A personal COVID-19 dendritic cell vaccine made at point-of-care: Feasibility, safety, and antigen- specific cellular immune responses

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RESEARCH ARTICLE

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A personal COVID-19 dendritic cell vaccine made at point-of-care: Feasibility, safety, and antigen-specific cellular immune responses

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a world-wide pandemic. Internationally, because of availability, accessibility, and distribution issues, there is a need for additional vaccines. This study aimed to: establish the feasibility of personal dendritic cell vaccines to the SARS-CoV-2 spike protein, establish the safety of a single subcutaneous vaccine injection, and determine the antigen-specific immune response following vaccination. In Phase 1, 31 subjects were assigned to one of nine formulations of autologous dendritic cells and lymphocytes (DCL) incubated with 0.10, 0.33, or 1.0 µg of recombinant SARS-CoV-2 spike protein, and admixed with saline or 250 or 500 µg of granulocyte-macrophage colony-stimulating factor (GM-CSF) prior to injection, then assessed for safety and humoral response. In Phase 2, 145 subjects were randomized to one of three formulations defined by incubation with the same three quantities of spike protein without GM-CSF, then assessed for safety and cellular response. Vaccines were successfully manufactured for every subject at point-of-care. Approximately 46.4% of subjects had a grade 1 adverse event (AE); 6.5% had a grade 2 AE. Among 169 evaluable subjects, there were no acute allergic, grade 3 or 4, or serious AE. In Phase 1, anti-receptor binding domain antibodies were increased in 70% of subjects on day-28. In Phase 2, in the 127 subjects who did not have high levels of gamma interferon-producing cells at baseline, 94.4% had increased by day 14 and 96.8% by day 28. Point-of-care personal vaccine manufacturing was feasible. Further development of such subject-specific vaccines is warranted.

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Introduction

An international pandemic caused by the SARS-CoV-2 virus has been ongoing since late 2019.[1,2] Because of challenges related to manufacturing, storage, new variants, and public perceptions of side-effects and efficacy, there is a need for additional types of Covid-19 vaccines.[3,4]

Available SARS-CoV-2 vaccines have had a favorable impact by reducing the rates of symptomatic infection, and decreasing the morbidity and mortality associated with COVID-19 infection.[5–16] The major target of COVID-19 vaccines is the SARS-CoV-2 S-protein which consists of an S1 subunit containing a receptor-binding domain (RBD) that engages the angiotensin-converting enzyme 2 (ACE2) receptor on host cells, and an S2 subunit that mediates membrane fusion between virus and host cells.[17] The S-glycoprotein mediates viral entry through pH-dependent endocytosis while intercellular virus transmission into immune cells occurs through dendritic cell cell-mediated transfer.[18] The S protein appears to be the most important antigen for inducing anti-viral antibodies and *T*-cell responses to SARS-CoV-2 infection, and subsequent protective immunity.[19,20] Other than attenuated virus vaccines,[16] all other vaccines utilize S-protein as their antigen, most via mRNA and DNA synthesis *in vivo*[5–15]

Although the benefits from immunization of existing vaccines have been clearly documented, there is room for additional improvement. Some improvements might include (1) less toxicity, (2) better point-of-care access (3) better long-term efficacy results, (4) availability of personal (autologous cell-based) vaccines, and (5) better memory T cell responses. As of mid-2022, the U.S. Centers for Disease Control estimated that about 90% of the U.S. adult population had received at least one injection of the currently available vaccines, 77% were fully vaccinated, 50% had received at least one booster, and 30% two boosters.[21] Reluctance to receive recommended vaccines was related to concerns about vaccine excipients and fear of near- and delayed adverse events. Vaccines associated with fewer and less severe adverse events might increase the willingness to be vaccinated. In some countries, particularly in emerging nations, high costs, cold-chain shipping and storage requirements, and limited capacity for large-

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scale distribution have limited access to vaccination. A vaccine that could be produced at the point-of-care would be advantageous in many parts of the world. In fact, the eradication of small pox is attributed to the ability to produce vaccines at the point-of-care, regardless of geographic location.[22,23] Some individuals are reticent to take vaccines because of the presence of excipients such as antibiotics, preservatives, and stabilizers that are important for stability and shelf-life for vaccines that are manufactured in large batches. In the Muslim world, Sharia (Islamic law) and the concept of "halal," which translates to "permissible" in English, leads some individuals to refuse vaccines that include excipients such as pork gelatin hydrolyzed collagen, which has been widely used as a stabilizer for storage and transport of vaccines. Any attenuated vaccine involves foreign biological material, and some individuals consider the RNA and DNA in modern vaccines to be foreign. A personal vaccine based on *ex vivo* manipulation of one's own immune cells could overcome these fears. In addition, over time variants have emerged that might be better addressed with vaccines that can be rapidly modified to take into account the additional mutations, rather than relying on boosters of vaccines that are increasingy less effective against evolving mutated variants. Once the new mutated S-protein has been sequenced, mRNA can be used to manufacture purified protein antigen per good manufacturing practices, which could then be used in the production of personal vaccines. This could be accomplished more quickly than via the current large-batch methods that take many months to develop and standardize. With this approach antigen-specific vaccines could have been quickly available for immunization against variants such as delta[24] and omicron[25] rather than relying on boosters of an increasingly less efficacious vaccine. Approved vaccines were designed to induce a strong humoral antigen-specific antibody response rather than a T cell response, although they also do induce T cell immunity. Vaccines that induce a stronger cellular immune response may be better at preventing recurrent infections months later, and may be needed if there are subsets of COVID-19 infected patients who harbor chronic infection that might benefit from a vaccine that is more therapeutic than preventive.[26] Thus, there are numerous reasons to investigate additional COVID-19 vaccines.

