Newcastle Disease Virus Vector-Based SARS-CoV-2 Vaccine Candidate AVX/COVID-12 Activates T Cells and Is Recognized by Antibodies from COVID-19 Patients and Vaccinated

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36 **Abstract**

37 Several effective vaccines for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have

38 been developed and implemented in the population. However, the current production capacity falls

39 short of meeting global demand. Therefore, it is crucial to further develop novel vaccine platforms

40 that can bridge the distribution gap. AVX/COVID-12 is a vector-based vaccine that utilizes the

41 Newcastle Disease virus (NDV) to present the SARS-CoV-2 spike protein to the immune system.

42 This study analyses the antigenicity of the vaccine candidate by examining antibody binding and T-

43 cell activation in individuals infected with SARS-CoV-2 or variants of concern (VOCs), as well as in

44 healthy volunteers who received coronavirus disease 2019 (COVID-19) vaccinations. Our findings

45 indicate that the vaccine effectively binds antibodies and activates T-cells in individuals who

46 received 2 or 3 doses of BNT162b2 or AZ/ChAdOx-1-S vaccines. Furthermore, the stimulation of T-

47 cells from patients and vaccine recipients with AVX/COVID-12 resulted in their proliferation and

48 secretion of interferon-gamma (IFN-γ) in both CD4+ and CD8+ T-cells. In conclusion, the

49 AVX/COVID-12 vectored vaccine candidate demonstrates the ability to stimulate robust cellular

50 responses and is recognized by antibodies primed by the spike protein present in SARS-CoV-2

51 viruses that infected patients, as well as in the mRNA BNT162b2 and AZ/ChAdOx-1-S vaccines.

52 These results support the inclusion of the AVX/COVID-12 vaccine as a booster in vaccination

53 programs aimed at addressing COVID-19 caused by SARS-CoV-2 and its VOCs.

54 **Introduction**

55 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its variants of concern have

56 caused billions of infections and millions of deaths worldwide since 2020. Despite the incredible

57 speed of coronavirus disease 2019 (COVID-19) vaccine development since 2020, there have been

58 more than 7 million deaths from COVID-19, with an estimated excess mortality of 28.4 million

59 deaths (1,2)**.** The unequal distribution of vaccines globally, especially impacting low- and middle-

60 income countries (LMICs), underscores the urgency for further advancements in new vaccine

61 platforms to address the disparity in vaccine access (3)**.**

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62   The AVX/COVID-12 vaccine candidate is a Newcastle disease virus (NDV) LaSota vector-based
63   vaccine that expresses the stabilized form of the SARS-CoV-2 spike protein. The stabilization is
64   achieved by introducing six prolines (HexaPro-S), ensuring the protein remains in its closed
65   conformation (4). This vaccine has demonstrated safety, immunogenicity, and protective efficacy in
66   preclinical models (5,6), and has the advantage of being produced in embryonated egg industrial
67   facilities similarly to influenza vaccine production which is cheap and widely available (7). In a
68   phase I clinical trial, the AVX/COVID-12 vaccine demonstrated safety, good tolerability, and
69   immunogenicity in volunteers (8), supporting further clinical development of the vaccine. However,
70   high rates of infections in the population and the availability of approved vaccines in Mexico made
71   the development of a phase II placebo-controlled clinical trial unethical. To provide evidence
72   supporting the testing of this vaccine as a booster dose, we analysed the capacity of the
73   AVX/COVID-12 vaccine to bind antibodies and stimulate T-cell responses from individuals
74   previously infected or vaccinated with approved vaccines.

75   **Material and methods**

76   **Study groups**

77   Samples were collected from 41 volunteers who read and signed the informed consent for this study.
78   The subjects were categorized into five groups: patients with COVID-19 in the acute phase of the
79   disease (7 days after hospital admission; n=12) and a subgroup infected with the Omicron variant
80   (Omicron peak, December 2021; n=10) who reported no previous infection or vaccination, patients
81   who had recovered from COVID-19 during the first peak of the pandemic (June-December 2020;
82   n=6), healthy volunteers vaccinated with two doses of Pfizer-BioNTech BNT162b2 (4 to 8 months
83   after the second dose; n=6) and volunteers with two doses of Pfizer-BioNTech BNT162b2 boosted
84   with AstraZeneca AZ/ChAdOx-1-S (4 to 8 months after the second dose; n=7).

