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Phase 1 trial of a Candidate Recombinant Virus-Like Particle Vaccine for Covid-19 Disease Produced in Plants — [Source link](#)

Brian J. Ward, Philippe Gobeil, Annie Séguin, Judith Atkins ...+15 more authors





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1 Title: Phase 1 trial of a Candidate Recombinant Virus-Like Particle Vaccine for Covid-19
2 Disease Produced in Plants

3

4 **Authors:** Brian J Ward^{1,2}, Philippe Gobeil¹, Annie Séguin¹, Judith Atkins¹, Iohann Boulay¹
 5 Pierre-Yves Charbonneau¹, Manon Couture¹, Marc-André D'Aoust¹, Jiwanjeet
 6 Dhaliwall¹, Carolyn Finkle¹, Karen Hager¹, Asif Mahmood¹, Alexander Makarkov¹,
 7 Matthew Cheng², Stéphane Pillet¹, Patricia Schimke¹, Sylvie St-Martin¹, Sonia
 8 Trépanier¹, Nathalie Landry^{1*}

9

10 ¹ Medicago Inc., 1020 route de l'Église office 600, Québec, QC, Canada, G1V 3V9

11 ² Research Institute of the McGill University Health Centre, 1001 Decarie St, Montreal, QC
 12 H4A 3J1

13 * *Corresponding author:*

14 Nathalie Landry

15 1020 Route de l'Église, Bureau 600

16 Québec, Qc, Canada

17 G1V 3V9

18 Tel. 418 658 93931

19 Fax. 418 658 6699

20 Landryn@medicago.com

21

22

23 **Keywords**

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 25 *tumefaciens*, safety and immunogenicity, cell-mediated immunity,

26

27

28 Other Material Study Protocols (1), Statistical Analysis Plans (1) Supplemental
 29 material (1)

30

31

32 Research In Context

33

34 Evidence before this study

35 The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was recognized as the
 36 causative agent of COVID-19 in early 2020. Since that time, >150 candidate vaccines are
 37 reported to be under development of which 47 have entered clinical trials
 38 (<https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines>
 39 accessed Nov 4, 2020). No vaccine to prevent COVID-19 has been licensed yet for either
 40 emergency or general use in North America or Europe. We searched PubMed for research
 41 articles published between July 2019 and November 4, 2020, using the terms “SARS-CoV-2”,
 42 “vaccine”, “clinical trial” OR “human”, AND “phase”. The same terms were used to search
 43 ClinTrials.gov. No language restrictions were applied. We identified 10 peer-reviewed studies,
 44 describing phase 1 or 1/2 trials using a range of novel (eg: RNA, DNA, non-replicating virus
 45 vectored) and more traditional vaccine approaches (eg: inactivated virus or recombinant protein
 46 ± adjuvants). None of these candidate vaccines was produced in plants. These reports
 47 demonstrate that several different vaccination strategies (typically delivered in two doses 14-28
 48 days apart) are capable of eliciting neutralizing antibody responses. In several cases, vaccine-
 49 induced cellular responses against SARS-COV-2 antigens - predominantly the spike (S) protein
 50 - can also be demonstrated. Although local and systemic adverse events following vaccination
 51 have varied between reports, the trials published to date suggest that each of these candidate
 52 vaccines is well-tolerated in the context of an evolving pandemic.

53

54 Added value of this study

55 We report the results of the first clinical study of CoVLP, a virus-like particle (VLP) vaccine
 56 that is produced by transient transfection of *Nicotiana benthamiana* plants. These VLPs
 57 spontaneously assemble at the plant cell membrane and display SARS-COV-2 trimers of
 58 stabilized pre-fusion S protein on their surface. The vaccine was administered as two
 59 intramuscular doses 21 days apart at three dose levels (S protein content 3.75, 7.5 or 15µg)
 60 alone or adjuvanted with either CpG1018 or AS03. All formulations were well-tolerated
 61 although both adjuvants increased reactogenicity. Humoral (anti-S IgG and neutralizing
 62 antibodies) as well as cellular responses (IFNγ and IL4 ELISpots) were detectable in almost all

63 subjects who received adjuvanted formulations 21 days after the second dose at all COVLP
 64 dose levels. Both antibody and cellular responses were highest in subjects who received AS03-
 65 adjuvanted formulations. Even at the lowest dose level (3.75µg), the neutralizing antibody titers
 66 21 days after the second dose in subjects who received the AS03-adjuvanted vaccine were 10-
 67 50-fold higher than those seen in subjects recovering from COVID-19 infection.

68

69 **Implications of all the available evidence**

70 Effective vaccines against SARS-CoV-2 are urgently needed to reduce the burden of disease
 71 and contribute to ending the global pandemic. Although no immune correlates for SARS-CoV-
 72 2 have been defined, it is likely that both arms of the immune system contribute to protection.
 73 After two doses of CoVLP (3.75µg+AS03), strong humoral and cellular responses were
 74 induced supporting the further clinical development of this vaccine.

75

76 Longer Abstract

77 **Background:** The stabilized prefusion form of the SARS-CoV-2 spike protein is produced by
78 transient expression in *Nicotiana benthamiana*. The trimeric spike glycoproteins are displayed
79 at the surface of self-assembling Virus-Like-Particles that mimic the shape and the size of the
80 virus. The candidate vaccine (CoVLP) administered alone or with AS03 or CpG1018
81 adjuvants was evaluated in a Phase 1 trial in healthy adults. (ClinicalTrials.gov number
82 NCT04450004)

83
84 **Methods:** The study was a randomized, partially-blinded, prime-boost 21 days apart, dose-
85 escalation Phase 1 study intended to assess the safety, tolerability, and immunogenicity of
86 CoVLP at three dose levels (3.75 µg, 7.5 µg, and 15 µg) unadjuvanted or adjuvanted with
87 either CpG 1018 or AS03 in 180 SARS-CoV-2 seronegative healthy adults 18 to 55 years of
88 age. Enrollment was staggered for dose-escalation. At each dose level, the vaccine was initially
89 administered to a small number of subjects. Vaccination of the remaining subjects at the same
90 dose level and the next higher vaccine dose level was administered with approval of an
91 Independent Data Monitoring Committee (IDMC). The same procedure was followed for the
92 second vaccine administration. Monitoring of safety signals was performed throughout the
93 study with pre-determined pausing/stopping rules if there was clear evidence of harmful effects
94 such as severe adverse events (AEs) related to the treatment. The primary endpoints were the
95 safety and tolerability of the vaccine after each dose and the immunogenicity as assessed by
96 neutralizing antibody responses assessed using a vesicular stomatitis virus (VSV) pseudovirion
97 assay and interferon-gamma (IFN-γ) and interleukin-4 (IL-4) ELISpot assays at Days 0, 21 and
98 42. Secondary endpoints were anti-spike antibody responses by ELISA and neutralizing
99 antibodies measured by live virus plaque reduction neutralization test (PRNT) assay at Days 0,
100 21 and 42 and immunogenicity with additional safety and immunogenicity endpoints planned
101 for 6-months following the last vaccination. The anti-spike and neutralizing antibody responses
102 were compared with 23 convalescent serum samples from symptomatic Covid-19 patients. We
103 performed a primary analysis at day 42.

104
105 **Results:** A total of 180 subjects (102 females: 78 males: average 34.3 years) were recruited to
106 the study and interim safety and immunogenicity data up to day 42 after the first dose are
107 reported here. There was no obvious CoVLP dose effect in safety outcomes for any of the
108 formulations tested and all formulations were generally well-tolerated. Most solicited local and
109 systemic AEs were mild-moderate and transient. Reactogenicity was increased in all adjuvanted

formulations and was generally highest in the CoVLP+AS03 groups. Local and systemic adverse events were reported with similar frequency after the first and second doses in subjects who received either CoVLP alone or CoVLP+CpG1018 but increased in both frequency and severity after the second dose in the CoVLP+AS03 groups. CoVLP alone only elicited a weak total anti-spike IgG response at the highest dose level and little-to-no neutralization antibody response, even after the second dose. Cellular responses in the CoVLP alone groups (IFN γ and IL-4) were detectable after the second dose but were still only marginally above background levels. The addition of either adjuvant substantially increased both antibody and cellular responses at most CoVLP dose levels and changes were most pronounced after the second dose. However, a substantial neutralizing antibody response after the first dose was only seen in all CoVLP+AS03 groups. After the second dose, both total anti-spike IgG and neutralizing antibody titers in the CoVLP+AS03 groups were higher than those in the CoVLP+CpG1018 groups. The antibody titers achieved were either similar to (CoVLP+CpG1018) or at least 10-times higher (CoVLP+AS03) than those seen in convalescent plasma. Administration of CoVLP with either adjuvant also significantly increased the cellular responses. After 2 doses, both IFN- γ and IL-4 responses were significantly increased in the CoVLP+CpG1018 groups. In the CoVLP+AS03 groups, significant increases in the cellular responses were observed after the first dose while IFN- γ and IL-4 increased further in both magnitude and number of subjects responding after the second dose. Again, the cellular responses in the CoVLP+AS03 groups were higher than those seen in the CoVLP+CpG1018 groups.

Conclusion: These data demonstrate that CoVLP administered with either CpG1018 or AS03 has a safety profile similar to other candidate vaccines for SARS-CoV-2. When administered with either AS03 or CpG1018, several of the CoVLP dose levels elicited strong humoral and T cell responses after the second dose. When administered with AS03, even the 3.75 μ g CoVLP dose elicited neutralizing antibody titers that were ~10-times higher than those observed in individuals recovering from Covid-19 as well as consistent and balanced IFN- γ and IL-4 responses. Although many CoVLP formulations were immunogenic, in the absence of established correlates of protection and given the advantages of dose-sparing in the context of the on-going pandemic, these findings suggest that CoVLP (3.75 μ g)+AS03 has a good benefit/risk ratio and support the transition of this formulation to studies in expanded populations and to efficacy evaluations

143

144 **Shorter Abstract (Word count: 242)**

145 **Background:** Virus-like particles (VLP) displaying recombinant SARS-CoV-2 spike protein
146 trimers were produced by transient expression in *Nicotiana benthamiana*. This candidate
147 vaccine (CoVLP) was evaluated in healthy adults 18-55 years of age alone or with AS03 or
148 CpG1018 (NCT04450004).

149

150 **Methods:** This randomized, partially-blinded, two-dose, dose-escalation study assessed
151 CoVLP (3.75, 7.5 or 15 µg/dose) administered intramuscularly alone or with CpG1018 or
152 AS03 in SARS-CoV-2 seronegative adults (18-55 years). Primary endpoints of safety and
153 immunogenicity are reported to day 42. Neutralizing antibodies (NtAb) were assessed using a
154 VSV pseudovirus assay and cellular responses by ELISpot (IFN γ , IL-4).

155

156 **Results:** 180 subjects (avg.34.3yrs) were recruited. All formulations were well-tolerated but
157 adjuvants increased reactogenicity. Adverse events were highest in the CoVLP+AS03 groups
158 and increased in frequency/severity after dose two. CoVLP alone elicited weak humoral
159 responses but modest cellular responses were detectable after dose two. Both adjuvants
160 increased immunogenicity significantly, particularly after dose two. A significant NtAb
161 response after dose one was only seen in CoVLP+AS03 groups. The vaccine dose had little
162 impact on levels of NtAb responses achieved in the CoVLP+AS03 groups. Both adjuvants also
163 increased IFN γ and IL-4 responses but these cellular responses also tended to be highest in the
164 AS03-adjuvanted groups.

165

166 **Conclusion:** CoVLP \pm adjuvants was well-tolerated. Several adjuvanted formulations
167 elicited strong humoral and T cell responses after dose 2. Even at the lowest CoVLP+AS03
168 dose, NtAb titers were ~10-times higher than in convalescent serum with a balanced IFN γ and
169 IL-4 response. These findings support-the transition of CoVLP (3.75µg+AS03) to further
170 clinical evaluation.

171

172 Introduction (Word count: 458)

173

174 A novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)
 175 jumped across a species barrier in China in late 2019 ^{1,2} and spread rapidly around the globe
 176 leading to the World Health Organization's declaration of a pandemic on March 11, 2020 ³. As
 177 of October 11th, 2020, more than 37 million cases of Covid-19 have been reported with >1
 178 million deaths ⁴. Although many therapeutic strategies has been tried ^{5,6,7}, the current options
 179 remain limited in both number and efficacy. Simultaneously, there has been a massive global
 180 effort to develop vaccines. This effort was primed to some extent by prior experience with other
 181 highly pathogenic human coronaviruses, SARS and MERS⁸ and the far-sighted efforts of the
 182 Coalition for Epidemic Preparedness Innovations (CEPI) to develop vaccines for a 'short-list'
 183 of pathogens with pandemic potential ⁹. At the time of writing, >150 vaccine candidates have
 184 been announced, >30 are in clinical trials and a small number have been authorized for limited
 185 use in some countries ¹⁰. These candidates are based on a wide range of traditional and novel
 186 platforms including mRNA, DNA, inactivated virus, live viral vectors, recombinant proteins,
 187 peptides, and virus-like particles (VLPs) ^{11,12}. No vaccine has been approved for general use yet
 188 in North America.

189

190 Adding to the complexity of this situation is the facts that no correlate of immunity has been
 191 defined for any highly pathogenic coronavirus ^{13,14} and that such correlates might differ
 192 between vaccines¹⁵. Nonetheless, a protective role for both humoral and cell-mediated
 193 immunity against coronaviruses has been suggested ^{16,17}. Antibody responses against the spike
 194 (S) protein have the potential to protect from infection ¹⁸⁻²⁰ and convalescent plasma with high
 195 titers of anti-S antibody have therapeutic benefit in selected patients ^{7,21}. However, a substantial
 196 proportion of people who develop Covid-19 fail to generate antibodies, and data from the
 197 SARS-CoV-1 outbreak of 2002–2003 ²² and the current pandemic suggest that antibody
 198 responses can be short-lived, disappearing within months of infection in some patients²³. In
 199 contrast, T cell immunity may be critical for recovery from Covid-19^{24,25} and was shown to
 200 persist for up to 11 years after SARS-CoV-1 infection ²⁶. T cells can provide substantial
 201 protection in animal models of highly pathogenic coronavirus infection.