It is believed that all vaccines induce humoral and cellular responses, but perhaps not always to an equal extent, and that adaptive immune memory is the major goal.[27] The favored strategy for preventive vaccines is induction of neutralizing antibodies to block virus before they enter cells. Interaction between antigen and B-cell receptors on rare cognate B cells is the basis for activation, clonal expansion, and differentiation into plasma cells that secrete antigen-specific immunoglobulin.[28] In addition, lymph node follicular dendritic cells (DC) facilitate interaction with cognate B cells to select for antibodies that maximize affinity and avidity which optimizes neutralization. This process can happen independent of T cells, but interaction with rare antigen-cognate helper T cells that express the complementary *T*-cell receptor and interaction with antigen-presenting DC facilitate the process. Antibodies can neutralize free virus, but CD8+ cytotoxic T lymphocytes are needed to eliminate cells loaded with virus.

A different approach to vaccination, and one which may be more effective as a therapeutic vaccine for chronic viral infection, is a personal vaccine in which antigen is incubated *ex vivo* with autologous DC which then phagocytose and process antigens into peptides for presentation to other immune cells. This approach involves DC at two levels, first, the injection of the *ex vivo* antigen-loaded DC that may then traffic to lymph nodes, and second, engagement of local cutaneous and Langerhans dendritic cells reacting to the injected DC. DC strategies may be preferable for producing cytotoxic T cells that can lyse infected cells,[29] but DC also facilitate B cell immune responses.[30] DC vaccines (DCV) incubated with viral antigens *ex vivo* were effective in preventing Herpes simplex,[31,32] and influenza[33,34] in animal models, and diminished human immunodeficiency virus (HIV) infection in humans.[35,36] In a model of antigen-presenting cell-specific immunosuppression and influenza infection, DCV injections generated high levels of specific antibody titers while protein vaccination could not. [33] It has been suggested that the application of DCV for the prophylaxis of viral infections is feasible and may be cost-effective. [34] In human trials, subcutaneous injections of 1 to 10 million DC incubated with antigens from HIV demonstrated a good safety profile and decreases in viral load.[35,36] Our experience with over 1,100 injections of one to 30 million DC into more than 180 cancer patients suggests that DCV are both safe and efficacious.[37–41] DC vaccines are expected to induce both humoral and cellular immunity, but most of the interest in this approach is for its effects on cellular immunity as a therapeutic rather than preventive vaccine, and for immune memory. For example, in a leukemia prevention model in mice, the immune response to a DC vaccine loaded with tumor associated antigen was predominantly cellular rather than humoral, while direct injection of antigen yielded primarily a humoral response.[42] The DC vaccine was more effective in the prevention of leukemia. The superiority of antigen-loaded DC vaccines over direct exposure to antigen-expressing tumor cells has been demonstrated in murine cancer models.[43] There are limited published data regarding DC vaccine induction of antigen-specific antibodies in humans, but similar to what was described in mouse studies, in humans with metastatic cancer, DC loaded with antigens from tumor cells were associated with better clinical outcome compared to injection of tumor cells.[39] Principal component analyses of human data demonstrated that benefit was associated primarily with a Th1/Th17 cytotoxic T cell response, but changes in immunoglobulins were also noted.[44] Even if a COVID-19 vaccine did not induce a strong primary humoral response, it would be expected to enhance the immunoglobulin response on re-exposure because of induction of memory B cells.

The induction of neutralizing antibodies for immediate protection was the emphasis of early reports of the COVID-19 vaccines that are currently available.[5–16] However, there is increasing interest in the *T*-cell responses associated with these products. One impetus for this is that the concept of effective COVID-19 vaccination has evolved from total prevention to minimization of infection, which includes subclinical infection that would be considered prevention. *T*-cell responses to mRNA vaccination are less well characterized than anitibody induction, but early reports show that CD4+ T cells are primed

by existing vaccines as part of a combined humoral and cellular adaptive immune response.[45] As would be expected, the currently available vaccines can induce T cell immunity, but whether it is optimized for adaptive cellular immunity for minimization of future exposure and infection is not yet clear.[46]

We previously proposed that personal DC vaccines might be useful in prevention of COVID-19 infection.[47] This study aimed to: (1) establish the feasibility of preparing personal dendritic cell vaccines to the SARS-CoV-2 spike protein at the point-of-care, (2) establish short-term safety following a single subcutaneous vaccine injection, (3) determine the antigen-specific immune response following vaccination, and (4) select a preferred formulation for future trials.