85   **Serum collection**

86   Venous blood samples were obtained from participants, and these samples were collected using two
87   ethylenediaminetetraacetic acid (EDTA) tubes and one red tube (BD Vacutainer tubes, Franklin
88   Lakes, NJ, USA) following standard phlebotomy procedures. After collection, all blood samples and
89   their derivatives were processed in a biosafety level (BSL)-2 laboratory, with the use of appropriate
90   personal protective equipment and safety precautions. Serum isolation involved centrifuging venous
91   blood (collected in red tubes) at 2,000 x g for 10 minutes to separate the serum. The resulting serum

92   was carefully extracted from the upper portion of the tube, aliquoted, and subsequently stored at -20

93   °C until needed.

Peripheral blood mononuclear cell (PBMC) isolation

95   PBMCs were isolated from venous blood collected in EDTA tubes (BD vacutainer tubes, Franklin

96   Lakes, NJ, USA). Within 4 hours (h) of collection, PBMC isolation was conducted by density-

97   gradient sedimentation of whole blood diluted at a 1:2 ratio in phosphate-buffered saline (PBS) at

98   room-temperature. The diluted blood was layered over an appropriate volume of room-temperature

99   Lymphoprep (Serumwerk Bernburg AG, DEU; cat. 07851). Then, the PBMCs were recovered,

100 cryopreserved in a medium consisting of 10% dimethyl sulfoxide (DMSO; Sigma Aldrich, St. Louis,

101 MO, USA) and 90% heat-inactivated fetal bovine serum (FBS; GIBCO, California, USA; cat.

102 11560636), and stored at -80 °C until use.

Vaccine manufacturing

104 The AVX/COVID-12 vaccine was cultivated in 10-day-old specific pathogen-free (SPF) chicken

105 embryos through inoculation into the allantoic cavity, using $10^{3.3}$ 50% egg infectious dose

106 (EID_{50})/0.1 mL of the production seed. The embryos were incubated for 72 h at 37 °C with 60-

107 70% humidity. After incubation, the embryos were refrigerated for a minimum of 12 h, and the

108 allantoic fluid (AF) was then aseptically harvested. The AF underwent clarification through filters,

109 was concentrated by a factor of 10X using 300 kDa cassettes, and was subsequently diluted in 20

110 volumes of PBS. The resulting AF was frozen and stored at −70°C until needed. The experimental

111 AVX/COVID-12 vaccine, along with NDV-V (empty vector), was provided frozen in 2 mL vials.

112 The vaccine was produced in Avimex's good manufacturing practice facilities in Mexico City.

Antibody analysis by enzyme-linked immunosorbent assay (ELISA)

114 The analysis of specific anti-spike IgG antibodies was conducted using ELISA. Each plate was

115 coated with NDV-V, AVX/COVID-12, or the ancestral receptor binding domain (RBD) of SARS-

116 CoV-2 at a concentration of 10 µg/mL. Serum samples, prediluted at 1:40, were transferred to the

117 plate (200 µL), and then 1:2 serial dilutions were performed. The plates were incubated at 37 °C for

118 60 minutes and subsequently washed with tris-buffered saline (TBS)-Tween 20 buffer. A 1:4000

119 dilution of anti-human IgG coupled to horseradish peroxidase (HRP; MyBioSource's, San Diego,

120 USA; cat. MBS440121) was added to the plates, followed by an incubation at 37 °C for 60 minutes.

121 To stop the reaction, 2.5 N sulfuric acid was added, and the optical density (OD) was read at 450 nm

122 using the Epoch Microplate Spectrophotometer (BioTek Laboratories, Seattle, Washington, USA)

123 within 10 minutes after adding the stop solution. The antibody titre was determined as three times the

124 value of the negative controls, and titres were represented as -Log2x40.

