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Supplementary appendix 3

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Ella R, Reddy S, Jogdand H, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: interim results from a double-blind, randomised, multicentre, phase 2 trial, and 3-month follow-up of a double-blind, randomised phase 1 trial. *Lancet Infect Dis* 2021; published online March 8. https://doi.org/10.1016/S1473-3099(21)00070-0.

Supplementary Appendix to Manuscript Entitled

Safety and immunogenicity clinical trial of an inactivated SARS-CoV-2 vaccine, BBV152 (a phase 2, double-blind, randomised controlled trial) and the persistence of immune responses from a phase 1 follow-up report

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This supplementary material has been provided by the authors to give readers additional information about their work.

Supplemental methods

i. RT-PCR (conducted at Dr. Dangs Lab, New Delhi) at Screening

TRUPCR SARS-CoV-2 RT qPCR (V 3.2) is a single tube, in vitro nucleic acid amplification test for the qualitative detection of severe acute respiratory syndrome coronavirus 2(SARS-CoV-2)specific RNA from respiratory specimens(nasopharyngeal or oropharyngeal aspirates,washes or swabs, bronchoalveolar lavage, sputum and tracheal aspirates)using Real-time PCR. The human RNasePgene serves as an endogenous internal positive control for human nucleic acid is also included in this kit.

E gene: For the detection of SarbecovirusRdRp gene and N genedual targets for the detection of SARS-CoV-2

RNase P gene:endogenous internal control, which qualifies the sample for testing and is essential for testing Viral RNA in host organism. According to the Centre for Daises Control and prevention, Respiratory Viruses Branch, Division of Viral Diseases, all clinical samples should be tested for human RNAse P gene to assess specimen quality for RT PCR for detection of 2019-Novel Coronavirus

The assay runs for 38 cycles;however for any interpretation,threshold cut off cycle Ct is 35.

Interpretation is as follows:

Amplification Signals In

a) RNAse P +/- , E Gene +, RdRp&N gene + : SARS-CoV-2 POSITIVE
b) RNAse P +/- , E Gene -, RdRp&N gene + : SARS-CoV-2 POSITIVE
c) RNAse P +/- , E Gene +, RdRp&N gene - : SARBECOVIRUS POSITIVE
d) RNAse P + , E Gene -, RdRp&N gene - : NEGATIVE
e) RNAse P - , E Gene -, RdRp&N gene - : INVALID

100 percent concordance of results for submitted samples with ICMR designated QC Lab(AIIMS for Dr. Dangs Lab)

ii. CLIA (conducted at Dr. Dangs Lab, New Delhi) at Screening

The liaison SARS-CoV-2 IgG assay performed on the Liaison XL analyzer is an indirect chemiluminescence Immunoassay (CLIA) for the Quantitative determination of anti-S1 and anti- S2 antibodies to SARS-CoV-2 in human serum or plasma.The sensitivity of the above assay is 97.4 percent for 15 days post-diagnosis and specificity 98.9 percent for Laboratory routine testing. Testing of assay-specific calibrators allows the detected Relative light unit (RLU)to adjust the assigned master curve.The analyzer automatically calculates SARS-CoV-S1/S2 IgG antibody concentrations as arbitrary units (AU/mL)and grades the results.

Results are interpreted as follows:

Less than 12 AU/mL: NEGATIVE (A negative result may indicate the absence or a very low level of IgG antibodies to the Pathogen. The test could score negative in infected patients during the incubation period and during the early stages of infection>or equal to 12 and less than 15 AU/mL: Equivocal (Retest in duplicate. Samples with 2 out of 3 results more than or equal to 15 or less than 12 should be reported as positive or negative, respectively.A second sample should be collected and tested no less than one to two weeks later if the results are repeatedly equivocal)>or equal to 15: Positive (A positive result generally indicates exposure of the subject to the pathogen)

iii. Enzyme-linked immunosorbent assay (ELISA) (conducted at Bharat Biotech)

ELISA tests were performed as per standard protocols. Briefly, Microtiter plates were coated with SARS-CoV-2 specific antigens (Whole inactivated SARS CoV-2 antigen, spike (S1) (Syngene, Bangalore, India, Batch No# PRB026913/Receptor Binding Domain (RBD), Syngene, Bangalore, India, Batch No#PRB025485/ nucleocapsid (N), Syngene, Bangalore, India, Batch No# PRB025627 at a concentration of 1µg/ml, 100µl/well in PBS pH 7.4). After overnight incubation, wells were blocked and added serially diluted sera. After incubation, wells were added with Goat anti-Human IgG HRP conjugate (Sigma-Aldrich, Cat# A8667, dilution 1:5000) and incubated for 1hr at RT. Tetramethyl benzidine used as a substrate and measured absorbance at 450/630nm. Threshold value (Mean + 3SD) was established by taking the absorbance of Day 0 sera samples and antigen-specific endpoint titers were

determined for Days 28, 42, and 56 sera samples. The reciprocal antibody dilution, at which absorbance is above the threshold, was taken as antigen-specific antibody endpoint titers. Since, Bharat Biotech is a vaccine manufacturing company, all serological assays such as ELISA/ PRNT50 were routinely performed for various vaccines before releasing every batch into the market. All methods were validated with respect to sensitivity and specificity. In the case of SARS-CoV-2 vaccine development, all these methods were used, while maintaining proper negative and positive controls.