202

203 We report here the results of a Phase 1 study initiated in July 2020 evaluating the safety,
 204 tolerability and immunogenicity of two doses, 21-days apart of 3.75, 7.5 or 15 µg of a virus-
 205 like-particle vaccine candidate for Covid-19 produced in plants (hereafter called CoVLP). This

recombinant platform has been used to produce hemagglutinin (HA)-bearing VLP vaccines for avian (monovalent) and seasonal (quadrivalent) influenza that induce balanced humoral and T cell responses²⁸⁻³¹. The CoVLP vaccine was administered alone or with AS03 or CpG1018 adjuvants in healthy adults 18-55 years of age.

Methods (Word count: 687)

The CoVLP Vaccine and Adjuvants

The full-length S glycoprotein of SARS-CoV-2, strain hCoV-19/USA/CA2/2020, corresponding in sequence to nucleotides 21563 to 25384 from EPI_ISL_406036 in GISAID database (<https://www.gisaid.org/>) was expressed in *Nicotiana benthamiana* plants as previously described³². The S protein was modified with R667G, R668S and R670S substitutions at the S1/S2 cleavage site to increase stability, and K971P and V972P substitutions to stabilize the protein in prefusion conformation. The signal peptide was replaced with a plant gene signal peptide and the transmembrane domain (TM) and cytoplasmic tail (CT) of S protein was also replaced with TM/CT from Influenza H5 A/Indonesia/5/2005 to increase VLP assembly and budding. The self-assembled VLPs bearing S protein trimers were isolated from the plant matrix and subsequently purified using a process similar to that described for the influenza vaccine candidates²⁸.

The AS03 adjuvant, an oil-in-water emulsion containing tocopherol and squalene, was supplied by GlaxoSmithKline. The CpG 1018 adjuvant, composed of cytosine phosphoguanine (CpG) motifs, was supplied by Dynavax. The CoVLP vaccine and adjuvants were mixed immediately prior to administration

Study design

The Phase 1 study was conducted at two sites in Quebec City and Montreal (Protocol available in Supplemental Material). At screening, health status was assessed by medical history, physical exam and clinical laboratory findings including detection of antibodies to SARS-CoV-2 (ElecSys: Roche Diagnostics). Healthy seronegative subjects 18-55 years of age who met all inclusion criteria and no exclusion criterion were enrolled and randomized into nine groups. Randomised subjects received two doses, 21 days apart of CoVLP at doses of 3.75, 7.5 or 15 µg unadjuvanted or adjuvanted with AS03 or CpG1018. The participants and the personnel collecting the safety information and testing laboratories remained blinded to treatment

allocation. On Day 0 (D0: pre-first dose), D21 (pre-second dose) and D42 (post second dose), serum and peripheral blood mononuclear cells (PBMC) were processed for immune outcomes as described previously ²⁸.

Safety

For details of safety monitoring, see the Protocol in Suppl. Materials. Briefly, enrollment was staggered for dose-escalation with sentinel subjects at each dose level (n=6) and independent data monitoring committee (IDMC) review of D3 safety data at 10% and 30% recruitment before each dose acceleration. The same process was followed for the second vaccine administration. Monitoring of safety signals was performed throughout the study (Suppl. Material: pp 2). Solicited adverse events (AEs) were assessed by the subjects as Grade 1 to 4 (mild, moderate, severe, or potentially life-threatening). Unsolicited AEs, and AEs leading to subject withdrawal were collected up to D21 after each vaccination. All serious AEs (SAE), Adverse Events of Special Interest (AESIs), and pregnancies will be collected for 6 months. Potential cases of vaccine enhanced disease (VED), hypersensitivity and potential immune-mediated diseases (pIMDs) are being monitored throughout the study (see Suppl. Material: pp 7, Tables S2, S3).

Immunogenicity assessments

The primary immunological outcomes were neutralizing antibody (NAb) responses measured using a VSV pseudovirion assay (Nexelis Inc, Laval, QC) and IFN γ and IL-4 cellular responses measured by ELISpot at D0, 21 and 42. Secondary immunological outcomes were total anti-spike IgG responses by ELISA and NAb responses by plaque reduction neutralization test (PRNT: Vismedri S.r.l., Siena, Italy) Details of these assays are provided in the Suppl. Material (pp 3-5).

Antibody responses were compared with those of individuals who had recovered from PCR-confirmed Covid-19 obtained from Solomon Park (Burien, WA)), Sanguine BioSciences (Sherman Oaks, CA) and M Cheng (McGill University Health Center, Montreal, Quebec). The severity of disease ranged from mild-moderate (n=23) to severe/critical (n=11) (Suppl. Material: Table S1 for patient characteristics: pp 6).

Analysis Populations and Statistical Analysis Plan

Overall, 180 healthy SATRS-COV-2 seronegative male and female subjects 18 to 55 years of age were randomized in a 1:1:1:1:1:1:1:1:1 ratio into nine treatment groups. The sample size made it possible to perform the initial evaluation of the vaccine immunogenicity and detect major differences in rates of AEs between groups. The sample size was not large enough to detect all types, including less frequent or rare, AEs.

The analyses of all immunogenicity endpoints were based on the Per Protocol set (PP) and are described in the Statistical Analysis Plan provided as Supplementary Material.

Results (Word count: 1090)

Demographic and baseline clinical characteristics

Participant demographics are presented in Table 1 and subject disposition up to D42 is presented in Table 2 and in Suppl. Materials: Fig. S1: pp. 19. More women (56.7%) than men (43.3%) were enrolled but the female:male ratio was the same for each CoVLP dose level and in each of the overall groups (unadjuvanted CoVLP, CoVLP+AS03, CoVLP+CpG1018). Subjects were mostly White (96%) with 2% each of Black or African American and Asian subjects. The average age was 34.3 years. A total of 180 subjects received the first dose of vaccine and 178 subjects received both doses. See Fig. S1 in Suppl. Materials for details.

Safety

Reactogenicity for all formulations was generally mild in severity (Figure 1) and of short duration. Both adjuvants increased the frequency of reported AEs. The frequency and severity of AEs were similar after the first and second doses in the unadjuvanted CoVLP and CoVLP+CpG1018 groups but increased after the second dose in subjects who received AS03-adjuvanted formulations. Details of solicited AEs and TEAEs by treatment group are provided in Suppl. Materials: Tables S4-S9).

There was no consistent impact of CoVLP dose level on safety outcomes in any group. After the first dose, 74.3% of participants reported >1 solicited AE, 66.5% reporting a local reaction

and 39.7% reporting >1 systemic event. Pain at the injection site was the most common local reaction (66.5%) while headache and fatigue were reported by 25.7% and 20.7% respectively. The incidence of headache and fatigue were generally higher in the adjuvanted treatment groups. AEs were mostly mild-moderate Grade 1-2) and only one Grade 3 report of fatigue that started the evening following vaccination and resolved the same day. After the second dose, 68.5% of participants reported >1 solicited AE, 62.9% reporting a local reaction and 47.8% reporting >1 systemic event. Pain at the injection site was again the most reported local reaction (61.2%) while headache and fatigue were reported by 33.1% and 33.1% respectively. Again, most symptoms were mild but there were more moderate AEs after the second dose. Nine Grade 3 solicited severe AEs (fatigue, redness at injection site, swelling at injection site, feeling of general discomfort or uneasiness) were reported in 6 subjects after the second dose. All but one of the Grade 3 reactions were reported by subjects who received AS03-adjuvanted formulations. One Grade 3 reaction occurred after the second dose in the CoVLP (7.5 µg)+CpG1018 group. All Grade 3 AEs resolved in one to four days. No clinically significant lab abnormalities were reported after any dose. No SAEs, AESIs or pregnancy exposures have been reported at the time of writing.

Immunogenicity: Antibody Response

A small number of subjects (12/180; 6.7%) had detectable pre-existing antibodies for the spike protein in one or more of the assays used. As illustrated in Figure 2, unadjuvanted CoVLP elicited no detectable antibody response after the first dose and humoral responses after even the second dose were modest and inconsistent. Although a minor dose-effect for the unadjuvanted CoVLP was seen on the anti-spike IgG response (ELISA) after the second vaccination, the responses in the two NAb assays remained low and variable even at the highest dose tested. Both adjuvants had a significant impact on antibody responses at all dose levels. Although there was no convincing effect of increasing CoVLP dose for either adjuvant, there was a trend towards increasing consistency of response in the CoVLP+CpG1018 groups at the higher CoVLP doses (ie: a greater response and a larger proportion of subjects responding). Although both adjuvants elicited modest IgG titers after the first dose, only the groups that received COVLP+AS03 formulations mounted significant NAb responses at D21 (36/60; 60%) and across all dose levels (ie: overall GMT of 33.3 in the pseudovirus assay). Both adjuvants induced more robust responses after the second dose with the large majority of subjects at all dose levels mounting a ≥ 4 -fold rise in total IgG (117/118; 99.1%) and in both NAb assays

(105/112; 93.8% in the pseudovirion assay and 106/116; 91.3% in the PRNT). At all dose levels, both anti-spike IgG and pseudovirion NAb titers at D42 were 10- to 50- fold higher in subjects who had received AS03-adjuvanted formulations compared to those who had received CpG1018-adjuvanted formulations. For example, after the second CoVLP(3.75 µg) dose, the GMTs in the PRNT were 7.2 for CoVLP alone, 56.6 for CoVLP+CpG1018 and 811.3 for CoVLP+AS03. Overall, the levels of NAb induced in the groups that received two doses of CoVLP with an adjuvant were either similar to (CoVLP+CpG1018) or substantially greater (CoVLP+AS03) than those seen in subjects 3-4 weeks after recovering from natural Covid-19 infection. Details of serologic response results by treatment group are presented in Suppl. Materials: Tables S10-S12.

Correlation between Neutralizing Antibody Assays

There was a strong correlation between the VSV pseudovirion neutralization assay and the PRNT for responses in all groups: $r=0.85$ ($p < 0.0001$) after the first (Figure 3A) and $r = 0.68$ ($p < 0.0001$) second vaccinations (Figure 3B).

Immunogenicity: T Cell Response

As illustrated in Figure 4, the IFN γ and IL-4 responses in PBMC (ELISpot) elicited by CoVLP \pm adjuvants were more variable than the antibody responses. A substantial minority of subjects in all groups had what appeared to be pre-existing IFN γ responses to the S protein peptide pool that were, in some subjects, substantial (ie: >200 spots)³³. Although low level ‘background’ IL-4 activity was seen in a small number of subjects, these responses were close to the limit of detection of the assay used (generally <10 spots). Unlike antibody responses, CoVLP alone was able to induce a substantial IFN γ response and, to a lesser extent, IL-4 response in many subjects after the second doses at all dose levels but that was most consistently seen at the highest dose (CoVLP 15µg). Both adjuvants generally increased IFN γ and IL-4 responses above background levels after the first dose that were further and substantially increased in magnitude and consistency by the second dose. Compared to unadjuvanted CoVLP, IFN γ responses were slightly higher and IL-4 responses were slightly lower in the CpG1018-adjuvanted groups but most of these differences did not reach statistical significance. Once again, the IFN γ and IL-4 responses to the CoVLP+AS03 formulations at all dose levels were 10 to 50-fold higher than those seen in the equivalent CoVLP+CpG1018 groups. For example, at

the 3.75 µg dose level: median IFN γ and IL-4 responses at D42 were 628 and 445 in the CoVLP+AS03 group and 49 and 4 in the CoVLP+CpG1018 group. Details of cellular response results by treatment group are provided in Suppl. Materials: Tables S13 - S14.

Discussion (Word count: 1223)

This phase 1 study was designed to select the CoVLP formulation (ie: dose level \pm adjuvant) and the number of doses needed to generate a consistent immune response in healthy adults with an acceptable safety profile. Although unadjuvanted CoVLP had the lowest reactogenicity and was recognized by the immune system after the second dose, the immune responses measured (i.e.: anti-spike IgG, NAb, IFN γ and IL-4 ELISpots) were generally modest. These observations are consistent with studies of plant-derived VLP vaccines bearing HA trimers of avian influenza strains^{30,34} in which serologic and T cell responses were observed with unadjuvanted formulations at doses >15 µg but were seen at lower doses when administered with an oil-in-water adjuvant (GLA-SE)³⁴. Although we observed no dose effect with unadjuvanted CoVLP for antibody or cellular responses, it is possible that higher doses of unadjuvanted CoVLP would have induced stronger responses. However, two different adjuvants were incorporated into this study because of their potential to induce more robust responses at a lower CoVLP dose (ie: dose-sparing).

While sharing some characteristics (ie: documented immune enhancement, large safety databases, previously licensed)³⁵⁻³⁸, the inclusion of both CpG1018 and AS03 permitted us to ask multiple questions about CoVLP performance simultaneously. In the case of CpG1018, by targeting the innate toll-like receptor 9 we hoped that both arms of the adaptive immune system would be engaged with induction of humoral and cellular responses³⁹. Although the mechanism of action of oil-in-water adjuvants like AS03 is still under active investigation⁴⁰, the combination of squalene and tocopherol (vitamin E) in AS03 has also been shown to enhance both humoral and cellular responses with several different vaccine antigens^{41,42}.