Materials and methods

Preclinical animal studies

Prior to initiating human trials, studies were conducted in mice to test the safety and efficacy of the AV-COVID-19 vaccine made with murine peripheral blood mononuclear cells (PBMCs) and murine granulocyte-macrophage colony stimulating factor (GM-CSF). The methods are described in Supplement 1.

Clinical study oversight

Studies were sponsored by the Indonesia Ministry of Health. Collaborators included AVITA Biomedical, Inc. (Irvine, CA.), PT AIVITA Biomedika Indonesia, the National Institute of Health Research and Development, Ministry of Health Republic of Indonesia, Dr. Kariadi Hospital Semarang, Indonesia, the Medical Faculty of the University of Diponegoro, Indonesia, and Gatot Soebroto Army Hospital (RSPAD) in Jakarta, Indonesia. The Phase 1 and 2 trials were approved by the Ethics Committees of the respective hospitals. All trials were conducted per the Helsinki Doctrine. The safety trial data was reviewed by two independent data safety monitoring boards (DSMB), one in Indonesia and one in the U.S. Oversight of the phase II trial was provided by Biometrik Riset Indonesia (Biometric Research Indonesia). Clinicaltrials.gov Phase 1: NCT04690387; Phase II: NCT05007496.

Trial design and participants

The objective of these studies was to provide safety and immune-response data for single injections of different formulations of the AV-COVID-19 SARS-CoV-2 vaccine. Eligible volunteers were over 18 years of age, in good health without serious medical diagnoses that required ongoing care or medication, non-pregnant, not previously vaccinated against COVID-19 and provided written informed consent. Subjects were excluded if they tested positive for SARS-CoV-2 antibodies per a rapid lateral flow chromatography test, if they had been diagnosed with COVID-19 in the previous 3 months or if they had symptoms of active COVID-19 infection. The first study was a double-blind randomized Phase 1 trial with a focus on safety and humoral immune reponse. This trial included nine different vaccine formulations per a 3×3 matrix testing the quantity of S-protein (0.10, 0.33, or 1.0 μg) incubated with dendritic cells during manufacturing, and the amount of GM-CSF (0, 250 or 500 μg) admixed with the cell product at the time of injection (Supplement 2). The frequency and grade of solicited and unsolicited local and systemic AE were monitored during the 28-day period after vaccination, with frequency and characterization noted for any AE or SAE that required medical intervention, and any new medical diagnoses. The second study, which was performed at a different institution 4 months later, was a Phase 2 trial testing three vaccine formulations with a focus on *T*-cell immune response and confirmation of safety. In the phase 2 study GM-CSF was omitted from the formulations, which were defined by the quantity of S-protein incubated with DCL.

The first three subjects were enrolled on 12/07/20 and vaccinated on 12/14/20 with DCL that had been incubated with 0.1 μg of S-protein with the consecutive assignment of one each to DCL in saline, DCL in 250 μg GM-CSF, and DCL in 500 μg GM-CSF. After a DSMB confirmed safety in these three subjects, an additional 28 subjects were randomized among the nine formulations between 12/15/20 and 01/06/21 (Supplement 2). After completion of the Phase 1 trial (n = 31) and review by the DSMB, between 04/14/21 and 04/30/21 the double-blind Phase 2 study randomized 149 subjects to receive one of three formulations in which DCL were incubated with 0.10, 0.33, or 1.0 μg of S-protein without GM-CSF.

Vaccine

The vaccine was developed by AVITA Biomedical, Inc. (Irvine, CA). AIVITA employees manufactured vaccine for the Phase 1 trial and supervised trained non-company technicians during manufacturing for the Phase 2 trial. 40 ml blood samples were taken to a laboratory in the same hospital where peripheral blood mononuclear cells (PBMC) were isolated using Ficoll density centrifugation. After removal of red cells, platelets, and plasma, the PBMC pellet was resuspended in media to which interleukin-4 and GM-CSF were added to differentiate monocytes into DC during 5 days of culture. Based on randomization assignment, the DCL product was incubated for 2 days with 0.10, 0.33, or 1.0 μg of recombinant S-protein (Lake Pharma Biologicals, San Carlos, CA). The product was cleared for use based on visual inspection and negative gram stain. Residual spike protein in the final product was assayed by ultra-sensitive ELISA.

Trial procedures

Potential participants were screened, consented and enrolled in outpatient clinics. Subjects provided a 40 ml blood sample from which PBMC were obtained to initiate vaccine manufacturing. Subjects returned to the clinic one week later and were reassessed to be sure they had not recently developed symptoms of COVID-19 infection. Blood was drawn for hematologic and chemistry studies, and for immune monitoring tests. In the phase 1 trial, the final DCL product was resuspended in

0.33 ml of autologous serum and either 0.67 ml of saline or saline containing 250 or 500 µg of GM-CSF. In the phase 2 trial GM-CSF was not admixed with the DCL.

On the day of vaccination, the DCL vaccine, which had been refrigerated overnight, was drawn into a 3 ml syringe with a 25-gauge needle that was capped and hand-carried to the clinic for injection within 5 hours. Each syringe was labeled with subject identifier. In the Phase 1 trial, each participant was injected with his/her personal vaccine SC in the back of the upper arm. In the Phase 2 trial, each participant was injected with his/her personal vaccine SC in either forearm to facilitate inspection and avoid confusion of local post-injection reactions or shoulder pain. Injections were administered by nurses or physicians in the clinic.