Known unvaccinated and uninfected individual serum was used as a negative control. Simultaneously, ELISA blank (without coating antigen) was also maintained as a negative control. Apart from this, cut off (Mean+3SD) was drawn from the absorbance obtained at various dilutions (1:1000 to 1:32000) of sera collected on day 0 (before vaccination), who is found negative for RT-PCR and Serology test.

iv. Plaque Reduction Neutralisation Test ($PRNT_{50}$) (conducted at Bharat Biotech)

The Plaque reduction neutralisation test was performed in a biosafety level 3 facility. To perform $PRNT_{50}$, Vero CCL-81 cell suspension (1.0 x 10^5 /mL/well)was added in duplicates in 24-well tissue culture plates and cultured in a CO_2 incubator at 37°C for 16-24 hrs. Serum samples from all enrolled participants were inactivated by keeping in a 56ºC-water bath for 30 min. Serial dilutions (4 fold) of serum samples were mixed with the virus, which can form 50 plaque-forming units and then incubated for 1 h at 37°C. The virus–serum mixtures were added onto the preformed Vero CCL-81 cell monolayers and incubated 1 h at 37°C in a 5% CO_2 incubator. The number of plaques was counted, and the Neutralizing antibody titer was determined based on the 50% reduction in the number of plaque count, which was further analyzed using 50% ProbitAnalysis(10.4103/ijmr.IJMR_2382_20). Inoculation dose used was 100 $CCID_{50}$ in 50 microlitre.

v. Microneutralisation assay (MNT_{50}) (conducted at Bharat Biotech)

The serum collected from all enrolled participants were inactivated at 56ºC in a water bath for 30 min. Serum was successively diluted with start dilution 1:8 to the required concentration by a 2-fold series, and an equal volume of challenge virus solution containing 100 $CCID_{50}$viruses was added. After neutralisation in a 37°C incubator for two hours, a 1.0 x 10^5 /mL cell suspension was added to the wells (0.1 mL/well) and cultured in a CO_2 incubator at 37ºC for 3-5 days. The Karber method (Ramakrishnan, 2016) by observing the CPE was used to calculate the neutralisation endpoint (MNT_{50}) (convert the serum dilution to logarithm), which means that the dilution of serum that can protect 50% of cells from infection by challenge with 100 $CCID_{50}$ virus. Inoculation dose used was 100 PFU in 100 microlitre

During each assay, known antibody titre from animal sera is used as a positive control. Pre-immune sera used as a negative control. T-cell Memory Response (Phase 1 PBMCs collected on Day 104):

PBMCs were cultured in 24 well plate with 0.5x10^6 cells/ml/well and stimulated with Whole virion Inactivated SARS CoV2 antigen (1.2 µg/ml) for 6days by keeping the plate at 37°C in 5% CO2 incubator. Cells stimulated with phorbol 12-myristate 13-acetate (PMA)(25 ng/ml, Sigma) and Ionomycin (1 µg/ml, Sigma) were used as positive control and unstimulated cells and PBMCs collected on Day 0 stimulated with inactivated SARS CoV 2 antigen were used as negative control. On Day 6, cells were harvested and stained with human specific fluorochrome conjugated B & T cell surface marker antibodies for 30 minutes at 4°C. The list of fluorochrome conjugated cell surface marker antibodies procured from BD Biosciences, USA are as follows, Mouse anti- human CD3 BV450 (clone# UCHT1, Cat#560365), Mouse anti- human CD4 PE-Cy 7 (clone# SK3, Cat# 348789), Mouse anti- human CD8 APC-H7 (clone# SK1, Cat# 560179), Mouse anti- human CD19 PE (clone# SJ25C1, Cat# 340364) and Mouse anti- human CD27 APC (clone# M-T271, Cat# 558664). Mouse anti- human CD45RO PerCp Cy5.5 (clone# UCHL1, Cat# 130-113-552) was procured from Miltenyi Biotec, Germany). Cells were again washed twice with PBS and resuspended in 500µl FACS buffer (BD Biosciences). All samples were acquired using BD FACSVerse (BD Biosciences, CA, USA) and data was analyzed using FlowJo software (Tree Star). To assess the T cell memory responses, gating was done from $CD3^+$T cell population and to $CD4^+$ T cell population and to assess the B cell memory response, gating was done on $CD3^-$ lymphocyte population. Additionally, on Day 3, 50µl cell culture supernatant was tested to determine Th1/Th2/Th17 cytokine profile using human CBA kit (Cytokine Bead Array Kit, Cat# 560484, BD Biosciences, USA). Further, Cell culture supernatant collected on Day 6 was also tested for secreted IgG antibodies by ELISA.

vi. T-cell Memory Response (Phase 1 PBMCs collected on Day 104):

PBMCs were cultured in 24 well plate with $0.5x10^6$ cells/ml/well and stimulated with Whole virion Inactivated SARS CoV2 antigen (1.2 µg/ml) for 6days by keeping the plate at 37°C in 5% CO2 incubator. Cells stimulated with PMA (25 ng/ml, Sigma) and Ionomycin (1 µg/ml, Sigma) were used as positive control and unstimulated cells and PBMCs collected on Day 0 stimulated with inactivated antigen were used as negative control. On Day 6, cells were harvested and stained with human specific fluorochrome conjugated B & T cell surface markers for 30 minutes at 4°C. The list of fluorochrome conjugated cell surface marker antibodies procured from BD Biosciences, USA are as follows, Mouse anti- human CD3 BV450 (clone# UCHT1, Cat#560365), Mouse anti-human CD4 PE-Cy 7 (clone# SK3, Cat# 348789), Mouse anti- human CD8 APC-H7 (clone# SK1, Cat# 560179), Mouse anti- human CD19 PE (clone# SJ25C1, Cat# 340364) and Mouse anti- human CD27 APC (clone# MT271, Cat# 558664). Mouse anti- human CD45RO PerCp Cy5.5 (clone# UCHL1, Cat# 130-113-552) was procured from MiltenyiBiotec, Germany). Cells were again washed twice with PBS and resuspended in 500µl
FACS buffer (BD Biosciences). All samples were acquired using BD FACSVerse (BD Biosciences, CA, USA) and data was analyzed using FlowJo software (Tree Star). To assess the T cell memory responses, gating was done from $CD3^+$T cell population and to $CD4^+$ T cell population and to assess the B cell memory response, gating was done on $CD3^-$ lymphocyte population. Additionally, on Day 3, 50µl cell culture supernatant was tested to determine Th1/Th2/Th17 cytokine profile using human CBA kit (Cytokine Bead Array Kit, Cat# 560484, BD Biosciences, USA). Further, Cell culture supernatant collected on Day 6 was also tested for secreted IgG antibodies by ELISA.