399

400 In the current study, the adjuvants performed as expected with enhancement of humoral
401 and cellular responses to the S protein and in promoting responses at lower CoVLP
402 doses. In both dose-sparing and enhancing immune responses, AS03 appeared to be
403 more effective than CpG1018. For example, a substantial minority of the subjects who
404 received even the lowest dose of CoVLP+AS03 (3.75 µg) mounted detectable NAb
405 responses after the first dose but no similar response was seen in the CpG1018-
406 adjuvanted groups. Furthermore, the anti-spike IgG and NAb responses were
407 consistently higher at all dose levels in the AS03-adjuvanted groups compared to the
408 CpG1018-adjuvanted groups although differences did not reach statistical significance
409 at higher doses of CoVLP. The humoral response to the AS03-adjuvanted formulations
410 demonstrated no CoVLP dose effect, particularly after the second dose. Overall, the
411 differences between unadjuvanted and adjuvanted formulations and between the two
412 adjuvants were less pronounced for Th1-type cell-mediated responses than for antibody
413 responses. Nonetheless, IFN γ responses to adjuvanted formulations were consistently
414 higher at most dose levels than responses to CoVLP alone, particularly after the second
415 dose. Overall, IFN γ responses were strongest in the AS03-adjuvanted groups,
416 particularly at lower CoVLP dose levels. Of course, it is possible that the IFN γ
417 responses observed were mediated in part by other cells (eg: NK or CD8 T cells),
418 since the ELISpot assay captures cell-mediated responses indiscriminately⁴⁴. Additional
419 flow cytometry evaluations are warranted to characterize the phenotype of these
420 cellular responses more comprehensively and in particular, to better understand the
421 relative contribution of CD4 T cells to the observed responses. The IL-4 response was
422 also consistently higher in the AS03-adjuvanted groups compared to the unadjuvanted
423 or CpG1018-adjuvanted groups. In the AS03-adjuvanted groups, the IL4 response was
424 10-fold lower than the IFN γ responses after the first dose but rose to near equivalence
425 with the second dose suggesting a balanced Th1- and Th2-type response.

426

427 It is noteworthy that a substantial proportion of the subjects in this study appeared to
428 have pre-existing IFN γ responses to the S protein peptide pool used for PBMC

restimulation. Such cross-reactive T cell memory, possibly due to prior exposure to common human coronaviruses has been seen in 40-60% of adults and may provide some protection against highly pathogenic strains³³. Prior exposure to circulating coronaviruses may also explain the small number of subjects in this study (6.7%) who were ‘seronegative’ at screening based upon a commercial N-based ELISA but who were sero-positive at D0 in our assays targeting the S protein.

Together, these immune outcome data suggest that CoVLP alone can elicit both humoral and cellular responses that are Th1-biased to some extent and that this pattern of response is reinforced when adjuvanted with CpG1018. In contrast, when CoVLP is adjuvanted with AS03, the response was faster and more balanced with evidence of both Th1- and Th2-type activation. Although Th2-deviated responses have been implicated in VED (e.g.: early RSV vaccine)⁴⁵ and are a theoretical risk for vaccines targeting pathogenic coronaviruses⁴⁶, the engagement of T cells is also critical for B-cell maturation and the induction of strong and durable antibody responses^{47,48}.

The safety profile of CoVLP alone was relatively benign at all dose levels but, as expected, both the frequency and the intensity of local and systemic AEs was increased with either adjuvant. Compared to the first dose, the frequency of Grade 2 or Grade 3 AEs also increased after the second dose in all groups that received adjuvanted formulations. All AEs reported, regardless of dose level, adjuvant or initial intensity, were transient and resolved rapidly. Although one subject did not receive a second dose of vaccine due to a grade 3 AE (per Protocol), no subject voluntarily withdrew from the study due to any AE/SAE. Overall, the reactogenicity of the adjuvanted CoVLP formulations was similar to that reported for other candidate SARS-CoV-2 vaccines in early-phase clinical studies. Although hypersensitivity to plant material is a theoretical risk with any plant-derived product, no subject in the current study had an allergic-type reaction, consistent with Medicago’s safety database of >14,000 subjects who have received one or two doses of plant-derived influenza vaccine candidates⁴⁹.

Like any early-phase clinical trial, this study has several limitations beyond the obvious concern regarding small group size when testing multiple dose levels and formulations (n=20/group). For comparisons between formulations however, the small group risk was mitigated by the fact that results across the three CoVLP dose levels were highly consistent (n=60 for CoVLP alone or with each adjuvant). Another obvious concern is the relatively limited range of immune response parameters available at the time of writing. Although the data presented herein provide a ‘first look’ at the immune response induced by the different formulations, the immune profiles will be further investigated in the coming months. Finally, like all other early-phase trials, our study assessed only the short-term (day 42) response to vaccination and the durability of these responses will only become apparent as subjects are followed for longer periods of time. However, based on the robust high antibody and balanced cellular responses to the adjuvanted CoVLP formulations and our previous experience with plant-derived VLP vaccines targeting influenza, we anticipate that the CoVLP-induced responses are likely to last for at least 6 months^{30, 50}.

In conclusion, this first trial of CoVLP alone or adjuvanted with either CpG1018 or AS03 suggests that our plant-derived candidate vaccine is well-tolerated and immunogenic. Its immunogenicity, particularly at low doses, is dramatically enhanced by the presence of an adjuvant. Based upon the totality of non-clinical and clinical results available and the obvious advantages of dose-sparing in a pandemic, a two-dose schedule of CoVLP at 3.75 µg/dose adjuvanted with AS03 will be carried forward into further studies planned for Canada, the USA and other countries.

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Appendix

Author Contributions

All authors contributed significantly to the research work. All company authors had full access to the Tables and Figure data. BJW and NL made the final decision to submit the manuscript.

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TABLES

Table 1: Summary of demographics and baseline characteristics (NCT04450004)

	CoVLP 3.75 µg			CoVLP 7.5 µg			CoVLP 15 µg			All
	Unadjuvanted	Adjuvanted with CpG 1018	Adjuvanted with AS03	Unadjuvanted	Adjuvanted with CpG 1018	Adjuvanted with AS03	Unadjuvanted	Adjuvanted with CpG 1018	Adjuvanted with AS03	
Subjects	20	20	20	20	20	20	20	20	20	180
Sex, n (%)										
Male	9 (45.0)	10 (50.0)	5 (25.0)	10 (50.0)	8 (40.0)	8 (40.0)	7 (35.0)	10 (50.0)	11 (55.0)	78 (43.3)
Female	11 (55.0)	10 (50.0)	15 (75.0)	10 (50.0)	12 (60.0)	12 (60.0)	13 (65.0)	10 (50.0)	9 (45.0)	102 (56.7)
Race, n (%)										
White	18 (90.0)	20 (100.0)	20 (100.0)	20 (100.0)	18 (90.0)	19 (95.0)	20 (100.0)	18 (90.0)	19 (95.0)	172 (95.6)
Black or African American	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.0)	4 (2.2)
Asian	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.0)	0 (0.0)	1 (5.0)	0 (0.0)	4 (2.2)
Ethnicity, n (%)										
Hispanic/Latinx	0 (0.0)	2 (10.0)	0 (0.0)	1 (5.0)	3 (15.0)	1 (5.0)	0 (0.0)	1 (5.0)	0 (0.0)	9 (5.0)
Age at Vaccination										
Mean ± SD	34.9 ± 8.3	35.3 ± 11.0	34.7 ± 9.1	35.6 ± 8.0	32.4 ± 9.5	37.2 ± 7.8	34.1 ± 9.6	32.0 ± 9.0	32.7 ± 9.1	34.3 ± 9.0
Median (range)	35 (18-49)	36 (18-53)	36 (19-49)	36 (20-50)	31 (19-52)	37 (21-55)	31.5 (22-54)	30 (19-51)	32.5 (18-52)	34 (18-55)

SD: Standard Deviation

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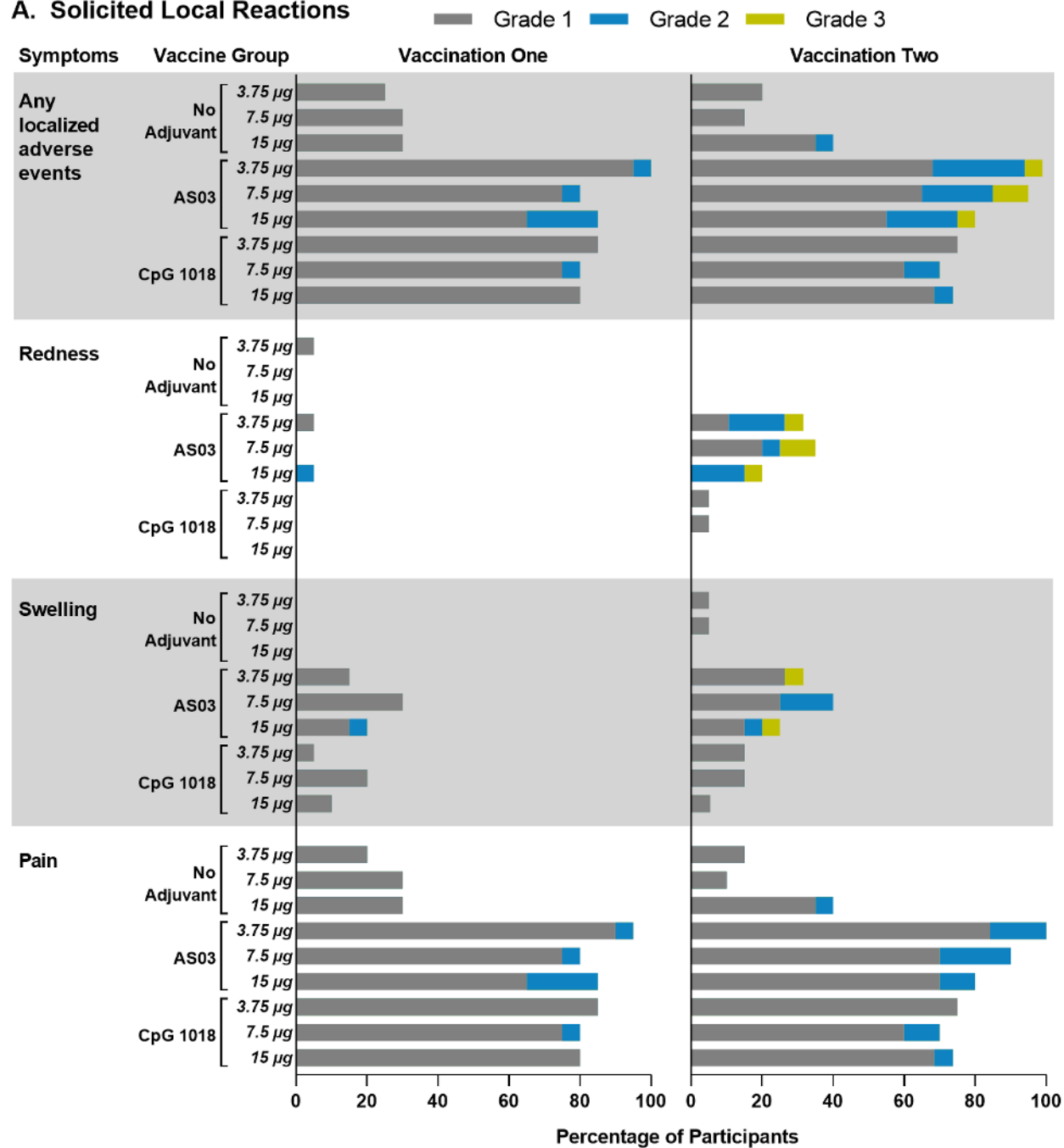
637 **Table 2: Subject Disposition**

	CoVLP 3.75 µg			CoVLP 7.5 µg			CoVLP 15 µg			All
	Unadjuvanted n (%)	Adjuvanted with CpG 1018 n (%)	Adjuvanted with AS03 n (%)	Unadjuvanted n (%)	Adjuvanted with CpG 1018 n (%)	Adjuvanted with AS03 n (%)	Unadjuvanted n (%)	Adjuvanted with CpG 1018 n (%)	Adjuvanted with AS03 n (%)	
Screened Subjects										413
Screened Failure										233
Randomized Subjects	20	20	20	20	20	20	20	20	20	180
Vaccinated at Day 0	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	180 (100.0)
Vaccinated at Day 21	20 (100.0)	20 (100.0)	19 (95.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	19 (95.0)	20 (100.0)	178 (98.9)
Safety Analysis Set	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	180 (100.0)
Per Protocol Set										
Day 21	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	19 (95.0)	20 (100.0)	179 (99.4)
Day 42	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	19 (95.0)	20 (100.0)	20 (100.0)	19 (95.0)	20 (100.0)	178 (98.9)
Completed Through Day 21	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	19 (95.0)	20 (100.0)	179 (99.4)
Early Withdrawal from Study	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	1 (0.6)
Adverse Event	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Death	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Lost to	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Follow-up										
Physician	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Decision										
Pregnancy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Protocol	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Deviation										
Site	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Terminated by										
sponsor										
Study	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Termination by										
Sponsor										
Withdrawal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	1 (0.6)
by Subject										
Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