Safety assessments

In both trials, subjects were observed for 30 min after vaccine injection and instructed to maintain a symptom diary for the next 7 days. They returned to the clinic on each of the 2 days following the vaccine for soliciting of symptoms. One week after injection they returned to the clinic with the diary at which time they were again questioned regarding symptoms, were examined as needed based on symptoms and had blood drawn for safety laboratory testing. Laboratory tests included complete blood count (CBC), glucose, electrolytes, calcium, cholesterol, triglycerides, HgA1C, and tests of renal and hepatic function and urinalysis. 28 days after vaccination, subjects were reassessed for delayed adverse events that may have occurred during the ensuing 3 weeks, and laboratory tests were repreated. Treatment emergent adverse events (TEAE) were described using terms in the *Medical Dictionary for Regulatory Activities*, version 23.0, and classified and graded per Common Terminology Criteria for Adverse Events (CTCAE v 4.03). Results were entered by clinical site staff trained to enter data into the REDCap Cloud electronic data capture system.[48] Immune monitoring blood samples were obtained at baseline and 14 and 28 days after vaccination.

Immune response assessments

In Phase 1, an enzyme-linked immunosorbent assay (ELISA) was used to measure antigen-specific immunoglobulin levels specific for the S-protein RBD relative to positive control sera, and reported in ELISA units per milliliter because control samples for converting to the World Health Organization (WHO) Biinding Antibody Units had not yet been established at the time of analysis. Comparison was made between results at day-0 and day-28. In Phase 2 an enzyme-linked assay (ELISPOT) was used to assess *T*-lymphocyte responses against the spike protein *in vitro* via intracellular gamma interferon detection under both S-protein antigen-stimulated and unstimulated conditions. Baseline activity was defined as a minimum of 100 ELISPOTS. Results were presented as the number of ELISPOT-positive cells per 150,000 cells and an increase was considered significant if the average number increased by 25% or more and by at least seven ELISPOTS. Differences between unstimulated and *in vitro* antigen-stimulated were characterized as "reactive" or "non-reactive" based on a significant difference between the average number of interferon-gamma ELISPOTS counted ($p < 0.05$, Student's *T*-Test). All assays were performed by the same laboratory technician.

Statistical analyses

For each study, safety analysis was presented as number and proportion of subjects experiencing adverse events by vaccine formulation and all formulations, and by toxicity and grade. Immune response data was presented by cohort and overall as averages and standard error of the mean, and by number and proportion of subjects who had an increase in response. Comparisons of proportions were made by Fisher's exact test; means were compared using the Student's *T*-Test. There were no corrections made for multiple tests.

Results

Pre-clinical studies

Sera from subjects who had recovered from COVID-19 infection was used as a positive control for S-protein-specific antibodies. In a 60-animal mouse study, a vaccine consisting of 30,000 murine DCL that had been incubated with the SARS-CoV-2 S-protein was safe and induced immunoglobulins to the S-protein, as well as a Th1 (CD8+) cytotoxic-*T*-cell response and a Th2 (CD4+) helper-*T*-cell response to the S-protein. The antibody response was not correlated with S-protein quantity, whereas the Th1 and Th2 response correlated with S-protein quantity (see Supplement 1).

Personal vaccines

Residual spike protein in the final product was undetectable by ultra-sensitive ELISA. Vaccines were produced for each of 176 participants, all of whom were injected. Characterization of 172 of the products that were injected into 172 randomized subjects (27 in Phase 1, 145 in Phase 2) are shown in Table 1. Each

Table 1. Cell number and viability in final vaccine products.

	Mean # (SD) and range DCL x 10^6	Mean # (SD) and range DC x 10^6	Mean # (SD) and range L x 10^6	Mean # (SD) and range MC x 10^6	Mean viability, (SD) and range
Phase 1 (n = 27)	52.2 (29.9) (10.8 to 128)	4.9 (3.1) (1.6 to 13.4)	47.3 (28.3) (8.0 to 118)	0.02 (0.02) (0 to 1.1)	79% (8.0) (52% to 87%)
Phase 2 (n = 145)	15.1(8.9) (1.2 to 44.4)	2.1 (2.2) (0.2 to 16.5)	12.7 (7.4) (1.0 to 39)	0.08 (0.09) 0 to 2.4	84% (9.5) (50% to 96%)

DCL=dendritic cells and lymphocytes, DC=dendritic cells, L=lymphocytes, MC=monocytes.
DC were identified by the combination of CD11c+ and CD14- staining. Monocytes were identified by the combination of CD11c+ and CD14+ staining. All products had a cell viability of greater than 50%. Viabilities greater than 70% were recorded for 25/27 (93.6%) in the Phase I trial, and for 133/145 (91.7%) in the Phase II trial. The proportions with viability less than 60% were 1/27 (3.7%) and 2/145 (1.4%).

Table 2. Demographics and baseline co-morbidities by vaccine formulation (amount of S-protein) in Phase 2.