vii. SARS-CoV2 spike (S1) Antibody (IgG1/IgG4) Isotyping:

Th1-dependent IgG1 vs. Th2 -dependent IgG4 antibody subclasses were determined by ELISA from sera collected from all vaccinated groups as described earlier. Briefly, 96 well microtiter plates were coated with spike (S1) protein (Cat: SYNG-PRB026913, Make: Syngene), at a concentration of 1µg/ml, in PBS pH 7.4) and blocked with 1% BSA in PBS, pH 7.4. Two fold serially diluted (1:50 to 1:204800) individual sera were added and incubated for 2hrs at 37 C followed by the addition of mouse anti-human IgG1 (Cat No: 409904, Make: Biolegend) or IgG4 (Cat No: 411202, Make: Biolegend) antibodies at a concentration of 25ng/well. After incubation of the plate for 1hr at RT, wells were again washed, and added Anti Mouse IgG HRP Conjugate (Cat No: A4416, Make: Sigma Aldrich) at a dilution of 1:2500. Later, 3,3′,5,5′-tetramethylbenzidine (TMB) solution
(Cat No: AR1002, make: deNovo Biolabs) was added as a substrate to develop color. Absorbance was read at 450nm. Cut off was determined as 1:50 dilution, by calculating threshold (Mean+3SD) of absorbance obtained at remaining all dilutions of known negative control (unvaccinated and uninfected sera). Th1:Th2 index was calculated by taking ratios of end point antibody titer (sera dilution at which absorbance was above the cut off) of IgG1 & IgG4. Th1/Th2 Immunophenotyping (conducted at Indoor Biotechnologies, Bangalore):

viii. Luminex Based Multiplex Assay

PBMCs from the study subject were thawed as per the SOP and washed with RPMI containing 10% FBS twice. Subsequently cells were washed twice with AIM-V Media with 10% Human AB Serum and resuspended at a density of $6x\ 10^6$ cells per ml. 300,000 cells (50µl) were aliquoted to individual wells in a U bottom tissue culture plate (Nunclon Delta-Treated cat no: 163320) and added with 50ul of plain media (for unstimulated condition) or 50µl of COVID peptide cocktail (peptides from S,N & M protein at 2ug/ml(for each peptide))(stimulated condition) or anti-CD3 antibody (Positive Control). Peptides and anti-CD3 were diluted in AIM-V media with 10% AB serum. Each condition was performed in duplicates. The cells were incubated in CO_2 incubator at 37°C for 48 hours.

At the end of 48 hours of incubation the culture supernatant from the wells were collected and analyzed for cytokine levels using a Bio-plex Pro Human Cytokine panel (BIORAD, cat no: M5000031YV). Briefly, array of beads specific for the analytes (via the capture antibody) were either incubated with culture supernatant or standards. After washing the beads, biotinylated detection antibody was added and after washing the bead were incubated with Streptavidin PE. After washing the beads were acquired in a Bio plex 200 (BIORAD) machine. The data was analyzed using the Bioplex Manager software 6.1 **ix.** Cytokine Bead Array based Multiplex Assay

PBMCs collected on Day 56 of Phase II & Day 104 of Phase I from vaccinated individuals were stimulated with whole virion inactivated SARS CoV-2 antigen (1.2 g/ml) for 72hrs. Cell culture supernatants were collected and used for the simultaneous detection of multiple cytokines using Human Th1/Th2/Th17 BD CBA Kit (BD Bioscience, San Jose, CA, USA). Supernatant samples were processed as per the manufacturer's instructions. Briefly, the kit was used for the simultaneous detection of human IL-2, IL-4, IL-6, IFN-γ, TNF, IL-17A, and IL-

10 cytokines in a single sample. For each sample, 50 µL of the mixed captured beads, 50 µL of the unknown serum sample or standard dilutions, and 50 µL of phycoerythrin (PE) detection reagent were added consecutively to each assay tube and incubated for 3 h at room temperature in the dark. The samples were washed with 1 mL of wash buffer for 5 min and centrifuged. The bead pellet was resuspended in 300 µL buffer after discarding the supernatant. Samples were measured on the BD FACS Verso and analyzed by FCAP Array Software (BD Bioscience). Unstimulated cells or PBMCs collected on Day 0 were maintained as a negative control. PBMCs stimulated with PMA & ionomycin were maintained as a positive control.

x. Database Handling/Procedures and Data Management Plan

The database used for this study is a fully validated, FDA 21 CFR Part 11 compliant system, proprietary, SAS (software as a Service) based clinical data management system. Error rates for clinical database are controlled and do not exceed 0.5% as an industry-wide standard. For critical data zero errors based on a 100% review of data are obtained. This is controlled and documented by database audits against the study CRFs. Based on Data Management Plan (DMP) document a road map to handle the data under projected circumstances and describes the CDM activities was followed in the trial. The DMP describes the annotations, database design, data entry and data tracking guidelines, quality control measures, SAE reconciliation guidelines, discrepancy management, data transfer/extraction, and database locking guidelines. Along with the DMP, a Data Validation Plan (DVP) containing all edit-checks was performed and the calculations for derived variables are also prepared. The edit check programs in the DVP help in cleaning up the data by identifying the discrepancies.

xi. Definition of Reactogenicity and Safety.

