	1	2.86	1	1	2.78	1	1	2.78	3	3	2.80
Subjects with any related AEs, Total	16	11	31.43	16	10	27.78	11	5	13.89	43	26	24.30
Infections and Infestations, Total	4	3	8.57	10	9	25.00	5	5	13.89	19	17	15.89
Mild	3	3	8.57	5	4	11.11	3	3	8.33	11	10	9.35
Moderate	1	1	2.86	5	5	13.89	2	2	5.56	8	8	7.48
Related	2	2	5.71	3	3	8.33	0	0	0·00	5	5	4.67
Nasopharyngitis, Total	3	3	8.57	3	2	5.56	2	2	5.56	8	7	6.54
Mild	2	2	5.71	3	2	5.56	1	1	2.78	6	5	4.67
Moderate	1	1	2.86	0	0	0·00	1	1	2.78	2	2	1.87
Gastrointestinal Disorders, Total	3	3	8.57	9	8	22.22	3	3	8.33	15	14	13.08
Mild	1	1	2.86	9	8	22.22	2	2	5.56	12	11	10·28
Moderate	2	2	5.71	0	0	0·00	1	1	2.78	3	3	2.80
Related	2	2	5.71	5	4	11.11	1	1	2.78	8	7	6.54

Dyspepsia, Total	2	2	5.71	3	3	8.33	0	0	0·00	5	5	4.67
Mild	1	1	2.86	3	3	8.33	0	0	0·00	4	4	3.74
Moderate	1	1	2.86	0	0	0·00	0	0	0·00	1	1	0·93
Related	2	2	5.71	2	2	5.56	0	0	0·00	4	4	3.74
General Disorders and Administration Site Conditions, Total	4	4	11.43	4	2	5.56	5	3	8.33	13	9	8.41
Mild	3	3	8.57	4	2	5.56	3	2	5.56	10	7	6.54
Moderate	1	1	2.86	0	0	0·00	2	1	2.78	3	2	1.87
Related	4	4	11.43	4	2	5.56	5	2	5.56	13	8	7.48
Musculoskeletal and Connective Tissue Disorders, Total	2	2	5.71	5	5	13.89	3	2	5.56	10	9	8.41
Mild	2	2	5.71	3	3	8.33	2	1	2.78	7	6	5.61
Moderate	0	0	0·00	2	2	5.56	0	0	0·00	2	2	1.87
Severe	0	0	0·00	0	0	0·00	1	1	2.78	1	1	0·93
Related	2	2	5.71	1	1	2.78	2	1	2.78	5	4	3.74
Nervous System Disorders, Total	1	1	2.86	2	2	5.56	4	4	11.11	7	7	6.54
Mild	0	0	0·00	0	0	0·00	4	4	11.11	4	4	3.74
Moderate	1	1	2.86	2	2	5.56	0	0	0·00	3	3	2.80
Headache, Total	1	1	2.86	1	1	2.78	2	2	5.56	4	4	3.74
Mild	0	0	0·00	0	0	0·00	2	2	5.56	2	2	1.87
Moderate	1	1	2.86	1	1	2.78	0	0	0·00	2	2	1.87

Reproductive System and Breast Disorders, Total	2	2	5.71	2	2	5.56	1	1	2.78	5	5	4.67
Mild	1	1	2.86	1	1	2.78	1	1	2.78	3	3	2.80
Moderate	0	0	0·00	1	1	2.78	0	0	0·00	1	1	0·93
Severe	1	1	2.86	0	0	0·00	0	0	0·00	1	1	0·93
Respiratory, Thoracic and Mediastinal Disorders, Total	5	2	5.71	2	1	2.78	3	2	5.56	10	5	4.67
Mild	5	2	5.71	2	1	2.78	1	1	2.78	8	4	3.74
Moderate	0	0	0·00	0	0	0·00	2	1	2.78	2	1	0·93
Related	5	2	5.71	2	1	2.78	1	1	2.78	8	4	3.74

AEs sorted via System Organ Class (SOC) and Preferred Terms (PT).



Supplementary Material Figure S1. Gating strategy and representative scatterplots for the stimulation with ID93. A. Gating strategy. On FSC-A versus FSC-H plot, aggregated cells were excluded. (Alternate: single cell population was selected). Single cells were viewed on a time versus SSC-A plot to screen out flow

rate perturbations. Following exclusion of CD14+, CD19+, and dead cells, lymphocytes were selected on an FSC-A versus SSC-A plot. The lymphocyte population was gated on CD3+ T cells, then CD4+ and CD8+ T cell populations. CD4+ T cells were subsequently examined for expression of IFN-γ, IL-2 and TNF-α. B. Cytokine production of representative responder and non-responder. Left side: high(er) responder (S1029); right side nonresponder (S1033). Top panel: PBS stimulation of PBMCs; subsequent panels: cytokine production upon ID93 stimulation for Days 0, 28, 56, 84, and 421

Consort 2010 Flow Diagram