FIGURES

A. Solicited Local Reactions



B. Solicited Systemic Reactions

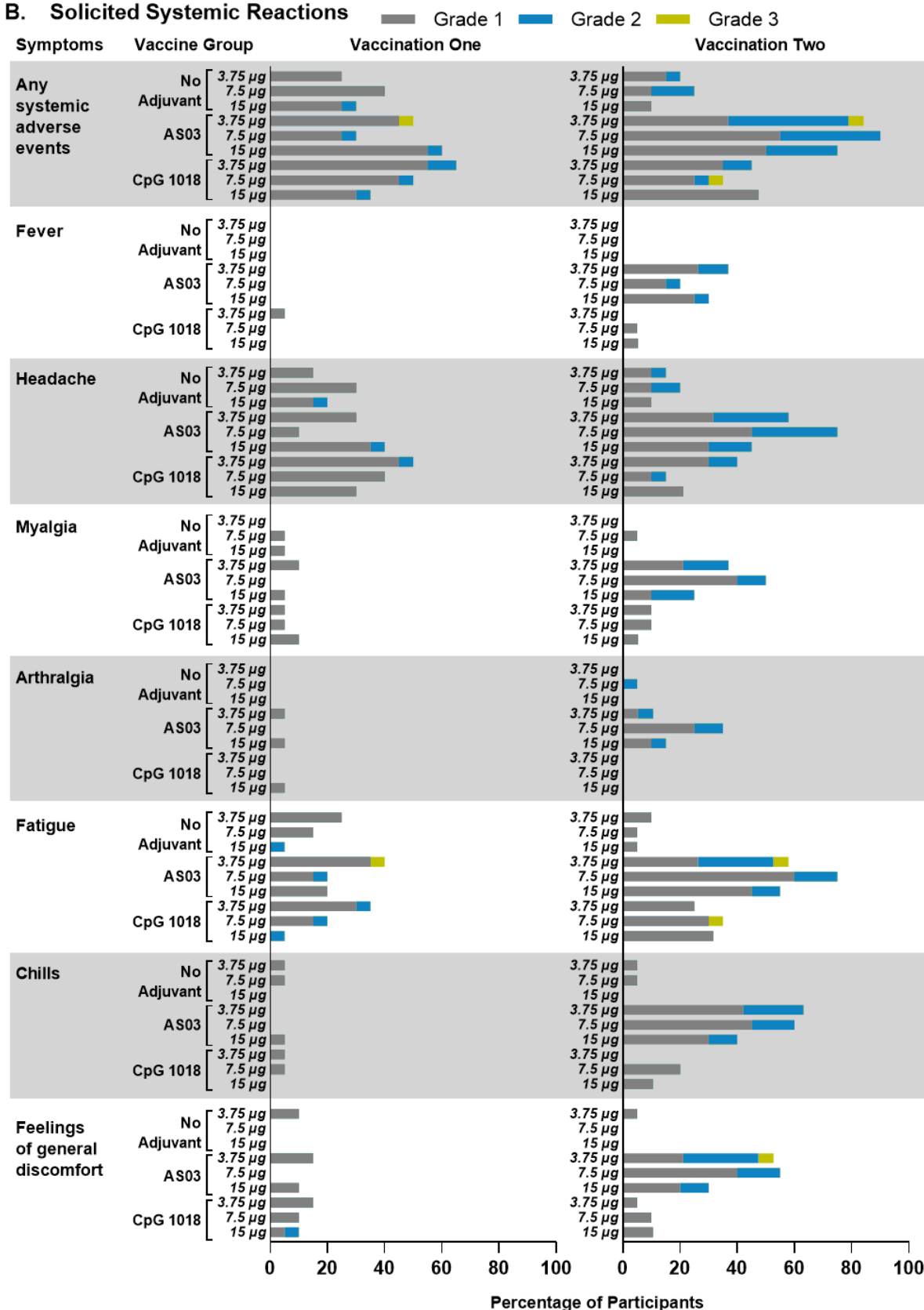
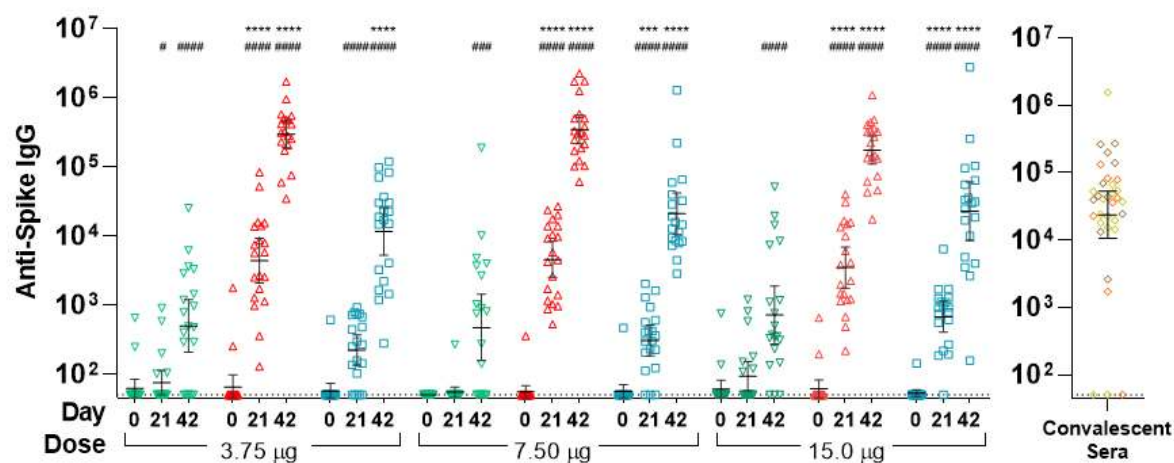


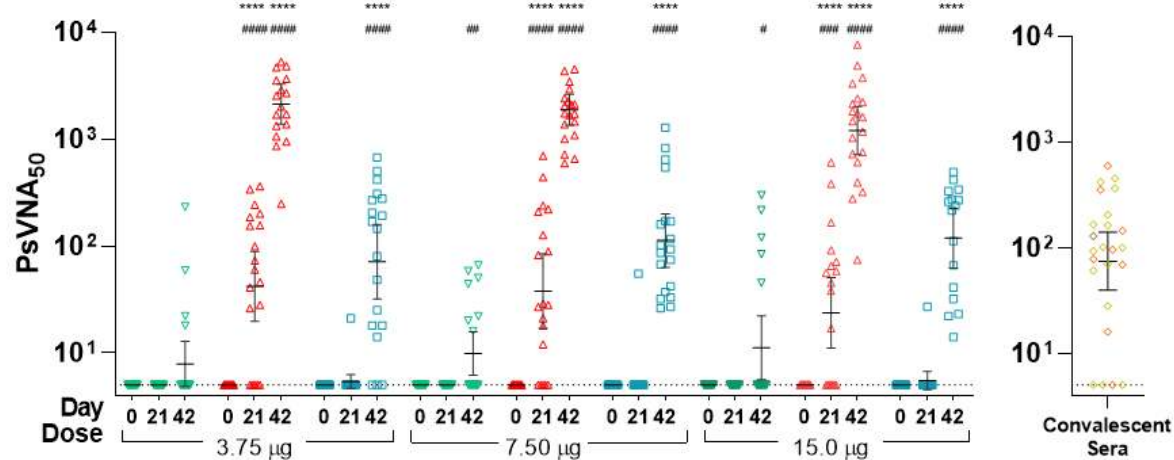
Figure 1 Solicited Local and Systemic AEs 7-Days After the First or Second Vaccine Dose.

Subjects were monitored for solicited local (panel A) and systemic (panel B) AEs from the time of vaccination through 7 days after vaccine administration. There was no grade 4 (life-threatening) events. Participants who reported 0 events make up the remainder of the 100% calculation (not shown). If any of the solicited AEs persisted beyond Day 7 after each vaccination (when applicable), it was recorded as unsolicited AEs.

A. SARS-CoV-2 Anti-Spike IgG Titers



B. Pseudovirus Neutralizing Antibody Titers



C. Live Virus Neutralizing Antibody Titers

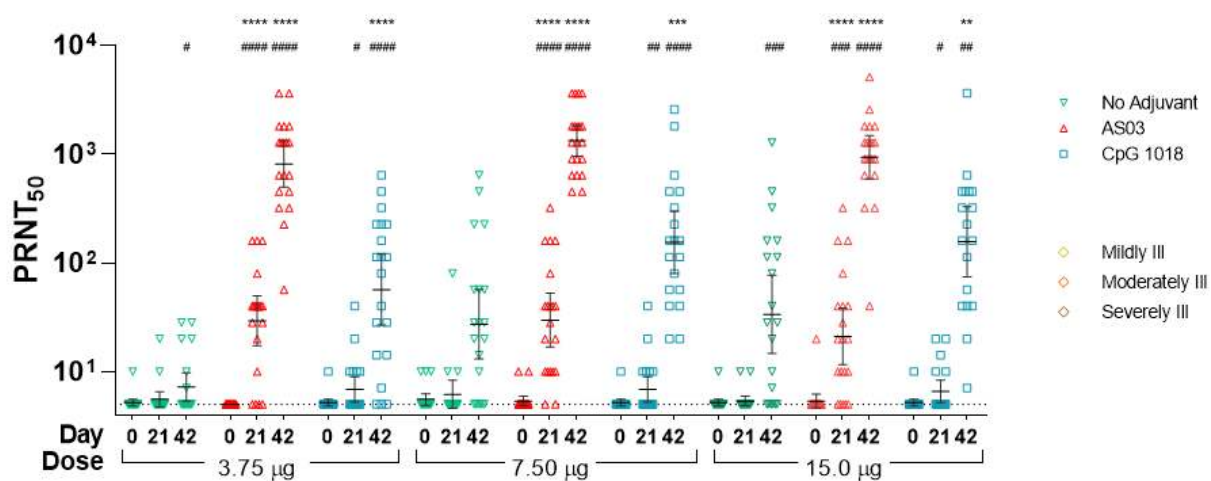
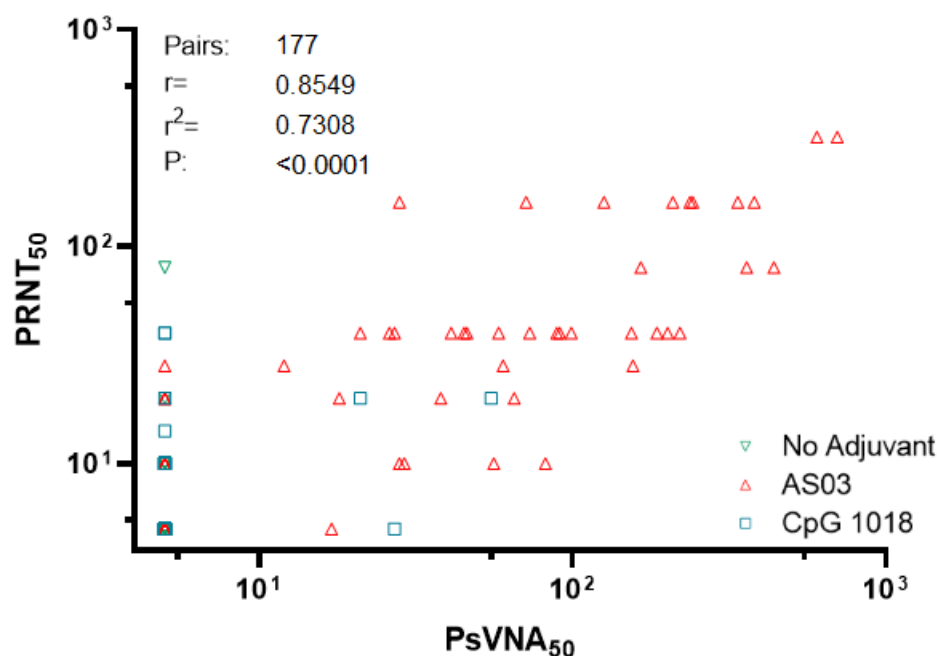


Figure 2 CoVLP Humoral Response.

Serum antibodies of subjects vaccinated with 3.75, 7.5, or 15 μ g CoVLP with or without AS03 or CpG1018 adjuvant, were measured to spike protein by ELISA (panel A) or by neutralization of pseudovirus (panel B), or live virus (panel C). Convalescent sera from recovered COVID-19 infected mildly, moderately, or severely ill patients were analyzed by anti-spike ELISA (n=35), and sera from mildly or moderately ill patients and one severely ill patient were analyzed by pseudovirion neutralization assay (n=21); results are shown in the right panels. All results are presented as reciprocal mid-point titers. Bars indicate geometric means. Error bars indicate 95% confidence intervals. Significant differences between days 0 and 21, or 0 and 42, for each vaccine regimen are indicated by # (#p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001; unpaired T-test of log-transformed values, GraphPad Prism, v8.1.1). Significant differences between unadjuvanted and adjuvanted regimens for days 21 and 42 are indicated by * (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; 2-way ANOVA of log-transformed values, GraphPad Prism, v8.1.1).

A. Vaccination One (Day 21)



B. Vaccination Two (Day 42)

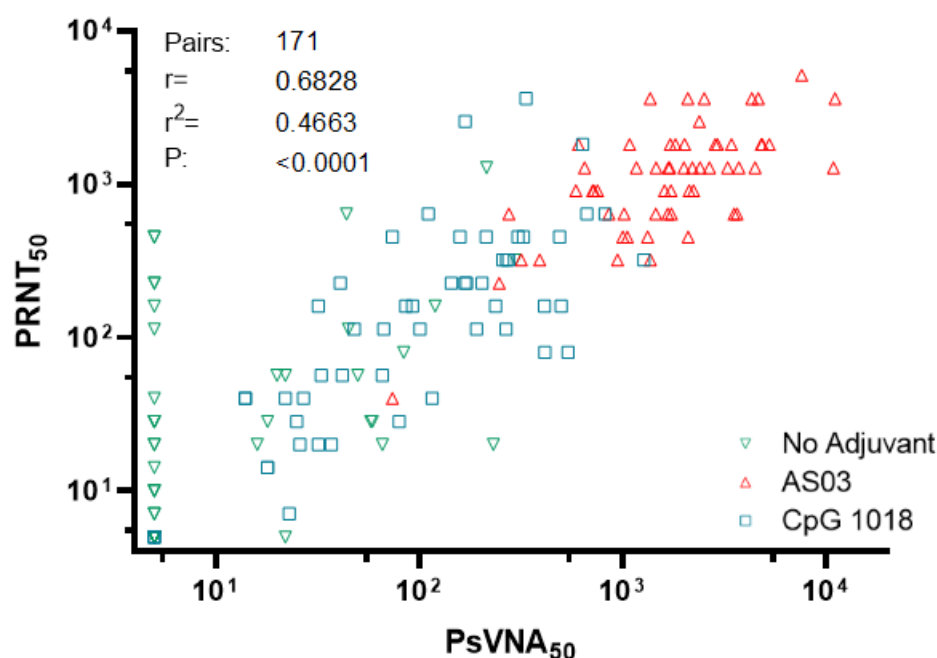
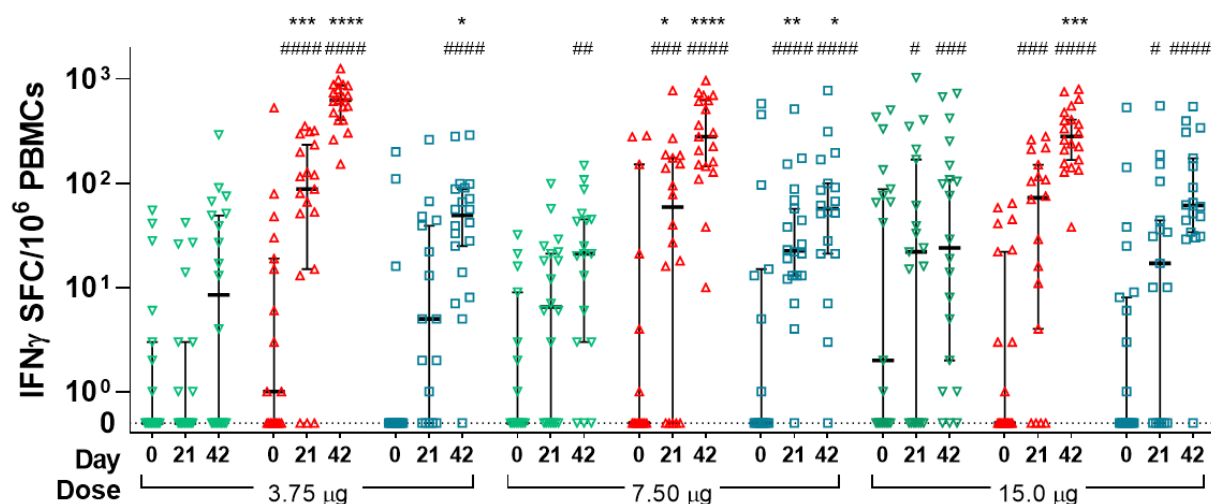


Figure 3 Correlation Between Assays Quantifying Neutralizing Antibody Titers

Neutralizing serum antibodies of subjects vaccinated with 3.75, 7.5, or 15 μ g CoVLP with or without AS03 or CpG1018 adjuvant, were measured by neutralization of pseudovirion (x-axis) or live virus (y-axis)

twenty-one days after the first vaccination (panel A) or twenty-one days after the second vaccination (panel B). Results are presented as reciprocal titers. R are Pearson correlation coefficients (GraphPad Prism, v8.1.1).