T-cell proliferation assay

126 PBMCs were washed twice with 1X PBS to eliminate the cryopreservation medium, and the cell

127 button was resuspended and adjusted to 5 x 10^6 cells/mL. Subsequently, proliferation staining was

128 carried out using the CellTrace Violet reagent (Invitrogen, Massachusetts, USA; cat. C34557) at 37

129 °C for 20 min. Every 5 min, the cells were vortexed to ensure uniform staining. Following staining,

130 the cells were washed with 1X PBS and then with Roswell Park Memorial Institute (RPMI) 1640

131 culture medium (GIBCO, Thermo Fisher, Massachusetts, USA) without supplementation. After this

132 incubation period, the stained cells were harvested, washed with fresh medium, counted, and

133 cultivated in 96-well plates (5 x 10^5 cells/well). Subsequently, they were stimulated separately with

134 10 µg of NDV-V, AVX/COVID-12 or 30 nM of a mixture of 10-15 amino acid long peptides derived

135 from the SARS-CoV-2 spike protein (PepTivator, Miltenyi Biotec, North Rhine-Westphalia,

136 Germany). In parallel, stained and unstimulated cells were cultivated at the same concentration as the

137 controls. After this step, the cells were cultured in RPMI 1640 with 10% FBS and incubated for 72 h

138 at 37 °C. The cells were harvested and surface-stained with anti-CD3 mAb APC (BioLegend, San

139 Diego, California, USA; cat. 344812), anti-CD4 mAb APC-Cy7 (BioLegend, San Diego, California,

140 USA; cat. 317418), and anti-CD8 mAb FITC (BioLegend, San Diego, California, USA; cat. 344704)

141 for PBMCs from critically ill patients hospitalized during the first wave of COVID-19. For PBMCs

142 from the other study groups, cells were stained with anti-CD3 mAb AF647 (BioLegend, San Diego,

143 California, USA; cat. 300322), anti-CD4 mAb APC/Cy7 (BioLegend, San Diego, California, USA;

144 cat. 317418), and anti-CD8 mAb PerCP/Cy5.5 (BioLegend, San Diego, California, USA; cat.

145 300924). The staining mixture was incubated for 15 minutes at room-temperature in the dark,

146 followed by an additional washing step with cell staining buffer. Subsequently, the cells were fixed

147 and permeabilized using Cytofix/Cytoperm for 20 minutes at room-temperature. After centrifugation,

148 Perm/Wash was added. Intracellular staining was then performed using anti-interferon (IFN)-γ mAb

149 PE (BioLegend, San Diego, California, USA; cat. 506507) for critically ill patients hospitalized and

150 anti-IFN-γ mAb FITC (BioLegend, San Diego, California, USA; cat. 502506) for PBMCs from the

151 other study groups in Perm/Wash solution (BD, San Jose, USA) for 30 minutes at room-temperature

152 in the dark. After washing with BD Perm/Wash buffer (BD, San Jose, USA), the cells were

153 resuspended in PBS and kept at 4 °C in the dark until acquisition and analysis. Flow cytometric

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154 analysis was performed using the FACS Canto II flow cytometer (BD Biosciences, New Jersey,
155 USA), and the data were analysed using FlowJo V10 software (Fig. 1S).

Statistical analysis

157 Descriptive statistics were employed to analyse the variables of interest. For discrete or continuous
158 quantitative variables, the mean, median, and standard deviation were determined as appropriate for
159 each case. The choice between parametric or non-parametric statistics was made based on the
160 distribution and variance of the data. Regarding the analysis of antibody titres and cell percentages,
161 calculations were performed for frequencies, means, or distributions. Student's t-test or Mann-
162 Whitney U test was used for two categories, while 2-way ANOVA and Bonferroni post-test or
163 Kruskal-Wallis were employed for the comparison of more than two groups. The results were
164 processed using Excel, the GraphPad Prism analysis program, and STATA v.14 software.