	0.10 mg (n=49)	0.33 µg (n=49)	1.00 µg (n=47)	Total (n=145)
Mean Age in years ± SD	45.3 ± 13.3	47.5 ± 12.3	44.9 +12.4	45.9
Median Age in years (IQR)	44.2 (34.4, 55.1)	48.7 (40.2, 54.0)	46.0 (36.0, 53.6)	46.1 (38.0, 54.0)
Range of years	20.2, 78.9	19.5, 82.8	20.5, 74.2	19.5, 82.8
Male	27 (55.1%)	22 (45.9%)	28 (59.6%)	77 (53.1%)
Female	22 (45.9%)	27 (55.1%)	19 (40.4%)	68 (46.9%)
*Race/Ethnicity**				
Javanese	21 (42.9%)	16 (32.7%)	23 (48.9%)	60 (41.4%)
Tionghoa	13 (26.5%)	18 (36.7%)	12 (25.5%)	43 (29.7%)
Sundanese	4 (8.2%)	4 (8.2%)	3 (6.4%)	11 (7.6%)
Minangkabau	3 (6.1%)	2 (4.1%)	3 (6.4%)	8 (5.5%)
Bataknese	1 (2.0%)	3 (6.1%)	1(2.2%)	5 (3.4%)
Sumatran	2 (4.1%)	1 (2.0%)	0	3 (2.1%)
Malaysian	1 (2.0%)	0	2 (4.3%)	3 (2.1%)
Buginese	1 (2.0%)	1 (2.0%)	1 (2.2%)	3 (2.1%)
Co-Morbidities				
Hypertension	6 (12.2%)	5 (10.2%)	4 (8.5%)	15 (10.3%)
Hypercholesterolemia	1 (2.0%)	1 (2.0%)	2 (4.3%)	4 (2.8%)
Diabetes	1 (2.0%)	1 (2.0%)	8 (17%)	10 (6.9%)
Asthma	3 (6.1%)	3 (6.1%)	1 (2.2%)	7 (4.8%)
Hyperuricemia, Gout	0	2 (4.1%)	1 (2.2%)	3 (2.1%)
Gastric Reflux	0	2 (4.1%)	1 (2.2%)	3 (2.1%)

*Others included 2 Minahasan, and 1 each Acehnese, Balinese, Batak, Hollanders, Kupang, Palembang, Torajanese.

vaccine consisted of 85 to 90% lymphocytes and 10% to 15% DC, with only a small number of residual monocytes that did not differentiate into DC.

Participants

The 31 subjects in the Phase I safety study included 18 men and 13 women with an age range of 20–62 years. The mean age was 40.3 (±12.0) years; median age was 39.7 years (IQR 31.7 to 50.5). For the Phase 2 trial, 227 subjects were screened, 149 were randomized, and 145 were vaccinated; characteristics of Phase 2 participants are summarized in Table 2.

Vaccine safety

All formulations were well-tolerated. There were no acute allergic events, serious adverse events (SAE), or grade 3 or 4 adverse events (AE). AE and their grades are summarized in Tables 3 and 4. In the Phase I trial, 33 AE were reported by 20 of 31 subjects (64.5%). In Phase I there was no association between AE and the quantity of spike protein incubated with DCL, or amount of GM-CSF administered as an adjuvant, or age or gender. AE were experienced by 10/11 whose DCL were incubated with 1.0 µg S-protein vs. 11/20 for the two lesser quantities ($p = 0.055$). Following vaccination, 4/31 had mild, clinically insignificant elevations of cholesterol that were in the normal range at baseline. In the Phase II trial safety data were available for 138 of the 145 vaccinated subjects (Tables 3 and 4). One hundred and thirty AE were reported by 73/138 subjects (53.6%); 65 (46.4%) reported no AE. The number of AE per subject was one in 44, two in 15, three in 8, four in 5, and seven in 1 subject. The highest AE grade experienced by individual subjects were 84 grade-1 (49.7%), and 10 grade-2 (5.9%). Investigators considered only 24 AE as "definitely" related to the vaccine; 23/24 were grade-1 local injection site reactions (ISR). These consisted of 15 reporting pain, three ecchymoses, three erythema, one local pruritus, one induration. Of 1,611 abnormal laboratory tests recorded, 82.2% were abnormal at baseline, 8.4% were abnormal the first time a blood test was obtained other than the day-0 baseline, 9.3% were first detected at day-7 or day-28 after being normal at day-0. None of the abnormal tests were considered clinically significant by the attending physicians.

Table 3. Adverse Events and Grade in Phase 1 trial (n = 33 events occurring in 31 subjects).

Type/Grade	1	2	3	4	
Injection Site Reaction	6 (18.2%)	0	0	0	6 (18.2%)
Fatigue/Drowsiness	0 (0%)	1 (3.0%)	0	0	1 (3.0%)
Headache	6 (18.2%)	1 (3.0%)	0	0	7 (21.2%)
Myalgias/Muscle Pain	3 (9.1%)	1 (3.0%)	0	0	4 (12.1%)
Arthralgia, Joint or Bone Pain	7 (21.2%)	0	0	0	7 (21.2%)
Chills	3 (9.1%)	0	0	0	3 (9.1%)
Nausea	1 (3.0%)	1 (3.0%)	0	0	2 (6.1%)
All others (n = 1)	3 (9.1%)	0	0	0	3 (9.1%)
TOTAL	28 (84.8%)	5 (15.2%)	0	0	33 (100%)

Table 4. Adverse Events and Grade in Phase 2 trial (n = 130 events occuring in 73 subjects).