A. IFN γ Cellular Immune Response



B. IL4 Cellular Immune Response

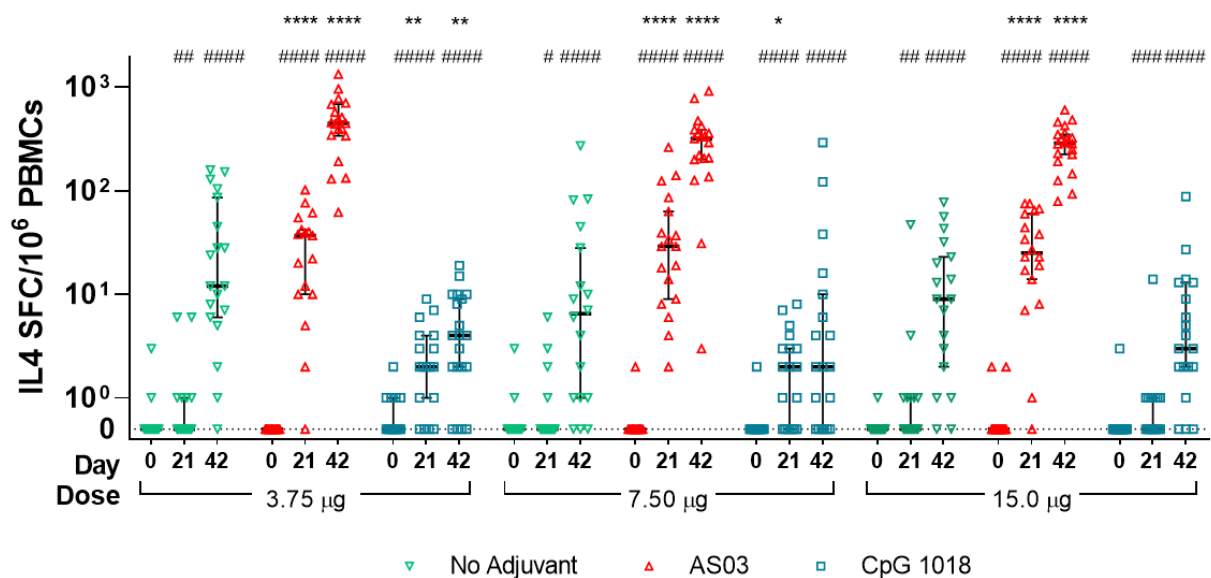


Figure 4 IFN- γ and IL-4 secreting cells as measured by ELISpot at Day 0, 21, and 42

Frequencies of antigen-specific T-cells producing interferon-gamma (Panel A) and interleukin-4 (Panel B) cellular immune responses at baseline (day 0) and 21 days after one immunization (day 21) or two immunizations (day 42) with 3.75, 7.5 or 15 μ g doses of CoVLP with or without adjuvants (CpG1018 and AS03) after restimulation ex vivo with recombinant spike peptide pool. Bars indicate medians and error bars indicate 95% CI. Significant differences between days 0 and 21 or between day 0 and 42 for each vaccine regimen are indicated by # (* P <0.05, ** P <0.01, *** P <0.001, **** P <0.0001; Unpaired T-test. Comparisons for each test to day 0 data are to the same pre-vaccination data set that include all subjects. The figure illustrates matched subject data. GraphPad Prism, v8.1.1). Significant difference between adjuvanted vaccine and unadjuvanted vaccine regimens at day 21 and 42 are indicated by * (* P <0.05, ** P <0.01, *** P <0.001, **** P <0.0001; Kruskal-Wallis, GraphPad Prism, v8.1.1).

Supplementary Appendix to

**Phase 1 trial of a Candidate Recombinant Virus-Like Particle Vaccine for Covid-19 Disease
Produced in Plants**

Supplementary Methods:

Vaccination Stopping Rules

Safety monitoring of safety signals were and will be performed throughout the study. Stopping rules or conditions for stopping this clinical trial would occur if there was clear evidence of harm or harmful effects such as SAEs related to the treatment (study vaccine). A SAE which was thought to be unrelated to the study vaccine would not warrant stopping the trial.

The following event(s) may result in a halt to the study, for further review and assessment of the event(s):

- Any death occurring during the study;
- Any vaccine-related SAE during the study;
- Any life-threatening (Grade 4) vaccine-related AE during the study;
- If 10 % or more of subjects in a single treatment group, experience the same or similar listed event(s) that cannot be clearly attributed to another cause:
 - a severe (Grade 3 or higher) vaccine-related AE during the study;
 - a severe (Grade 3 or higher) vaccine-related vital sign(s) abnormality;
 - a severe (Grade 3 or higher) vaccine-related clinical laboratory abnormality.

In the case that a pre-defined safety signal is met in any treatment group, subsequent dosing will result in at least a transient halt in the study to permit a complete evaluation of the reported event(s) and to consult an IDMC. A decision as to whether the study can progress as planned must be made and documented in the event of any safety signal. If a stopping rule has been met once all subjects have been vaccinated in the study, the IDMC will be notified by a Note To File (NTF) for their information purposes.

Immunological Assay Method Details

Evaluation of SARS-CoV-2 Humoral Response

SARS-CoV-2 Spike Protein ELISA

Briefly, SARS-Cov2 Spike protein (SARS-Cov2/Wuhan/2019, Immune Technology Corp.) was coated onto a flat-bottom 96-well microplate at a concentration of 1 μ g/mL in sodium carbonate 50mM (overnight; 4°C). Following washing steps (PBS-Tween), plates were blocked using Blotto 5% (Rockland Inc.) in PBS (1-2 hours; 37°C). Following washing steps, serially diluted sera (starting dilution 1/100, 4-fold dilutions, 8 dilutions, in PBS-Tween-Blotto) were added to the wells, in duplicates, and incubated at 37°C for 1 hour. Plates were washed and incubated with secondary antibody (anti-Human IgG (H+L) antibody, Peroxidase-labeled, Seracare), diluted at 1/20 000 in PBS-Tween-Blotto and incubated at 37°C for 1 hour. Plates were washed and incubated with peroxidase substrate (SureBlue TMB, Seracare) for 20 minutes at room temperature. Reaction was stopped using hydrochloric acid and absorbance was read at 450nm, within 2 hours (Variaskan Flash microplate reader, Thermo Scientific). Optical density (OD) results for the serial dilutions were used to generate a 4-parameter logistic regression (4PL). The titer was defined as the reciprocal dilution of the sample for which the OD is equal to a fixed cut-point value. Samples below cut-point were attributed a value of 50 (half the minimum required dilution).

SARS-CoV-2 Pseudovirus Neutralisation Assay (PNA)

Neutralizing antibody analysis was performed using a cell-based pseudotyped virus neutralisation assay (Nexelis, Quebec, Canada). Pseudotyped virus particles were first generated using a genetically modified Vesicular Stomatitis Virus backbone from which the glycoprotein G was removed and luciferase reporter introduced (rVSVΔG-luciferase, Kerafast) to allow quantification using relative luminescence units (RLU). This rVSVΔG–Luc virus was transduced into HEK293T cells that had previously been transduced with SARS-Cov-2 Spike glycoprotein (NXL137-1 in POG2 containing 2019-nCOV Wuhan-Hu-1; Genbank: MN908947) from which the last nineteen amino acids of the cytoplasmic tail were removed (rVSVΔG-Luc-Spike ΔCT).

Serial dilutions (starting dilution of 1/10; 2-fold; 8 dilutions, in complete growth media) of the heat-inactivated human sera (56°C; 30min) were prepared in a 96-well plate, in duplicates. The SARS-Cov-2 pseudovirus (in complete growth media) was added to the sera dilutions to reach a target concentration equivalent to approximately 150,000 RLU/well and mixture was incubated at 37°C with 5% CO₂ supplementation for 1 hour. Serum-pseudovirus complexes were then transferred onto plates previously seeded overnight with Vero E6 cells (ATCC CRL-1586), expressing ACE-2 receptor, and incubated at

37°C with 5% CO₂ supplementation for 20-24 hours. Once incubation was completed, cells were lysed and samples equilibrated using ONE-Glo EX luciferase assay system (Promega), incubated for 3 minutes at room temperature, and luminescence level read using a luminescence plate reader (i3x plate reader, Molecular Devices). The resulting RLU was inversely proportional to the level of neutralizing antibodies present in the serum. For each sample, the neutralizing titer was established as the reciprocal dilution corresponding to the 50% neutralization (NT50), when compared to the pseudoparticle control. The NT50 was interpolated from a linear regression using the two dilutions flanking the 50% neutralisation. Samples below cut-off were attributed a value of 5 (half the minimum required dilution).

SARS-CoV-2 Microneutralization CPE-based assay

Neutralizing antibody analysis was also performed using a cell-based cytopathic effect (CPE) assay (VisMederi, Sienna, Italy). Sera sample were first heat inactivated (56°C; 30min) and then serially diluted (starting dilution of 1/10, 2-fold; 8 dilutions, in complete growth media). Wild-type SARS-Co-2 virus (2019 nCOV ITALY/INMI1, provided by EVAg; Genbank: MT066156) was then added at final concentration of 25 TCID₅₀/mL (in complete growth media), and plates were incubated for 1 hour at 37°C with 5% CO₂ supplementation. At the end of the incubation, the mixture was transferred onto duplicate 96-well microtiter plates pre-seeded overnight with Vero E6 cells (ATCC CRL-1586), expressing ACE-2 receptor. Plates were then incubated for 3 days at 37°C with 5% CO₂ supplementation. Cytopathic effect (CPE) was then quantified using an inverted optical microscope. The microneutralization titer (MNt) was defined as the reciprocal of the highest sample dilution that protects from CPE at least 50% of the cells. If no neutralization was observed, samples were attributed a titer value of 5 (half the minimum required dilution).

Evaluation of SARS-CoV-2 Cell-Mediated Immune Response

Interferon-γ ELISpot

Cell-mediated immune response was evaluated using an Interferon-γ ELISpot assay (Human IFN-γ ELISpot assay, Cellular Technology Limited (CTL), USA). Cryopreserved peripheral blood mononuclear cells (PBMC) were rapidly thawed and allowed to rest between 2 to 3 hours at 37°C with 5% CO₂ supplementation, in CTL-Test media supplemented with 1% Glutamine and 1% Penicillin/Streptomycin. Cells were enumerated and dispensed at 0.5x10⁶ cells per well, in duplicates, onto PVDF filter plates pre-coated with an IFN-γ specific capture antibody. Cells were stimulated using a pool of peptides (15-mer peptides) overlapping the full sequence of SARS-Cov-2 spike protein (USA-CA2/2020, Genbank: MN994468.1) at a concentration of 2.19 µg/mL, for 18-24 hours, at 37°C with 5% CO₂ supplementation.

After washes (PBS-Tween), biotinylated anti-interferon- γ detection antibody was added to the plates and incubated for 2 hours at room temperature after which, following another round of washes, a streptavidin-alkaline phosphatase conjugate was added and incubated for 30 minutes at room temperature. After washing steps, substrate solution was added, incubated at room temperature for 15 minutes, after which plate was rinsed and left to air-dry pending spot enumeration, using an ELISpot reader (ImmunoSpot S6 Universal Analyzer, Cellular Technology Limited). Mean of peptide pool stimulation duplicates was calculated and normalized using the mean of the negative control replicates (control media) and multiplied by a factor of 2 to express cells counts per million cells.

Interleukin-4 (IL-4) ELISpot

Cell-mediated immune response was evaluated using an IL-4 ELISpot assay (Human IL-4 ELISpot assay, Cellular Technology Limited (CTL), Cleveland, OH, USA). Cryopreserved peripheral blood mononuclear cells (PBMC) were rapidly thawed and allowed to rest between 2 to 3 hours at 37°C with 5% CO₂ supplementation, in CTL-Test media supplemented with 1% Glutamine and 1% Penicillin/Streptomycin. Cells were enumerated and dispensed at 0.5x10⁶ cells per well, in duplicates, onto PVDF filter plates pre-coated with an IL-4 specific capture antibody. Cells were stimulated using a pool of peptides (15-mer peptides) overlapping the full sequence of SARS-Cov-2 spike protein (USA-CA2/2020, Genbank: MN994468.1) at a concentration of 2.19 μ g/mL, for 32-48 hours, at 37°C with 5% CO₂ supplementation. After washes (PBS-Tween), biotinylated anti-IL-4 detection antibody was added to the plates and incubated for 2 hours at room temperature after which, following another round of washes, a streptavidin-alkaline phosphatase conjugate was added and incubated for 30 minutes at room temperature. After washing steps, substrate solution was added, incubated at room temperature for 15 minutes, after which plate was rinsed and left to air-dry pending spot enumeration, using an ELISpot reader (ImmunoSpot S6 Universal Analyzer, Cellular Technology Limited). Mean of peptide pool stimulation duplicates was calculated and normalized using the mean of the negative control replicates (control media) and multiplied by a factor of 2 to express cells counts per million cells.