Results

Analysis of antibody response for antigenicity evaluation of the AVX/COVID-12 vaccine

167 To evaluate the antigenicity of AVX/COVID-12, we performed *in vitro* tests with antibodies induced
168 after infection or vaccination against COVID-19. Utilizing ELISA, we assessed the binding of these
169 antibodies to the AVX/COVID-12 vaccine, NDV-V, and recombinant RBD (Fig. 1).

170 We observed specific AVX/COVID-12 IgG antibody titres ranging from 4 to 7 logarithmic units base
171 2 (log2 units) in sera from both acute and recovered SARS-CoV-2 infected patients. Notably, sera
172 from hospitalized patients during the Omicron wave, who had not received prior COVID-19
173 vaccination in Mexico, also exhibited binding to AVX/COVID-12, similar to sera from patients
174 infected with other VOCs. In vaccinated individuals, we observed statistically significant differences
175 with higher titres, ranging from 7 to 9. In contrast, NDV-V, which lacks SARS-CoV-2 spike protein
176 expression, exhibited only marginal binding with a titre of 1. Using RBD as a positive control for
177 coating the plates, we found no statistical differences between antibody titres binding to RBD or the
178 AVX/COVID-12 vaccine. These results suggest that the spike protein expressed in the
179 AVX/COVID-12 vaccine enables the binding of IgG antibodies triggered by the spike protein
180 expressed by the SARS-CoV-2 variant that infected patients, as well as by the BNT162b2 and
181 AZ/ChAdOx-1-S vaccines.

AVX/COVID-12 vaccine elicits T-cell proliferation and IFN-γ production in patients and
COVID-19 vaccinated volunteers

184 Next, we investigated whether the AVX/COVID-12 vaccine activates T-cells in individuals who had

185 COVID-19 or were vaccinated with anti-COVID-19 vaccines. We stimulated PBMCs from patients

186 (Fig. 2) and vaccinated volunteers (Fig. 3) with AVX/COVID-12 or the NDV-V empty vector. As a

187 positive control, we utilized a spike protein peptide cocktail mixture of 10-15 amino acid peptides

188 (PepTivator S) (Fig. 1S).

189 We observed that the AVX/COVID-12 vaccine induced specific proliferation and intracellular IFN-γ

190 production in both critical COVID-19 patients and vaccinated volunteers. In contrast, NDV-V

191 induced a basal response similar to that of non-stimulated cells (Fig. 2A and 3A). The positive

192 control, PepTivator, also triggered strong specific cell proliferation and IFN-γ intracellular

193 production, surpassing AVX/COVID-12 vaccine by 1-2%.

194 The production of intracellular IFN-γ in total $CD3^+$ T cells from patients was higher than in

195 vaccinated volunteers (p = 0.0169 and p = 0.0009). The same trend was observed for $CD4^+$ T-cells (p

196 = 0.0011 and p = 0.0437) and $CD8^+$ T-cells (p = 0.0005 and p = 0.0035). Additionally, we observed

197 an increase in the proliferation response in T-cells from critically ill acute patients compared to those

198 vaccinated with two doses of the BNT162b2 vaccine (Fig. 2A and 3A).

199 With a vaccine booster, one would anticipate a substantial increase in both antibody and cellular

200 responses, potentially ranging from 10 to 25 times greater (9,10). Therefore, we examined the

201 cellular response in PBMCs from volunteers who had received two doses of BNT162b2 and were

202 subsequently boosted with the AZ/ChAdOx-1-S vaccine, stimulated with the AVX/COVID-12

203 vaccine (Fig. 4). We detected specific proliferation and intracellular production of IFN-γ induced by

204 the AVX/COVID-12 vaccine in CD3+, CD4+, and CD8+ T-cells, while the NDV-V empty vector

205 induced basal activation similar to that of non-stimulated cells. Nevertheless, the response in

206 volunteers who received a booster dose with the AZ/ChAdOx-1-S vaccine is comparable to that of

207 volunteers who received only two doses of the BNT162b2 vaccine, with a slight tendency to be lower

208 when compared to the response observed in T-cells from critically ill COVID-19 patients.