Type/Grade	1	2	3	4	
Injection Site Reaction	23 (17.7%)	0	0		23/130 (17.7%)
Fatigue/Drowsiness	17 (13.1%)	0	0	0	17/130 (13.1%)
Cold/Sore Throat	13 (10.0%)	0	0		13/130 (10.0%)
Dyspepsia	10 (7.7%)	1 (0.8%)	0	0	11/130 (8.5%)
Headache	9 (6.9%)	2 (1.5%)	0	0	11/130 (8.5%)
Myalgias/Muscle Pain	8 (6.2%)	2 (1.5%)	0	0	10/130 (7.7%)
Dizziness	6 (4.6%)	3 (2.3%)	0	0	9/130 (6.9%)
Pruritus/Itching	6 (4.6%)	0	0	0	6/130 (4.6%)
Cough	6 (4.6%)	0	0	0	6/130 (4.6%)
Arthralgia, Joint or Bone Pain	2 (1.5%)	1 (0.8%)	0	0	3/130 (2.3%)
Chills	2 (1.5%)	1 (0.8%)	0	0	3/130 (2.3%)
Insomnia	3 (2.3%)	0	0	0	3/131 (2.3%)
Diarrhea	1 (0.8%)	1 (0.8%)	0	0	2/130 (1.5%)
Sneezing	2 (1.5%)	0	0	0	2/130 (1.5%)
All others	10 (7.7%)	1 (0.8%)	0	0	11/130 (8.5%)
TOTAL	118 (90.8%)	12 (9.2%)	0	0	130 (100%)

Grade 1=mild, 2=moderate, 3=severe, and 4=life-threatening.
For both studies combined, 90% were grade 1, 10% were grade 2. In Phase 1, the number of AE per subject were zero in 11 subjects, one in 18, two in 1, and five in 1. In Phase 2, the number of AE per subject were zero in 65 subjects, one in 44, two in 15, three in 8, four in 5, and seven in 1.

Table 5. Distribution of severity of AE within vaccine formulations (amount of S-protein).

S-protein incubation	Grade-0	Grade-1	Grade 2	Grade 3 or 4	Any Toxicity
0.10 µg (n = 48)	28 (58.3%)	18 (37.5%)	2 (4.2%)	0 (0%)	20 (41.7%)
0.33 µg (n = 47)	22 (46.8%)	21 (44.7%)	4 (8.5%)	0 (0%)	25 (53.2%)
1.00 µg (n = 43)	15 (34.9%)	25 (58.1%)	3 (7.0%	0 (0%)	28 (65.1%)
Total (n = 138)	65 (47.1%)	64 (46.4%)	9 (6.5%)	0 (0%)	73 (46.4%)

There was no correlation between the vaccine formulations and AE, except that the proportion of subjects with no AE was higher in the 0.10 µg cohort compared to the 1.00 µg cohort (28/48 vs. 15/43 p = 0.0354).

The distribution of AE by severity and vaccine formulation are shown in Table 5. In the Phase II trial, the proportions with local injections site reactions were 7/48 (14.6%) for the 0.10 µg formulation, 5/47 (10.6%) for the 0.33 µg formulation, and 8/43 (18.6%) for the 1.00 µg formulation. There was no correlation between the formulations and AE, except that a higher proportion in the 0.10 µg cohort had zero AE compared to the 1.00 µg cohort (28/48 vs. 15/43 p = 0.0354).

B-Cell responses to sars-cov-2 spike protein

These results are summarized in Supplements 3 and 4. For combined data for all 30 subjects, anti-RBD levels were higher on day-28 than day-0 (p = 0.037). 21/30 (70%) had an increase in anti-RBD antibody levels. Cohort analyses revealed that only the lowest quantity of S-protein used in manufacturing the vaccine was associated with an increase in anti-RBD levels (p = 0.037). Anti-RBD IgG levels were inceased in 10/12 (83%) whose DCL were incubated in 0.10 µg compared to 12/18 (67%) incubated with a higher quantity of S-protein (p = 0.149). Only the highest dose of GM-CSF admixed with DCL prior to injection was possibly associated with increased anti-RBD levels (p = 0.097). Anti-RBD IgG levels were inceased in 9/10 (90%) whose DCL were admixed with 500 µg GM-CSF just prior to injection compared to 13/20 (65%) whose DCL were incubated with 0 or 250 µg GM-CSF (p = 0.210).