Convalescent sera description

Sera from Covid-19 convalescent patients were collected from a total of 34 individuals with confirmed disease diagnosis. Time between the onset of the symptoms and sample collection varied between 27 and 105 days. Three samples were collected by Solomon Park (Burien, WA, USA), 20 by Sanguine BioSciences (Sherman Oaks, CA, USA) and 11 from McGill University Health Centre. Disease severity were ranked as mild (Covid-19 symptoms without shortness of breath), moderate (shortness of breath reported), and severe (hospitalized). These samples were analysed in parallel of clinical study samples, using the same assay as described above. Samples from severely ill were analyzed by serum IgG ELISA only. Demographic characteristics are provided in Supplementary Table 1.

Supplementary Table 1 Summary of Covid-19 Patients Providing Convalescent Sera

Characteristics	Mild	Moderate	Severe
Subjects	16	8	11
Sex, n (%)			
Male	10 (62.5%)	2 (25.0%)	8 (72.7%)
Female	5 (31.3%)	6 (75.0%)	3 (27.3%)
Race, n (%)			
White	7 (43.8%)	6 (75.04%)	5 (45.5%)
Black or African American	1 (6.3%)	0 (0.0%)	5 (45.5%)
Asian	4 (25.0%)	2 (25.0%)	0 (0.0%)
Ethnicity, n (%)			
Hispanic/Latinx	2 (12.5%)	1 (12.5%)	1 (9.1%)
Age:			
Mean \pm SD	42.7 \pm 13.6	37.8 \pm 13.0	51.9 \pm 16.0
Median (range)	39 (20-66)	40.5 (19-58)	50.0 (28-82)

SD: Standard Deviation

Adverse Event Severity Evaluation and the Monitoring of Adverse Events of Special Interest

Adverse Event of Special Interest (AESI)

AESI for CoVLP Vaccine – Vaccine Enhanced Disease (VED)

Safety signal of VED after exposure to the Coronavirus-Like Particle COVID-19 Vaccine was closely monitored and assessed by retrieving data for this AESI as follows: AEs within the system organ class (SOC): immune system disorders and high level group term (HLGT): lower respiratory tract disorders (excluding obstruction and infection), cardiac disorders, signs and symptoms not elsewhere classified (NEC), vascular disorders, heart failures NEC, arteriosclerosis, stenosis, vascular insufficiency and necrosis, cardiac arrhythmias, myocardial disorders, and vascular hemorrhagic disorders. High level term (HLT): renal failure and impairment and preferred term (PT): pericarditis, coagulopathy, deep vein thrombosis, pulmonary embolism, cerebrovascular accidents, peripheral ischemia, liver injury, Guillain-Barre syndrome, anosmia, ageusia, encephalitis, chilblains, vasculitis, erythema multiforme (based on standardized MedDRA® classification)^{1,2} that require inpatient hospitalization (≥ 24 hours) and have laboratory confirmed SARS-Cov-2 infection will be monitored for assessment of any potential case of VED.

AESI for CoVLP Vaccine – Hypersensitivity Reactions

All reported events were also monitored for hypersensitivity reactions after exposure to the Coronavirus-Like Particle COVID-19 Vaccine. In eight clinical studies conducted to date with the Quadrivalent VLP Influenza Vaccine (QVLP) produced using similar plant-based technology, all reported events were monitored for a possible hypersensitivity component (events were searched using both narrow and broad standardized MedDRA® queries). Based on these data, there was a single case of possible early anaphylactic reaction associated with use of QVLP in humans. A small number of subjects had potential hypersensitivity reactions judged to be related to vaccine administration (no more than 0.3 % of subjects in any given QVLP treatment group experienced one of these events) and the events were distributed fairly evenly among treatment groups, including the placebo and the active comparator groups. However, since severe reactions are considered to be an important potential risk (based on the theoretical risk that using plants for the production of biotherapeutics may induce hypersensitivity), Medicago required that appropriate medical treatment and supervision were available to manage any possible anaphylactic reactions in this study. To collect data on these events, Medicago closely monitored and assessed allergic reactions assessed by the site Investigators as related to the Investigational product as AESIs.

AESI for Adjuvant – Potential Immune-Mediated Diseases (pIMD)

Potential immune-mediated diseases are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune aetiology.

Supplementary Table 2 List of Potential Immune-Mediated Diseases

Neuroinflammatory disorders	Musculoskeletal disorders	Skin disorders
<ul style="list-style-type: none"> • Cranial nerve neuropathy, including paralysis and paresis (eg, Bell's palsy). • Optic neuritis. • Multiple sclerosis. • Transverse myelitis. • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants. • Acute disseminated encephalomyelitis, including site specific variants, eg, noninfectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis. • Myasthenia gravis, including Lambert-Eaton myasthenic syndrome. • Demyelinating peripheral neuropathies and plexopathies including: <ul style="list-style-type: none"> - Chronic inflammatory demyelinating polyneuropathy. - Multifocal motor neuropathy. - Polyneuropathies associated with monoclonal gammopathy. • Narcolepsy. • Tolosa Hunt syndrome. 	<ul style="list-style-type: none"> • Systemic lupus erythematosus and associated conditions. • Systemic scleroderma (systemic sclerosis), including: <ul style="list-style-type: none"> - Diffuse scleroderma. - CREST syndrome. • Idiopathic inflammatory myopathies, including: <ul style="list-style-type: none"> - Dermatomyositis. - Polymyositis. • Anti-synthetase syndrome. • Rheumatoid arthritis and associated conditions including: <ul style="list-style-type: none"> - Juvenile idiopathic arthritis. - Still's disease. • Polymyalgia rheumatica. • Spondyloarthropathies, including: <ul style="list-style-type: none"> - Ankylosing spondylitis. - Reactive arthritis (Reiter's syndrome). - Undifferentiated spondyloarthritis. - Psoriatic arthritis. - Enteropathic arthritis. • Relapsing polychondritis. • Mixed connective tissue disorder. • Gout. 	<ul style="list-style-type: none"> • Psoriasis. • Vitiligo. • Erythema nodosum. • Autoimmune bullous skin diseases (including pemphigus, pemphigoid, and dermatitis herpetiformis). • Lichen planus. • Sweet's syndrome. • Localized scleroderma (morphea). • Cutaneous lupus erythematosus. • Rosacea.
Vasculitis	Blood disorders	Others
<ul style="list-style-type: none"> • Large vessels vasculitis including: <ul style="list-style-type: none"> - Giant cell arteritis (temporal arteritis). - Takayasu's arteritis. • Medium sized and/or small vessels vasculitis including: <ul style="list-style-type: none"> - Polyarteritis nodosa. - Kawasaki's disease. - Microscopic polyangiitis. - Wegener's granulomatosis (granulomatosis with polyangiitis). 	<ul style="list-style-type: none"> • Autoimmune hemolytic anemia. • Autoimmune thrombocytopenia. • Antiphospholipid syndrome. • Pernicious anemia. • Autoimmune aplastic anemia. • Autoimmune neutropenia. • Autoimmune pancytopenia. 	<ul style="list-style-type: none"> • Autoimmune glomerulonephritis including: <ul style="list-style-type: none"> - IgA nephropathy. - Glomerulonephritis rapidly progressive. - Membranous glomerulonephritis. - Membranoproliferative glomerulonephritis. - Mesangioproliferative glomerulonephritis. - Tubulointerstitial nephritis and uveitis syndrome.

Vasculitis (continued)	Blood disorders (continued)	Others (continued)
<ul style="list-style-type: none"> - Churg–Strauss syndrome (allergic granulomatous angiitis or eosinophilic granulomatosis with polyangiitis). - Buerger’s disease (thromboangiitis obliterans). - Necrotizing vasculitis (cutaneous or systemic). - Antineutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified). - Henoch-Schonlein purpura (IgA vasculitis). - Behcet’s syndrome. - Leukocytoclastic vasculitis. 		<ul style="list-style-type: none"> • Ocular autoimmune diseases including: <ul style="list-style-type: none"> - Autoimmune uveitis. - Autoimmune retinitis. • Autoimmune myocarditis/cardiomyopathy. • Sarcoidosis. • Stevens-Johnson syndrome. • Sjögren’s syndrome. • Alopecia areata. • Idiopathic pulmonary fibrosis. • Goodpasture syndrome. • Raynaud’s phenomenon.
Liver disorders	Gastrointestinal disorders	Endocrine disorders
<ul style="list-style-type: none"> • Autoimmune hepatitis. • Primary biliary cirrhosis. • Primary sclerosing cholangitis. • Autoimmune cholangitis. 	<ul style="list-style-type: none"> • Inflammatory bowel disease, including: <ul style="list-style-type: none"> - Crohn’s disease. - Ulcerative colitis. - Microscopic colitis. - Ulcerative proctitis. • Celiac disease. • Autoimmune pancreatitis. 	<ul style="list-style-type: none"> • Autoimmune thyroiditis (including Hashimoto thyroiditis). • Grave’s or Basedow’s disease. • Diabetes mellitus type 1. • Addison’s disease. • Polyglandular autoimmune syndrome. • Autoimmune hypophysitis.

Severity of Grades for Solicited Local and Systemic Adverse Events

Supplementary Table 3 List of Potential Immune-Mediated Diseases

Symptoms	Severity				
	None	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Potentially life-threatening)
Injection Site Adverse Events (Local Adverse Events)					
Erythema (redness)	< 25 mm	25 - 50 mm	51 - 100 mm	> 100 mm	Necrosis or exfoliative dermatitis
Swelling	< 25 mm	25 - 50 mm and does not interfere with activity	51 – 100 mm or interferes with activity	> 100 mm or prevents daily activity	Necrosis
Pain	None	Does not interfere with activity	Repeated use of non-narcotic pain reliever for more than 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Results in a visit to emergency room (ER) or hospitalization
Solicited Systemic Adverse Events					
Fever (°C or °F)	< 38.0 °C < 100.4 °F	38.0 - 38.4 °C 100.4 - 101.1 °F	38.5 - 38.9 °C 101.2 - 102.0 °F	39.0 - 40.0 °C 102.1 - 104.0 °F	> 40.0 °C > 104.0 °F
Headache	None	No interference with activity	Repeated use of non-narcotic pain reliever for more than 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	Results in a visit to emergency room (ER) or hospitalization
Fatigue	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	Results in a visit to emergency room (ER) or hospitalization
Muscle aches	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	Results in a visit to emergency room (ER) or hospitalization
Joint aches, chills, feeling of general discomfort or uneasiness (malaise), swelling in the axilla, swelling in the neck, swelling in the groin, swelling in the chest wall	None	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	Results in a visit to emergency room (ER) or hospitalization

Source: FDA. Guidance for Industry: Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. 2007.

Solicited Adverse Events

Supplementary Table 4 Solicited Adverse Events by Severity Grades from Days 0 to 7 (Safety Analysis Set) – 3.75 µg

Symptoms	Severity	COVLP 3.75 ug unadjuvanted		COVLP 3.75 ug with CpG 1018		COVLP 3.75 ug with AS03	
		Vacc. 1	Vacc 2	Vacc. 1	Vacc 2	Vacc 1	Vacc 2
		N=20	N=20	N=20	N=20	N=20	N=19
Redness at injection site	Grade 1	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.0)	2 (10.5)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.8)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)
Swelling at injection site	Grade 1	0 (0.0)	1 (5.0)	1 (5.0)	3 (15.0)	3 (15.0)	5 (26.3)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)
Pain at injection site	Grade 1	4 (20.0)	3 (15.0)	17 (85.0)	15 (75.0)	18 (90.0)	16 (84.2)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	3 (15.8)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fever	Grade 1	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	5 (26.3)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (10.5)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Headache	Grade 1	3 (15.0)	2 (10.0)	9 (45.0)	6 (30.0)	6 (30.0)	6 (31.6)
	Grade 2	0 (0.0)	1 (5.0)	1 (5.0)	2 (10.0)	0 (0.0)	5 (26.3)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Muscle aches	Grade 1	0 (0.0)	0 (0.0)	1 (5.0)	2 (10.0)	1 (5.0)	4 (21.1)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.8)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Joint aches	Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.3)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fatigue	Grade 1	5 (25.0)	2 (10.0)	6 (30.0)	5 (25.0)	7 (35.0)	5 (26.3)
	Grade 2	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	5 (26.3)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.3)
Chills	Grade 1	1 (5.0)	1 (5.0)	1 (5.0)	0 (0.0)	0 (0.0)	8 (42.1)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (21.1)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Discomfort or uneasiness	Grade 1	2 (10.0)	1 (5.0)	3 (15.0)	1 (5.0)	3 (15.0)	4 (21.1)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (26.3)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)
Swelling in the neck	Grade 1	1 (5.0)	0 (0.0)	4 (20.0)	1 (5.0)	0 (0.0)	1 (5.3)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling in the axilla	Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling in the groin	Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling in the chest wall	Grade 1	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