Reactogenicity refers to a subset of reactions that occur soon after vaccination, and are a physical manifestation of the inflammatory response to vaccination. In clinical trials, information on expected signs and symptoms after vaccination is actively sought (or 'solicited'). These symptoms may include pain, redness, swelling or induration for injected vaccines, and systemic symptoms, such as fever, myalgia, headache, or rash. The broader term 'safety' profile refers to all adverse events (AEs) that could potentially be caused/ triggered or worsened at any time after vaccination, and includes AEs, such as anaphylactic reactions, diseases diagnosed after vaccination and autoimmune events. (Reference: Hervé C, Laupèze B, Del Giudice G, Didierlaurent AM, Tavares Da Silva F. The how's and what's of vaccine reactogenicity. NPJ Vaccines. 2019;4:39.)

Table S1: Ethic Committees from All Participating Trial Sites with Reference Numbers:

Site Name	Reference Number
Nizam's Institute of Medical Science, Hyderabad, Telangana	ECR/303/INST/AP/2013/RR-19
All India Institute of Medical Science, New Delhi	ECR/547/INST/DL/2014/RR-17
PGIMS, Rohtak, Haryana	ECR/293/Inst/HR/2013/RR-19
All India Institute of Medical Science, Patna	ECR/1387/INST/BR/2020
Redkar Hospital & Research Centre, Goa	ECR/902/INST/GA/2018
Jeevan Rekha Hospital, Belgaum, Karnataka	ECR/1242/INST/KA/2019
Gillukar Multispecialty Hospital, Nagpur	ECR/1374/INST/MH/2020
Prakhar Hospital, Kanpur, Utter Pradesh	ECR/1017/INST/UP/2017
SRM Medical College Hospital & Research Centre, Chennai Tamil Nadu	ECR/431/INST/TL/2013/RR-19

Table S2: SARS-CoV-2 Neutralising Antibody Responses and Cell-mediated responses.

Parameters		3 µg with Algel –IMDG (n=190)	6 µg with Algel-IMDG (n=190)
$PRNT_{50}$ GMT (95% CI)	**Day 0**	0.11 (0.10, 0.13)	0.10 (0.09, 0.11)
	Day 28	1.23 (0.78, 1.94)	1.54 (0.99, 2.4)
	Day 42	78.4 (54.8, 112.0)	161.8 (126.2, 207.4)
	Day 56	100.9 (74.1, 137.4)	197.0 (155.6, 249.4)
$PRNT_{50}$ SCR (95% CI)	**Day 28**	39.3 (32.2, 46.8)	47.5 (39.9, 55.1)
	Day 42	88.5 (83.0, 92.8)	97.2 (93.5, 99.1)
	Day 56	92.9 (88.2, 96.2)	98.3 (95.1,99.7)
MNT_{50} GMT (95% CI)	**Day 0**	6.3 (6.02, 6.68)	6.0 (5.8, 6.1)
	Day 28	12.6 (10.8, 14.7)	12.0 (10.2, 14.0)
	Day 42	78.5 (64.6, 95.2)	134.8 (144.4, 158.8)
	Day 56	92.5 (77.7, 110.2)	160.1 (135.8, 188.8)
MNT_{50} SCR (95% CI)	**Day 28**	23.5 (17.6, 30.3)	21.5 (15.7, 28.3)
	Day 42	84.7 (78.7, 89.6)	96.6 (92.8, 98.8)
	Day 56	88.0 (82.4, 92.3)	96.6 (92.6, 98.5)
SARS-CoV-2 Cell mediated Responses (on Day 42) Mean (95% CI) (pg/mL)	**IFN – gamma**	1167.2 (445.9, 1888)	1082.5 (110.9, 2054)
	IFN-Alpha	4577.1 (4015, 5139)	3612.7 (2918, 4307)
	IL-2	42.13 (31.0, 53.2)	28.1 (22.9, 33.3)
	IL-5	33.4 (29.2, 37.5)	30.9 (27.2, 34.6)
	IL-10	27.3 (21.5, 33.1)	72.2 (53.9, 90.5)
	IL-13	20.1 (14.6, 25.6)	16.3 (9.4, 23.1)
Th1: Th2 Ratio	**(IFN- gamma +TNFAlpha+ IL-2) / (IL-5 + IL-13)**	59.2 (48.5, 69.7)	42.5 (28.6, 56.3)

Shown are neutralising antibody and cell-mediated immunity results at baseline (day 0), 4 weeks after the first vaccination (day 28), 2 weeks after the second vaccination (day 42), and 4 weeks after the second vaccination (day 56) for the 3 µg (n=190) and 6 µg (n=190) with Algel-IMDG groups. Shown are the cytokine levels in 2day supernatants for 58 participants (n=29 in each the 3 µg- and 6 µg with Algel-IMDG groups) with proliferative responses to BBV152 vaccination whose PBMC were evaluated after stimulation with SARS-CoV-2 peptides.

Table S3: PRNT50 Neutralising antibody titres across age groups and gender on day 56 (four weeks after the second dose).

Parameter	3 µg with Algel-IMDG			6 µg wit Algel-IMDG		
Age Group	**n**	**SCR (95%CI)**	**Median (Q1, Q3)**	**N**	**SCR (95%CI)**	**Median (Q1, Q3)**
≥12-<18	10	100% (69.2,100)	231.8 (171.0,1036.5)	4	100% (39.8,100)	224.5 (76.6,840.7)
≥18-<55	166	92.2% (87.0,95.8)	137.7 (63.9,273.4)	164	98.17% (94.8,99.6)	182.4 (83.9,423.7)
≥55-≤65	7	100% (59,100)	70.7 (21.6,186.8)	9	100% (66.4,100)	199.5 (72.3,344.7)
Gender	**n**	**SCR (95%CI)**	**GMT (95%CI)**	**n**	**SCR (95%CI)**	**GMT (95%CI)**
Male	135	91.1% (85.0 95.3)	89.1 (60.3, 131.8)	137	97.8% (93.7,99.6)	211.3 (158.3, 282.2)
Female	48	97.9% (88.9,100.0)	143 (93.5, 218.8)	40	100% (91.2,100)	154.8 (109.9, 218)

Table S4: Phase 1 Long term Follow-up immunogenicity analysis.