T-Cell responses to sars-cov-2 spike protein

Supplement 5 shows the flow of patients from screening to randomization, injection, safety evaluation and assessment of immune response in Phase 2. Table 6 shows that there were no differences in cellular responses among the three formulations. In assays conducted without *in vitro* antigen stimulation, 127/135 had fewer than 100 ELISPOTS at baseline. For these 127, the average number of gamma interferon producing ELISPOTS increased from 15.4 at day-0 to 163.8 at day-14 (p < 0.0001); respective medians increased from 4.3 to 124.0. 68 (53.5%) subjects increased to more than 100 ELISPOTS on day-14. Of 126 subjects with less than 100 ELISPOT-positive cells at baseline and paired samples for day-0 and day-14, 119/126 (94.4.%) had increased ELISPOTS at day-14 and to 96.8% at day 28. The SARS-CoV-2 cellular immune response was enhanced in 92.5% of all 135 vaccinated subjects by 28 days after vaccination. The lower 95% CI for each formulation was well above the 50% threshold specified in the study. At baseline 98/133 subjects were non-reactive to stimulation with S-protein *in vitro*, suggesting no prior exposure to SARS-CoV-2 (naïve subjects); 35/133 were reactive (suggesting prior exposure). 14 days after vaccination, 38.8% of the naïve reacted to *in vitro* antigen stimulation, increasing to 62.2% on day-28. The cellular response induced by the DC vaccine was more pronounced than the humoral response, which may be typical for DC vaccines.[27–30]

Discussion

Study results by specific aims

We previously proposed that personal DC vaccines might be an alternative approach for preventing COVID-19 infection.[47] This is the first report of clinical studies with such vaccines. The results suggest that: (1) it is feasibile to prepare personal COVID-19 DC vaccines at the point-of-care based on successful manufacting of such vaccines for 176/176 subjects, (2) single subcutaneous COVID-19 DC vaccine injections are associated with minimal acute and subacute toxicity based on immediate, 7-day and 28-day observations of 169 subjects, (3) single injections induce desirable antigen-specific immune responses in most subjects,

Table 6. T-Lymphocyte reactivity to SARS-CoV-2 S-protein based on S-protein-antigen-stimulated and unstimulated ELISPOT assay for gamma interferon.

	Subjects whose cells were non-reactive to in vitro S-protein antigen stimulation at day-0,		Subjects with <100 unstimulated ELISPOTs at day-0	
Formulation by the quantity of S-protein incubated with DCL*	Proportion reactive by antigen-stimulated ELISPOT assay at day-14	Proportion reactive by antigen-stimulated ELISPOT assay test at day-14 or day-28	Proportion with increased unstimulated ELISPOTs at day-14	Proportion with increased unstimulated ELISPOTs at day-14 or day-28
0.10 µg	14/33 (42.4%)	22/33 (66.7%)	44/46 (95.7%)	45/46 (97.8%)
0.33 µg	13/31 (41.9%)	21/31 (67.7%)	39/41 (95.1%)	40/41 (97.6.%)
1.00 µg	11/34 (32.4%)	18/34 (52.9%)	36/39 (92.3%)	38/40 (95.0.%)
Total	38/98 (38.8%)	61/98 (62.2%)	119/126 (94.4%)	123/127 (96.8%)

*DCL=dendritic cells and lymphocytes.

There was no difference in any 2 × 2 comparison of proportions with increased ELISPOTS based on quantity of S-protein used in manufacturing. This included the proportions reactive to *in vitro* antigen stimulation in the 1.00 µg formulation compared to the combination of 0.10 µg and 0.33 µg formulations for day-14 results (27/64 vs. 11/34, p = 0.389, X^2 test) or for day-14 or day-28 positive results (43/64 vs. 18/34, p = 0.193, X^2 test. The combination of positive results from all formulations for day-14 or day-28 was higher than for day-14 alone for the *in vitro* stimulation tests (38/98 vs. 61/98, (p = 0.0016, X^2) but not for the unstimulated conditions (119/126 vs. 123/127, p = 0.376, X^2.

including *T*-cell responses within 14 days in 94% and humoral responses within 28 days in 70%, (4) incubation of DCL with 0.10 μg of S-protein and not admixed with adjuvant GM-CSF is an appropriate formulation for additional investigation. The rapid enhancement of SARS-CoV-2 cellular immune response may be due to the *ex vivo* exposure of DC to the viral protein such that DC are already primed to communicate with immune cells after injection. The limited acute toxicity associated with this vaccine may be due to the vaccine being composed of only the subject's primed immune cells, lacking viral antigen, virus, mRNA, or DNA or immune adjuvants. Safety and immune responses were similar regardless of the quantity of S-protein incubated with DCL; therefore, it appears appropriate to utilize DCL incubated with 0.10 μg of S-protein in Phase 3 trials to establish efficacy in the prevention of symptomatic COVID-19 infection.

Safety and immune response compared to other COVID vaccines

The data in Supplement 6 suggest that the immediate and short-term toxicity associated with this DC vaccine is less than reported after single doses of COVID vaccines currently available in the U. S.[5,11,12] This appears to be true in terms of both local and systemic toxicity and grade 3 systemic AE. This may not be surprising since this vaccine consists of autologous DCL and did not contain an adjuvant or foreign protein. No grade 3 AE were reported in our trial and were infrequent during the 28 days following a single intramuscular injections of available COVID vaccines.[5,11,12] During vaccine development for infectious disease, induction of neutralizing antibodies is accepted as a good predictor for efficacy of preventive vaccines. During phase 1–2 trials of these other vaccines, higher levels of anti-RBD antibodies were observed than we saw in our study, and we did not directly assay for neutralizing antibodies. The rate of T cell responses observed in our study appears to be higher than following a single dose of the available vaccines, but at this time it is unclear whether a rapid antigen-specific *T*-cell response is predictive of prevention.