**Supplementary Table 5 Solicited Adverse Events by Severity Grades from Days 0 to 7 (Safety Analysis Set)
– 7.5 µg**

Symptoms	Severity	COVLP 7.5 ug unadjuvanted		COVLP 7.5 ug with CpG 1018		COVLP 7.5 ug with AS03	
		Vacc. 1 N=20	Vacc 2 N=20	Vacc. 1 N=20	Vacc 2 N=20	Vacc 1 N=20	Vacc 2 N=20
Redness at injection site	Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	4 (20.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (10.0)
Swelling at injection site	Grade 1	0 (0.0)	1 (5.0)	4 (20.0)	3 (15.0)	6 (30.0)	5 (25.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pain at injection site	Grade 1	6 (30.0)	2 (10.0)	15 (75.0)	12 (60.0)	15 (75.0)	14 (70.0)
	Grade 2	0 (0.0)	0 (0.0)	1 (5.0)	2 (10.0)	1 (5.0)	4 (20.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fever	Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	3 (15.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Headache	Grade 1	6 (30.0)	2 (10.0)	8 (40.0)	2 (10.0)	2 (10.0)	9 (45.0)
	Grade 2	0 (0.0)	2 (10.0)	0 (0.0)	1 (5.0)	0 (0.0)	6 (30.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Muscle aches	Grade 1	1 (5.0)	1 (5.0)	1 (5.0)	2 (10.0)	0 (0.0)	8 (40.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (10.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Joint aches	Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (25.0)
	Grade 2	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (10.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fatigue	Grade 1	3 (15.0)	1 (5.0)	3 (15.0)	6 (30.0)	3 (15.0)	12 (60.0)
	Grade 2	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	3 (15.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)
Chills	Grade 1	1 (5.0)	1 (5.0)	1 (5.0)	4 (20.0)	0 (0.0)	9 (45.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Discomfort or uneasiness	Grade 1	0 (0.0)	0 (0.0)	2 (10.0)	2 (10.0)	0 (0.0)	8 (40.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling in the neck	Grade 1	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.0)	2 (10.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling in the axilla	Grade 1	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling in the groin	Grade 1	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling in the chest wall	Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Supplementary Table 6 Solicited Adverse Events by Severity Grades from Days 0 to 7 (Safety Analysis Set) – 15 µg

Symptoms	Severity	COVLP 15 ug unadjuvanted		COVLP 15 ug with CpG 1018		COVLP 15 ug with AS03	
		Vacc 1 N=20	Vacc 2 N=20	Vacc. 1 N=20	Vacc 2 N=19	Vacc 1 N=20	Vacc 2 N=20
Redness at injection site	Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	3 (15.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling at injection site	Grade 1	0 (0.0)	0 (0.0)	2 (10.0)	1 (5.3)	3 (15.0)	3 (15.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
Pain at injection site	Grade 1	6 (30.0)	7 (35.0)	16 (80.0)	13 (68.4)	13 (65.0)	14 (70.0)
	Grade 2	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.3)	4 (20.0)	2 (10.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fever	Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)	0 (0.0)	5 (25.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Headache	Grade 1	3 (15.0)	2 (10.0)	6 (30.0)	4 (21.1)	7 (35.0)	6 (30.0)
	Grade 2	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	3 (15.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Muscle aches	Grade 1	1 (5.0)	0 (0.0)	2 (10.0)	1 (5.3)	1 (5.0)	2 (10.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Joint aches	Grade 1	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	2 (10.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fatigue	Grade 1	0 (0.0)	1 (5.0)	0 (0.0)	6 (31.6)	4 (20.0)	9 (45.0)
	Grade 2	1 (5.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	2 (10.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chills	Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	2 (10.5)	1 (5.0)	6 (30.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	(10.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Discomfort or uneasiness	Grade 1	0 (0.0)	0 (0.0)	1 (5.0)	2 (10.5)	2 (10.0)	4 (20.0)
	Grade 2	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	2 (10.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling in the neck	Grade 1	2 (10.0)	0 (0.0)	1 (5.0)	1 (5.3)	2 (10.0)	3 (15.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling in the axilla	Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)	2 (10.0)	2 (10.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling in the groin	Grade 1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling in the chest wall	Grade 1	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Supplementary Table 7 Treatment- Emergent Unsolicited Adverse Events up to 21 days after last vaccination (Safety Analysis Set) – Dose 3.75 µg

Adverse Event SOC/PT	COVLP 3.75 ug unadjuvanted (N=20)	COVLP 3.75 ug with CpG 1018 (N=20)	COVLP 3.75 ug with AS03 (N=20)
Subjects with at least one unsolicited AE	8	9	8
Eye disorders			
Conjunctivitis allergic	1 (5.0)	0 (0.0)	0 (0.0)
Gastrointestinal disorders			
Abdominal pain upper	0 (0.0)	1 (5.0)	0 (0.0)
General disorders and administration site conditions			
Chest discomfort	1 (5.0)	0 (0.0)	0 (0.0)
Fatigue	0 (0.0)	1 (5.0)	0 (0.0)
Feeling hot	0 (0.0)	0 (0.0)	1 (5.0)
Injection site pain	0 (0.0)	0 (0.0)	1 (5.0)
Injection site swelling	0 (0.0)	0 (0.0)	1 (5.0)
Infections and infestations			
Bacterial vaginosis	0 (0.0)	0 (0.0)	1 (5.0)
Hordeolum	0 (0.0)	1 (5.0)	0 (0.0)
Vaginal infection	0 (0.0)	0 (0.0)	1 (5.0)
Injury, poisoning and procedural complications			
Ligament sprain	1 (5.0)	0 (0.0)	0 (0.0)
Tooth fracture	1 (5.0)	0 (0.0)	0 (0.0)
Investigations			
Blood glucose increased	0 (0.0)	1 (5.0)	0 (0.0)
Musculoskeletal and connective tissue disorders			
Plantar fasciitis	0 (0.0)	1 (5.0)	0 (0.0)
Nervous system disorders			
Dizziness exertional	0 (0.0)	1 (5.0)	0 (0.0)
Paraesthesia	1 (5.0)	0 (0.0)	0 (0.0)
Psychiatric disorders			
Depressed mood	0 (0.0)	1 (5.0)	0 (0.0)
Irritability	0 (0.0)	1 (5.0)	0 (0.0)
Reproductive system and breast disorders			
Dysmenorrhoea	0 (0.0)	0 (0.0)	1 (5.0)
Respiratory, thoracic and mediastinal disorders			
Asthma exercise induced	0 (0.0)	0 (0.0)	1 (5.0)
Skin and subcutaneous tissue disorders			

Adverse Event SOC/PT	COVLP 3.75 ug unadjuvanted (N=20)	COVLP 3.75 ug with CpG 1018 (N=20)	COVLP 3.75 ug with AS03 (N=20)
Rash maculo-papular	1 (5.0)	0 (0.0)	0 (0.0)

Supplementary Table 8 Treatment- Emergent Unsolicited Adverse Events up to 21 days after last vaccination (Safety Analysis Set) – Dose 7.5 µg

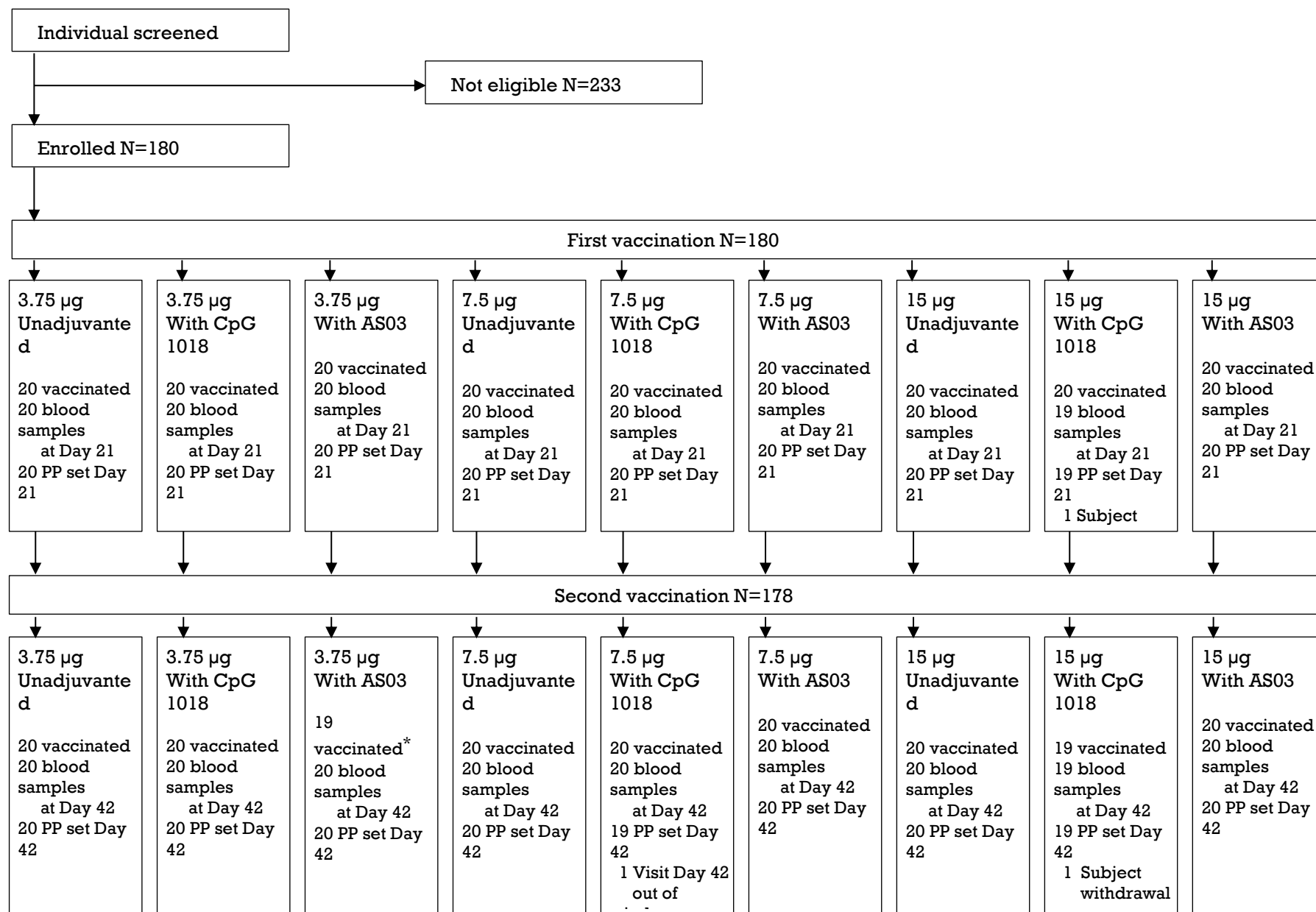
Adverse Event SOC/PT	COVLP 7.5 ug unadjuvanted (N=20)	COVLP 7.5 ug with CpG 1018 (N=20)	COVLP 7.5 ug with AS03 (N=20)
Subjects with at least one unsolicited AE	9	11	7
Blood and lymphatic system disorders			
Lymphadenopathy	0 (0.0)	0 (0.0)	1 (5.0)
Gastrointestinal disorders			
Diarrhoea	0 (0.0)	0 (0.0)	1 (5.0)
General disorders and administration site conditions			
Injection site papule	0 (0.0)	0 (0.0)	1 (5.0)
Investigations			
Blood pressure decreased	1 (5.0)	0 (0.0)	0 (0.0)
Blood pressure increased	1 (5.0)	0 (0.0)	0 (0.0)
Metabolism and nutrition disorders			
Hypercholesterolaemia	1 (5.0)	0 (0.0)	0 (0.0)
Musculoskeletal and connective tissue disorders			
Costochondritis	0 (0.0)	1 (5.0)	0 (0.0)
Limb discomfort	0 (0.0)	1 (5.0)	0 (0.0)
Pain in extremity	0 (0.0)	0 (0.0)	1 (5.0)
Nervous system disorders			
Dizziness	0 (0.0)	1 (5.0)	0 (0.0)
Psychiatric disorders			
Anxiety	0 (0.0)	1 (5.0)	0 (0.0)
Reproductive system and breast disorders			
Vulvovaginal pruritus	0 (0.0)	1 (5.0)	0 (0.0)
Respiratory, thoracic and mediastinal disorders			
Dry throat	1 (5.0)	0 (0.0)	0 (0.0)
Rhinitis allergic	1 (5.0)	0 (0.0)	0 (0.0)
Throat irritation	0 (0.0)	2 (10.0)	0 (0.0)
Skin and subcutaneous tissue disorders			
Dermatitis contact	0 (0.0)	1 (5.0)	0 (0.0)
Erythema	0 (0.0)	1 (5.0)	0 (0.0)
Rash	1 (5.0)	0 (0.0)	0 (0.0)

Supplementary Table 9 Treatment- Emergent Unsolicited Adverse Events up to 21 days after last vaccination (Safety Analysis Set) – Dose 15 µg

Adverse Event SOC/PT	COVLP 15 ug unadjuvanted (N=20)	COVLP 15 ug with CpG 1018 (N=20)	COVLP 15 ug with AS03 (N=20)
Subjects with at least one unsolicited AE	8	6	11
Ear and labyrinth disorders			
Ear pain	0 (0.0)	0 (0.0)	1 (5.0)
Gastrointestinal disorders			
Abdominal discomfort	0 (0.0)	1 (5.0)	0 (0.0)
Abdominal pain	1 (5.0)	0 (0.0)	0 (0.0)
Nausea	0 (0.0)	0 (0.0)	1 (5.0)
Toothache	1 (5.0)	0 (0.0)	0 (0.0)
Vomiting	0 (0.0)	0 (0.0)	2 (10.0)
General disorders and administration site conditions			
Feeling cold	0 (0.0)	0 (0.0)	1 (5.0)
Injection site bruising	0 (0.0)	0 (0.0)	1 (5.0)
Injection site erythema	0 (0.0)	0 (0.0)	1 (5.0)
Injection site warmth	0 (0.0)	0 (0.0)	1 (5.0)
Swelling	0 (0.0)	0 (0.0)	1 (5.0)
Infections and infestations			
Oral herpes	1 (5.0)	0 (0.0)	0 (0.0)
Injury, poisoning and procedural complications			
Joint injury	0 (0.0)	1 (5.0)	0 (0.0)
Scratch	0 (0.0)	1 (5.0)	0 (0.0)
Wound	0 (0.0)	1 (5.0)	0 (0.0)
Investigations			
Aspartate aminotransferase increased	0 (0.0)	0 (0.0)	1 (5.0)
Blood cholesterol increased	1 (5.0)	0 (0.0)	0 (0.0)
Blood creatine phosphokinase increased	0 (0.0)	0 (0.0)	2 (10.0)
Musculoskeletal and connective tissue disorders			
Back pain	0 (0.0)	0 (0.0)	1 (5.0)
Muscle spasms	0 (0.0)	0 (0.0)	1 (5.0)
Nervous system disorders			
Headache	0 (0.0)	0 (0.0)	1 (5.0)
Presyncope	2 (10.0)	0 (0.0)	0 (0.0)
Taste disorder	0 (0.0)	0 (0.0)	1 (5.0)
Psychiatric disorders			
Insomnia	0 (0.0)	0 (0.0)	1 (5.0)
Respiratory, thoracic and mediastinal disorders			
Oropharyngeal pain	0 (0.0)	0 (0.0)	2 (10.0)

Adverse Event SOC/PT	COVLP 15 ug unadjuvanted (N=20)	COVLP 15 ug with CpG 1018 (N=20)	COVLP 15 ug with AS03 (N=20)
Rhinorrhoea	0 (0.0)	1 (5.0)	0 (0.0)
Skin and subcutaneous tissue disorders			
Dyshidrotic eczema	1 (5.0)	0 (0.0)	0 (0.0)
Vascular disorders			
Hot flush	0 (0.0)	1 (5.0)	0 (0.0)

Supplementary Figure 1 Consort Flow Diagram



*One subject did not receive the second vaccination following Grade 3 adverse event but accepted to have blood collection for immunogenicity.