Total Parameters		3 µg with Algel –IMDG (n=100)	6 µg with Algel-IMDG (n=100)	6 µg with Algel (n=100)	Algel alone (n=75)
MNT_{50} GMT (95% CI)	**Day 0**	6.22 (5.9,6.5)	6.02 (5.8,6.2)	5.95 (5.8,6.1)	6.09 (5.1,6.4)
	Day 28	60.33 (48.5, 75.03)	65.96 (53.2,81.8)	48.37 (37.9,61.7)	7.20 (6.38,8.1)
	Day 42	45.96 (36.1, 58.5)	81.95 (64.6, 103.9)	64.93 (53.7,78.5)	11.79 (9.4,14.8)
	Day 104	39.96 (32.0,49.9)	69.52 (53.7,90.0)	53.34 (40.1,71.0)	20.67 (14.5,29.5)
MNT_{50} SCR (95% CI)	**Day 28**	87.88 (81.5,94.3)	91.92 (86.6,97.3)	82.80 (75.1,90.5)	8.22 (1.9,14.5)
	Day 42	74.23 (64.2,82.3)	88.42 (80.94.1)	91.21 (83.4,96.1)	18.13 (10.129.3)
	Day 104	73.47 (63.6,81.9)	81.05 (71.4,88.1)	73.12 (62.9.81.8)	32.88 (22.3,44.9)

Shown are geometric mean titers of the wild-type SARS-CoV-2 microneutralisation assay (MNT_{50}) at baseline (day 0), 2 weeks after the second vaccination (day 28), 4 weeks after the second vaccination (day 42), and 3 months after the second vaccination (day 104) for the 3 µg and 6 µg with Algel-IMDG groups, the 6 µg with Algel group, and the Algel-only control arm (from the immunogenicity cohort). Seroconversion rates (SCR) were defined by the proportion of titers achieving ≥4-fold above baseline.

Table S5: Secreted antibodies upon PBMC stimulation from samples collected on day 104 (three months after the second dose) in the phase 1 trial.

Mean	3 µg with Algel –IMDG	6 µg with Algel-IMDG	6 µg with Algel	Algel alone
	12.63	**16.60**	**19.73**	**2.33**

Values represented in Mean±SD indicates spike specific secreted IgG titers measured as end point antibody dilution by ELISA from cell culture supernatant, after stimulation of PBMCs with antigen for 6days (*Ex-vivo*). The value for pre-vaccination samples were 0.

Table S6: Further effector function of activated and differentiated T cells from PBMCs from the Phase 1 study (at day 104, three months after the second dose).

	3µg Ag+ Algel-IMDG (n=10)	6µg Ag+ Algel-IMDG (n=10)	6µg Ag+ Algel (n=10)	Algel Alone (n=6)	Pre-vaccination (n=5)
IL-17A	4.39±4.29	3.40±3.41	14.70±28.75	9.58±8.79	5.23±2.97
IFNg	82±172.8	124.69±224.73	13.03±16.50	26.77±60.27	0.01±0.02
TNF-alpha	3.99±4.93	3.25±5.63	1.30±0.58	3.37±6.54	0.00±0.00
IL-2	30.55±46.83	55.84±69.15	17.05±9.40	6.14±8.50	0.29±0.36
IL-4	0.2±0.29	0.35±0.48	0.13±0.13	0.08±0.16	0.00±0.00
IL-10	37.87±60.28	22.47±41.76	6.35±6.76	6.23±8.18	1.22±1.06
IL-6	297.15±410.52	464.24±470.51	218.54±327.33	544.56±1057.95	27.61±30.19

Values shown in Mean±SD represents cytokines levels measured in pg/ml by CBA method from culture supernatants, obtained after stimulation of PBMCs with antigen for 6days (*Ex-vivo*). Release of Th1 biased cytokines (IFNγ, TNF-α and IL-2) as a function of effector cells of $CD4^+$ T cells, which further supports towards T cell dependent memory response. No or negligible levels of Th2 cytokines (IL-4) and IL-17Acytokine levels were observed, whereas, IL-6 cytokine levels were observed in 3µg and 6µg with Algel-IMDG formulation predicted to be due to activation of both T & B cells.

Table S7: Number of unsolicited, non-serious, adverse events classified by MedDRA® System Organ Class, severity, and investigator-assigned relationship to study vaccine/control.

MedDRA SOC	Dose Group	Adverse Events	Severity			Relationship to IP	
			Mild N (%)	Moderate N (%)	Severe N (%)	Not Related	Related
Gastrointestinal Disorders	3µg with Algel-IMDG	Mouth Ulcer (1)	1(0.53%)	-	-	1(0.53%)	-
	6µg with Algel-IMDG	Gastric Problem (1)	1(0.53%)	-	-	1(0.53%)	-
General Disorders and administrative site conditions	3µg with Algel-IMDG	Weakness (2)	2(1.05%)	-	-	2(1.05%)	-
		Malaise (1)	1(0.53%)	-	-	1(0.53%)	
	6µg with Algel-IMDG	Fever(1)	-	1(0.53%)	-	-	1(0.53%)
		Body ache (1)	-	1(0.53%)	-	-	1(0.53%)
Nervous system disorders	3µg with Algel-IMDG	Headache (1)	1(0.53%)	-	-	1(0.53%)	-
	6µg with Algel-IMDG	Dizziness (1)	1(0.53%)	-	-	-	1(0.53%)
		Headache (1)	1(0.53%)	-	-	1(0.53%)	-
Respiratory, thoracic and mediastinal disorders	3µg with Algel-IMDG	Cold (3)	2(1.05%)	1(0.53%)	-	2(1.05%)	1(0.53%)
		Breathlessness (2)	2(1.05%)	-	-	1(0.53%)	1(0.53%)
		Cough (2)	2(1.05%)	-	-	1(0.53%)	1(0.53%)
		Heaviness in chest (1)	1(0.53%)	-	-	1(0.53%)	-
		Running nose/Rhinnorhea (2)	2(1.05%)	-	-	2(1.05%)	-
		Sore throat (1)	1(0.53%)	-	-	1(0.53%)	
		Throat pain (1)	1(0.53%)	-	-	-	1(0.53%)
	6µg with Algel-IMDG	Cold (2)	2(1.05%)	-	-	2(1.05%)	-
		Running nose (1)	-	1(0.53%)	-	-	1(0.53%)
		Sneezing (1)	1(0.53%)	-	-	1(0.53%)	-
Skin and subcutaneous tissue disorders	3µg with Algel-IMDG	Rashes (1)	-	1(0.53%)	-	-	1(0.53%)
	6µg with Algel-IMDG	-	-	-	-	-	-