As noted earlier, during infectious disease vaccine development, induction of neutralizing antibodies is considered the best predictor for efficacy of preventive vaccines. The existing COVID vaccines induced high levels of anti-S-protein RBD antibodies in nearly all subjects.[5–16] At this time it is unclear whether a rapid antigen-specific *T*-cell response is predictive of vaccine benefit. The major limitations of our phase I-2 study are the comparatively small sample size and the limited data on humoral immunity. A randomized phase 3 trial and long-term follow-up would be needed to confirm safety and establish the efficacy of AV-C19 for prevention of symptomatic COVID-19 infection in the year following vaccination.

Potential advantages and disadvantages for this DC COVID vaccine approach

Assuming similar efficacy among different vaccines, the major theoretical advantage of the personal DC approach is the ease and rapidity of preparing such a vaccine. The rate-limiting critical component for such DC vaccines is a source of pure sterile antigen that can be obtained from companies that manufacture such proteins. Once antigen and a small laboratory with incubator and refrigerator are available, and local personnel have been trained, personal vaccines can be manufactured within a week after obtaining a blood sample. Such laboratories may be supplied with kits that contain closed-system culture bags, media, growth factors, syringes, and needles to manufacture products for individual subjects. Simple sterile procedures are required, but it is more critical that a subject is not injected with a vaccine intended for another subject because of disease transmission risk. If effective, this approach may be ideal as a bridging technology for certain populations (such as medical personnel) while the lengthy process of developing and manufacturing traditional vaccines for mass distribution is in progress. This autologous approach may be acceptable to individuals who are otherwise opposed to currently available COVID vaccines, despite the abundance of safety and efficacy data that supports their use. A major disadvantage of this approach is that it is not practical for rapid mass inoculations because of limitations of space and personnel to produce such vaccines. This approach likely will never be as efficient as an off-the-shelf vaccine for rapid inoculation of masses of people, but it may be a suitable approach for limited numbers of subjects. Depending on the size of the population being vaccinated, it may be more, or less, cost-effective than large-batch production of vaccines that require more time and resources for development. Because of inter-subject biological heterogenity, the DCL approach does not yield a uniform product in terms of cell number and composition, but this does not seem to an issue for the range of cell doses that can be generated from a standardized blood collection. Another challenge for this approach is the regulatory path to approval. While there has been much interchange with regulatory authorities regarding the development of DC-based therapeutic cancer vaccines, and approval of one anti-cancer DCL product, sipuleucel-T for prostate cancer,[49] there is no precedent for such a preventive vaccine in the infectious disease field. While induction of neutralizing antibodies is established as a predictor of preventive vaccine efficacy, it remains to be seen whether induction of rapid T cell immunity is a useful predictor of preventing COVID infection.

Conclusions

The aims of these relatively small phase 1–2 studies were achieved. They confirmed manufacturing feasibility, short-term safety, induction of immune responses, and enabled selection of a formulation of DCL for future studies. Large randomized prospective trials are needed to establish the efficacy of the approach for the prevention of symptomatic COVID infection and to confirm safety.

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Disclosure statement

G. I. Nistor, R. O. Dillman, MD., R. M. Robles, J. L. Langford, A. J. Poole, are employed by AIVITA Biomedical, Inc. and own stock in the company. H. S. Keirstead is CEO and founder of AIVITA Biomedical, Inc. with a major equity position.

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Authors' contribution

From AIVITA Biomedical, Inc. Dr. Nistor supervised vaccine manufacturing in Indonesia, designed and interpreted the immune monitoring tests, and contributed to writing the manuscript. Dr. Dillman designed the clinical study, did the statistical analyses, composed the initial draft of the manuscript, and revised the manuscript in response to reviewer comments. Mrs. Robles and Mr. Langford collected laboratory and clinical data respectively, and assisted in data analysis. Dr. Poole assisted with project supervision, review, and editing of this manuscript. Dr. Keirstead arranged institutional and governmental relationships and financial support, influenced study design, and made revisions to the initial manuscript.

From Indonesia, Dr. Yetty Movieta Nency served as co-investigator on this trial. Drs. Retty Karisma Sari, Nur Alaydrus are acting physicians at Dr. Kariadi Hospital in Semarang, Indonesia, and they had an active role in the recruitment and screening of subjects in this trial. Ria Triwardhani, M.D. is the chief of the lab in which the vaccine was produced. At Gatot Soebroto Army Hospital (RSPAD), Jonny Jonny, M.D. was a principal investigator of this trial, Martina Lily Yana, M.D., and Terawan Angus Putranto, M.D., Ph.D. were co-investigator. Mujahidah, M.D. is an acting physician in the same hospital recruiting subjects for this trial. Daniel Tjen, M.D. is a general at Gatot Soebroto Army Hospital (RSPAD), assisting with the logistics of trial management. Muhammad Karyana, M.D. and Taruna Ikrar, M.D., from the Ministry of Health Republic of Indonesia, lead the lab testing of patients' blood samples. Gregory Sarkissian and Haryono Winarta provided project supervision and financial assistance.

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