Detailed Immunogenicity Results

Supplementary Table 10 CoVLP-induced Binding Antibody IgG Titers (Anti-Spike ELISA)

Vaccine Group (Dose and Adjuvant)	CoVLP 3.75 µg			CoVLP 7.50 µg			CoVLP 15 µg			Convalescent Serum
	-	+ CpG 1018	+ AS03	-	+ CpG 1018	+ AS03	-	+ CpG 1018	+ AS03	
Day 0										
N	20	20	20	20	20	20	20	20	20	Overall
GMT (95% CI)	61.5 (48.1, 78.6)	56.6 (44.3, 72.4)	64.8 (50.7, 82.8)	50.0 (39.1, 63.9)	55.9 (43.8, 71.4)	55.1 (43.1, 70.5)	60.2 (47.1, 77.0)	52.7 (41.2, 67.3)	60.9 (47.6, 77.8)	N=35 GMT: 19841 (8407, 46825)
Day 21										
N	20	20	20	20	20	20	20	19	20	Mild:
GMT (95% CI)	75.1 (45.4, 124.4)	220.8 (133.4, 365.6)	4354.0 (2629.5, 7209.7)	54.4 (32.8, 90.0)	305.7 (184.6, 506.3)	4550.3 (2748.0, 7534.7)	93.0 (56.2, 154.0)	673.9 (401.7, 1130.6)	3464.3 (2092.2, 5736.4)	N=16 GMT: 11638 (2465, 54952)
GMFR (95% CI)	1.29 (0.78, 2.14)	3.86 (2.33, 6.38)	73.99 (44.67, 122.56)	0.97 (0.59, 1.62)	5.36 (3.24, 8.86)	79.93 (48.31, 132.23)	1.6 (0.97, 2.66)	11.94 (7.12, 20.03)	59.63 (36.04, 98.67)	Moderate: N=8 GMT: 14989 (1617, 138966)
Day 42										
N	20	20	19	20	19	20	20	19	20	Severe:
GMT (95% CI)	496.1 (232.3, 1059.6)	11544.6 (5405.2, 24657.5)	295239.6 (137967.3, 631790.4)	471.0 (220.5, 1005.9)	20811.1 (9553.6, 45334.1)	342989.1 (160587.7, 732568.5)	711.5 (333.1, 1519.6)	22598.3 (10374.0, 49227.2)	173457.2 (81212.8, 370476.3)	N=11 GMT: 52857 (20556, 13)
GMFR (95% CI)	8.45 (3.96, 18.04)	201.85 (94.65, 430.44)	4943.72 (2307.32, 10592.56)	8.56 (4.0, 18.3)	364.73 (167.7, 793.25)	6046.89 (2835.03, 12897.54)	12.21 (5.72, 26.04)	403.77 (185.48, 878.98)	2965.83 (1390.17, 6327.40)	

GMT = Geometric Mean Titer; GMFR = Geometric mean Fold Rise; CI = Confidence Interval

Supplementary Table 11 CoVLP-induced Neutralization Antibody (Nab) Titers (Pseudovirion Neutralization Assay)

Vaccine Group (Dose and Adjuvant)	CoVLP 3.75 µg			CoVLP 7.50 µg			CoVLP 15 µg			Convalescent Serum
	-	+ CpG 1018	+ AS03	-	+ CpG 1018	+ AS03	-	+ CpG 1018	+ AS03	
Day 0										
N	20	20	20	20	20	20	20	20	20	
GMT (95% CI)	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	
Day 21										
N	20	20	20	20	19	19	19	18	20	Overall N=24 GMT: 86.14 (42.0, 176.7)
GMT (95% CI)	5.0 (3.2-7.7)	5.4 (3.5-8.3)	41.6 (27.0, 64.1)	5.0 (3.2, 7.7)	5.7 (3.6, 8.8)	37.8 (24.3, 58.9)	5.0 (3.2, 7.8)	5.5 (3.5, 8.7)	23.6 (15.3, 36.4)	
GMFR (95% CI)	1.00 (0.65, 1.54)	1.07 (0.70, 1.65)	8.33 (5.41, 12.82)	1.0 (0.65, 1.54)	1.13 (0.73, 1.77)	7.57 (4.86, 11.78)	1.0 (0.64, 1.56)	1.1 (0.7, 1.73)	4.72 (3.07, 7.27)	
SCR (%) (95%CI)	0 (0.0%) (0.0, 16.8)	0 (0.0%) (0.0, 16.8)	12 (60.0%) (36.1, 80.9)	0 (0.0%) (0.0, 16.8)	1 (5.3%) (0.1, 26.0)	8 (42.1%) (20.3, 66.5)	0 (0.0%) (0.0, 17.6)	0 (0.0%) (0.0, 18.5)	9 (45.0%) (23.1, 68.5)	
Day 42										
N	20	19	18	20	19	19	20	16	20	Moderate: N=8 GMT: 77.2 (21.1, 283.1)
GMT (95% CI)	7.9 (4.5-13.7)	71.3 (40.4, 125.9)	2118.3 (1228.7, 3651.9)	9.8 (5.7, 17.1)	112.2 (63.6, 198.0)	1883.8 (1082.9, 3277.0)	11.2 (6.4, 19.4)	118.1 (63.6, 219.4)	1200.9 (690.3, 2089.0)	
GMFR (95% CI)	1.57 (0.90, 2.74)	14.26 (8.08, 25.17)	423.66 (245.75, 730.39)	1.97 (1.13, 3.42)	22.44 (12.71, 39.60)	376.76 (216.58, 655.39)	2.23 (1.28, 3.88)	23.62 (12.72, 43.87)	240.18 (138.07, 417.81)	
SCR (%) (95%CI)	2 (10.0%) (1.2, 31.7)	12 (63.2%) (38.4, 83.7)	18 (100%) (81.5, 100)	4 (20.0%) (5.7, 43.7)	14 (73.7%) (48.8, 90.9)	20 (100.0%) (83.2, 100.0)	5 (25.0%) (8.7, 49.1)	12 (75.0%) (47.6, 92.7)	20 (100.0%) (83.2, 100.0)	

GMT = Geometric Mean Titer; GMFR = Geometric mean Fold Rise; SCR = Seroconversion ratio; CI = Confidence Interval

Supplementary Table 12 CoVLP-induced Neutralization Antibody (Nab) Titers (Live Wild-Type Virus Neutralization Assay)

	Vaccine Group (Dose and Adjuvant)	-	CoVLP 3.75 µg		-	CoVLP 7.50 µg		-	CoVLP 15 µg	
			+ CpG 1018	+ AS03		+ CpG 1018	+ AS03		+ CpG 1018	+ AS03
Day 0										
	N	20	20	20	20	20	20	20	20	20
	GMT (95% CI)	5.2 (4.8, 5.6)	5.2 (4.8, 5.6)	5.0 (5.0, 5.0)	5.5 (4.9, 6.2)	5.2 (4.8, 5.6)	5.4 (4.9, 5.9)	5.2 (4.8, 5.6)	5.2 (4.8, 5.6)	5.4 (4.6, 6.2)
Day 21										
	N	20	20	20	20	20	20	20	19	20
	GMT (95% CI)	5.5 (4.7, 6.5)	6.8 (5.2, 8.9)	29.3 (17.3, 49.7)	6.2 (4.6, 8.3)	6.8 (5.2, 8.9)	29.8 (16.9, 52.7)	5.4 (4.9, 5.9)	6.6 (5.2, 8.4)	21.1 (11.6, 38.4)
	GMFR (95% CI)	1.1 (0.9-1.3)	1.3 (1.0, 1.7)	5.9 (3.5, 9.9)	1.1 (0.9, 1.4)	6.8 (5.2, 8.9)	29.8 (16.9, 52.7)	1.3 (1.0, 1.7)	1.3 (1.0, 1.6)	3.9 (2.0, 7.6)
	SCR (%) (95%CI)	1 (5.0%) (0.1, 24.9)	2 (10.0%) (1.2, 31.7)	15 (75.0%) (50.9, 91.3)	1 (5.0%) (0.1, 24.9)	2 (10.0%) (1.2, 31.7)	13 (65.0%) (40.8, 84.6)	0 (0.0%) (0.0, 16.8)	2 (10.5%) (1.3, 33.1)	11 (55.0%) (31.5, 76.9)
Day 42										
	N	20	20	19	20	20	20	20	18	20
	GMT (95% CI)	7.2 (5.3, 9.7)	56.6 (26.8-119.5)	811.3 (496.0-1327)	27.3 (13.1, 57.2)	157.3 (84.3, 293.4)	1325 (962.6, 1824)	33.6 (14.7, 77.1)	156.9 (74.6, 330.3)	937.0 (592.4, 1482)
	GMFR (95% CI)	1.4 (1.0, 1.9)	10.9 (5.1, 23.5)	162.3 (99.2, 265.4)	4.9 (2.4, 10.1)	157.3 (84.3, 293.4)	247.3 (182.9, 334.4)	6.5 (2.9, 14.8)	30.2 (14.6, 62.6)	174.9 (104.4, 292.7)
	SCR (%) (95%CI)	4 (20.0%) (5.7, 43.7)	14 (70.0%) (45.7, 88.1)	19 (100.0%) (82.4, 100)	11 (55.5%) (31.5, 76.9)	20 (100.0%) (83.2, 100.0)	20 (100.0%) (83.2, 100.0)	12 (60.0%) (36.1, 80.9)	17 (94.4%) (72.7, 99.9)	20 (100.0%) (83.2, 100.0)

GMT = Geometric Mean Titer; GMFR = Geometric mean Fold Rise; SCR = Seroconversion ratio; CI = Confidence Interval.

Supplementary Table 13 CoVLP-induced IFN γ Cellular Response (ELISpot)

	Vaccine Group (Dose and Adjuvant)	CoVLP 3.75 µg				CoVLP 7.50 µg			CoVLP 15 µg	
		-	+ CpG 1018	+ AS03	-	+ CpG 1018	+ AS03	-	+ CpG 1018	+ AS03
Day 0										
	N	157 (day 0 samples treated as a single group)								
	GMT (95% CI)	0.0 (0.0, 1.0)								
Day 21										
	N	18	17	19	20	20	18	19	17	18
	M SFU (95% CI)	0.0 (0.0 - 3.0)	5.0 (0.0-39.0)	88.0 (51.0-233.0)	6.5 (0.0-21.0)	22.5 (13.0-57.0)	59.0 (0.0-175.0)	22.0 (0.0-168.0)	17.0 (0.0-44.0)	72.5 (4.0-151.0)
Day 42										
	N	20	20	19	18	19	19	20	18	20
	M SFU (95% CI)	8.5 (0.0-49.0)	49.0 (25.0-88.0)	628.0 (476.0, 862.0)	21.0 (3.0-45.0)	61.0 (28.0-100.0)	305.0 (160.0-623.0)	24.0 (2.0-108.0)	61.0 (34.0-174.0)	281.5 (167.0-407.0)

M SFU = Median Spot Forming Units per 10⁶ cells; CI = Confidence Interval.

Supplementary Table 14 CoVLP-induced IL4 Cellular Response (ELISpot)

	Vaccine Group (Dose and Adjuvant)	CoVLP 3.75 µg			CoVLP 7.50 µg			CoVLP 15 µg		
		-	+ CpG 1018	+ AS03	-	+ CpG 1018	+ AS03	-	+ CpG 1018	+ AS03
Day 0										
	N	137 (day 0 samples treated as a single group)								
	GMT (95% CI)	0.0 (0.0, 0.0)								
Day 21										
	N	16	18	17	17	19	18	17	17	18
	M SFU (95% CI)	0.0 (0.0-1.0)	2.0 (1.0-4.0)	37.0 (10.0-42.0)	0 (0.0- 0.0)	2.0 (0.0-3.0)	29.0 (9.0-63.0)	0.0 (0.0-1.0)	0.0 (0.0-1.0)	25.0 (14.0-60.0)
Day 42										
	N	19	20	19	18	19	19	18	19	20
	M SFU (95% CI)	12.0 (7.0-86.0)	4.0 (2.0-9.0)	445.0 (343.0, 680.0)	6.5 (1.0-28.0)	2.0 (0.0-10.0)	291.0 (200.0-358.0)	9.0 (2.0-23.0)	3.0 (2.0-13.0)	287.5 (224.0-348.0)

M SFU = Median Spot Forming Units per 10⁶ cells; CI = Confidence Interval.

Supplementary References

1. Law B, Sturkenboom M. Safety platform for emergency vaccines (SPEAC). D2.3 Priority list of adverse events of special interest: COVID-19. Report v1.1. 2020 Mar 5.
2. Law B, Sturkenboom M. Safety platform for emergency vaccines (SPEAC). D2.3 Priority list of adverse events of special interest: COVID-19. Report v2.0. 2020a May 25.