GERD: Gastroesophageal reflux disease, COLD: the lowest level term (LLT) in the MedDRA for the preferred term (PT) Nasopharyngitis is cold

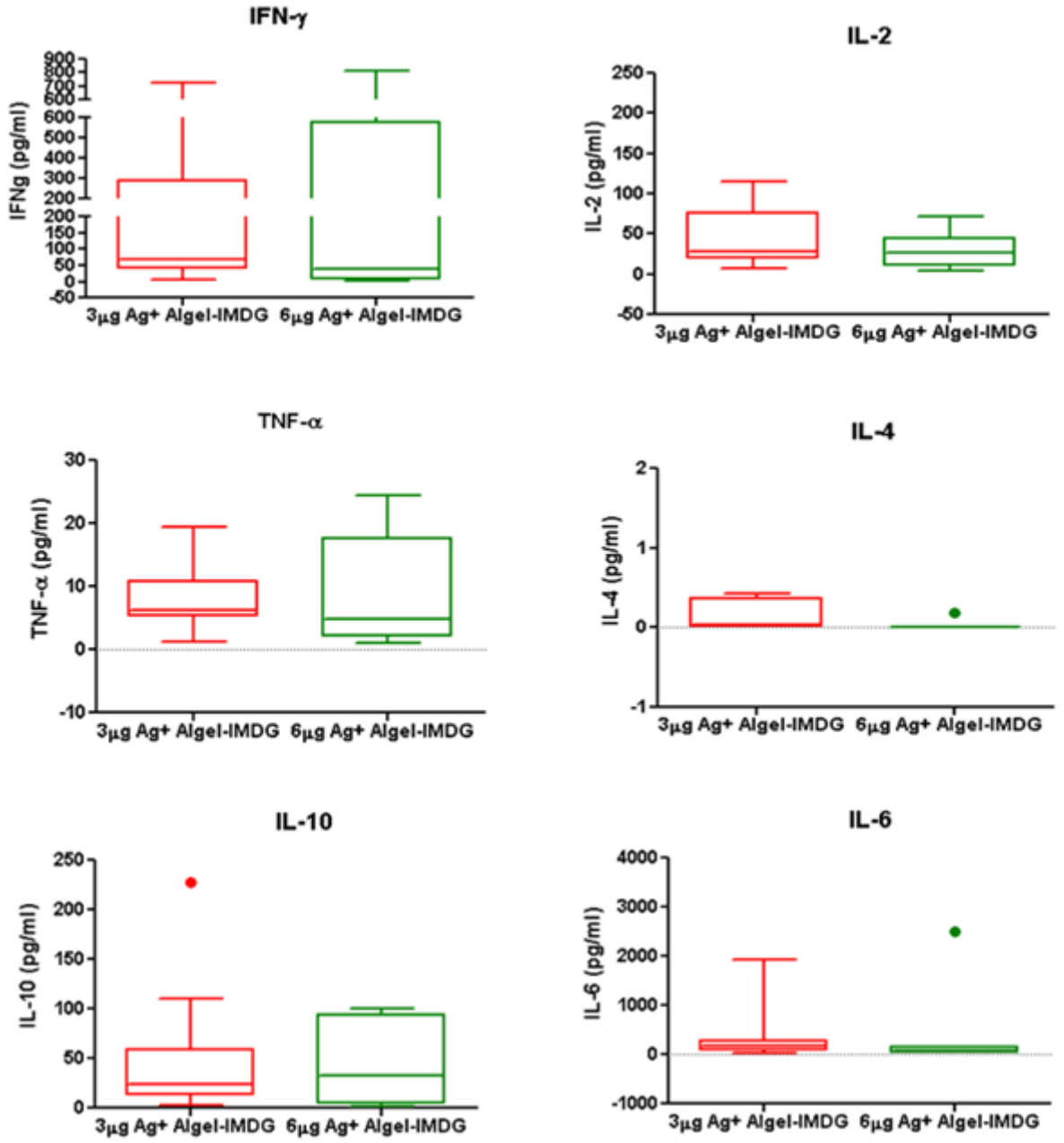
Table S8: Grading Scales for Local and Systemic Adverse Events.

Event Name	None=1	Mild =2	Moderate=3	Severe=4	Potentially life threatening=5
Local Adverse Events					
Pain at injection site	Absent	Does not interfere with activity	Interferes with activity or repeated use of non-narcotic pain reliever >24hrs	prevents daily activity or repeated use of narcotic pain reliever	Emergency room (ER) visit or hospitalization
Tenderness/ Soreness	Absent	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Emergency room (ER) visit or hospitalization
Redness/ Erythema	Absent	2.5-5cm	5.1-10cm	>10 cm	Necrosis or exfoliative dermatitis
Swelling/ Induration	Absent	2.5-5cm and does not interfere with daily activity	5.1-10cm or interferes with daily activity	>10 cm prevents daily activity	Necrosis
Pruritus associated with injection	Absence of any Pruritus	itching localized to injection site and relieved spontaneously or with<48 hours treatment	Itching beyond injection site not generalized or localized itching requiring >48 hours treatment	Itching causing inability to perform usual social & functional activities	NA
Any other Local AE's	Absent	Does not interfere with daily activity	interferes with daily activity,	prevents daily activity	Emergency room (ER) visit or hospitalization
Systemic Adverse Events					
Pain	Absent	Does not interfere with activity	Repeated use of nonnarcotic pain reliever > 24 hours or interferes with daily activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Fever	<38^{0}C (<100.4^0 F)	38.0-38.4^{0}C(100.4-^{0}F) 101.1	38.5-38.9^0 C (101.2-102.0^{0}F)	39.0-40^{0}C C(102.1104^{0}F)	> 40^{0}C (>104^{0}F)
Nausea/ Vomiting	Absent	No interference with daily activity or 1-2 episodes/24 hours	Some interference with daily activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	Emergency room (ER) visit or hospitalization for hypotensive shock
Headache	Absent	No interference with daily activity	some interference with daily activity or repeated use of nonnarcotic pain reliever	Significant, prevents daily activity or repeated use of narcotic pain reliever	Emergency room (ER) visit or hospitalization
Fatigue	Absent	No interference with daily activity	some interference with daily activity	Significant, prevents daily activity	Emergency room (ER) visit or hospitalization
Myalgia	Absent	No interference with daily activity	some interference with daily activity	Significant, prevents daily activity	Emergency room (ER) visit or hospitalization
Acute Allergic Reaction	Absent	No interference with daily activity	some interference with daily activity	Prevents daily activity	Emergency room (ER) visit or hospitalization

Rash	Rash Absent	rashes covering <10%BSA with or without symptoms (pruritus, burning, tightness)	rashes covering 10-30 %BSA (Body Surface Area) with or without symptoms (pruritus, burning, tightness), interferes with daily activity	rashes covering >30 %BSA with or without symptoms (pruritus, burning, tightness), prevents with daily activity	NA
Joint pain	Absent	Does not interfere with daily activity	Repeated use of nonnarcotic pain reliever > 24 hours or interferes with daily activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Any other Systemic AE's	Absent	Does not interfere with daily activity	interferes with daily activity	prevents daily activity	Emergency room (ER) visit or hospitalization

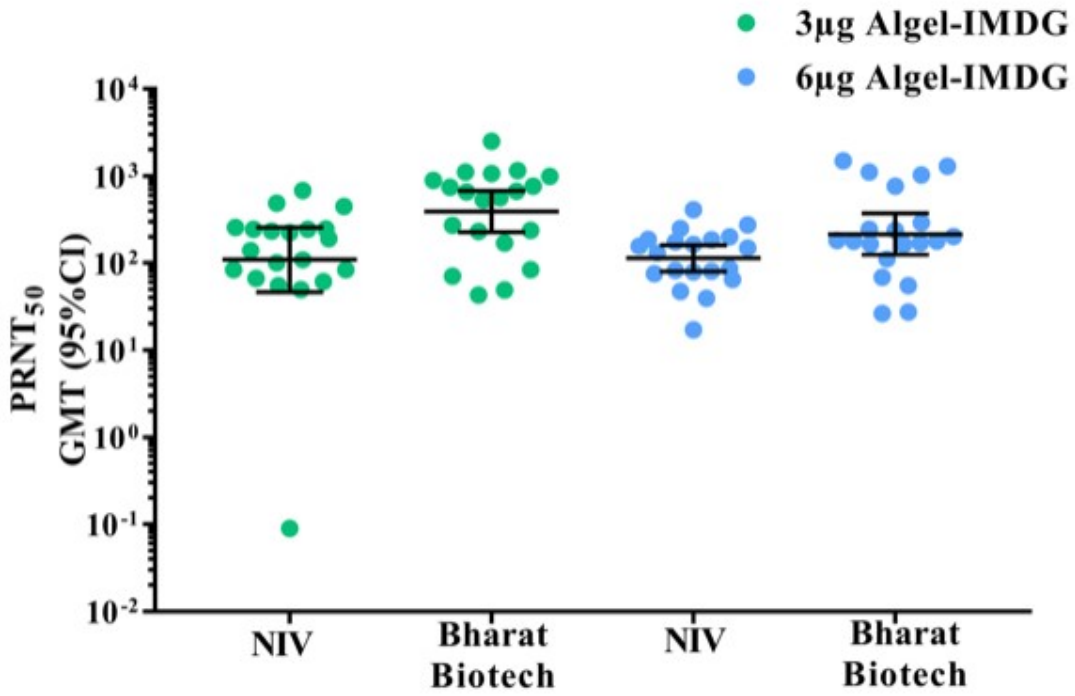
Abbreviation: NA, not available. The grading scale for most AEs was based on the FDA guidance document for Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. The causality assessment was done based on the investigator's taking the following factors into consideration: Temporal (time-based) relationship between the event and administration of the study vaccine. Possible alternative etiology for the AE, such as concurrent illness or natural history of underlying diseases, or concomitant medications. Adverse events of similar nature having previously been observed with the study vaccine. The adverse event having often been reported in literature for similar types of treatments.

Figure S1: Cytokine profile measured from culture supernatant upon stimulation of PBMCs with antigen



Box plot represents mean with 95% CI values of secreted cytokine levels measured in the cell culture supernatant after 72hrs of whole virion inactivated antigen stimulation. PBMCs collected on Day 56 were used to measure Th1/Th2/Th17 cytokines by CBA method. Graphs and statistics done using Tukey test.

Figure S2: comparison between PRNT50 assays at the two laboratories (Bharat Biotech and National Institute of Virology).



FigureS3: Solicited Adverse Events After Two Doses in the Safety Set

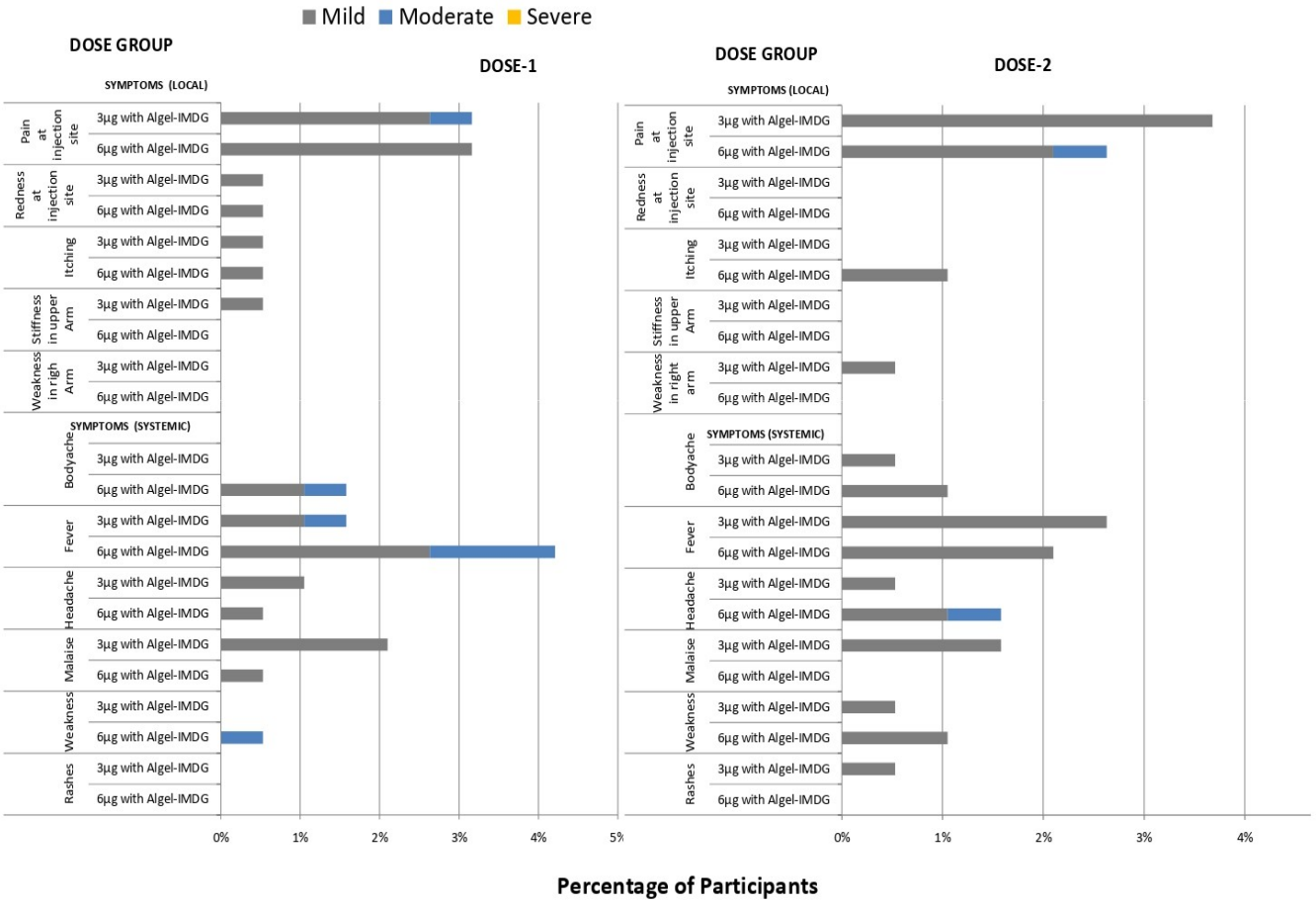
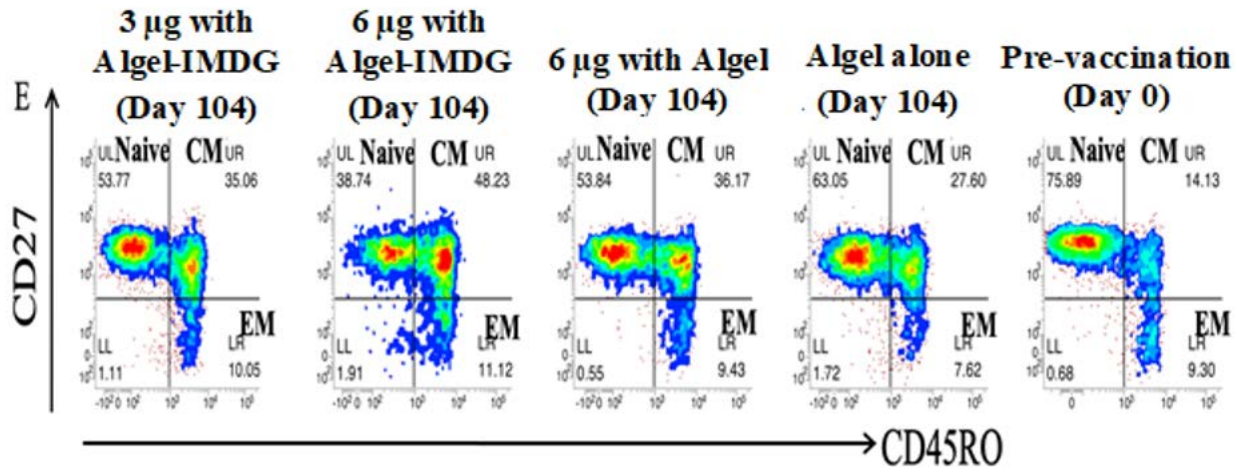


Figure S4: Frequency of effector memory T cells



Footnote: Gating was done on $CD4^+$ T cells illustrating the frequencies of effector memory ($_{EM}$) T_{EM}, CD45RO- $CD27^+$, central memory ($_{CM}$) T_{CM}, $CD45RO^+CD27$-, and T_{EM}, $CD45RO^+CD27^+$ $CD4^+$ T cells. The $_{EM}$ in the 6 µg with Algel-IMDG group was the most pronounced