209 **Discussion**

210 During the SARS-CoV-2 pandemic, there was a pressing need for accelerated technological

211 advancements in the development of new vaccine platforms. mRNA vaccines and viral vectors

212 emerged as frontrunners, receiving emergency use approvals, and demonstrating their efficacy in

213 alleviating hospital saturation and reducing deaths due to COVID-19. However, to achieve broader

214 vaccination coverage, especially in LMICs, there is a demand for vaccine candidates that are not only

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215 effective but also affordable and easily transportable. The NDV viral vector platform has proven to
216 be a safe and innocuous vector (5,6,11). It is easily developed for use, showing high effectiveness in
217 oncolytic treatment and preclinical models as a live vaccine vector (12,13). This makes it a promising
218 candidate for meeting the vaccine needs in diverse settings.

219 The use of the NDV-HXP-S (AVX/COVID-12-HEXAPRO) vector technology has facilitated the
220 development of the AVX/COVID-12 vaccine candidate, demonstrating effectiveness in eliciting
221 neutralizing antibodies and an IFN-γ^+ T-cell response crucial for combating SARS-CoV-2 infection
222 (5,7).

223 Due to the updated COVID-19 vaccine distribution inequities affecting mainly LMICs, it is crucial to
224 gather evidence on the efficacy of currently available vaccines and vaccine candidates. This should
225 focus on assessing the capacity of the spike protein from the original Wuhan-1 strain to generate
226 immunity against emerging VOCs such as Omicron and its subvariants.

227 If the epitopes constituting the spike protein of the SARS-CoV-2 or those from the first-generation
228 vaccines (BNT162b2 and AZ/ChAdOx-1-S) are efficiently expressed in the NDV vector, we
229 anticipate the development of antibodies targeting similar epitopes. These antibodies would play a
230 crucial role in protecting the host from the development of severe and critical pathology (14–16).

231 One approach to evaluate the capacity of a vaccine candidate in eliciting immune responses in
232 humans involves *ex vivo* tests assessing its antigenicity. This process entails examining the
233 candidate's interaction with pre-existing antibodies developed in patients who have recovered from
234 the targeted pathology or through the recognition and activation of the cellular immune response.

235 We observed the binding of antibodies to the spike protein in comparison to the empty vector NDV-
236 V or the RBD in different study groups during and after infection or post-vaccination with second
237 and third doses. Anti-spike IgG titres showed an increase of 4 to 6 log2 units across all study groups
238 (Fig. 1), indicating similar humoral responses between the two vaccination schemes. In this context,
239 the design of the vaccine protein S in BNT162b2 and AVX/COVID-12 shares the prefusion-
240 stabilized structure (6). However, there are differences in the substitutions made to maintain the
241 structure of the molecule, involving two prolines in BNT162b2 and six prolines in the S2 subunit of
242 AVX/COVID-12 (6,17). This suggests antibodies are targeting conformational epitopes allowing
243 both candidates to potentially share structural epitopes. When comparing the antigenicity against
244 antibodies developed with a third dose of the AZ/ChAdOx-1-S vaccine, where the structure of the
245 protein S resembles the native one (18), we observed similar activation (without significant

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246 differences, p > 0.9) compared to the complete scheme with BNT162b2 alone. Therefore, all three

247 designs appear to induce similar repertoires of antibodies against SARS-CoV-2.

248 Moreover, upon analysing the antibody response in patients during the acute phase and those who

249 have recovered from COVID-19, we observed that recovered patients had 3 log2 units higher anti-

250 spike IgG antibody titres than acute patients (p = 0.1533) (Fig. 1). In contrast, it has previously been

251 reported that there are no significant differences in antibody titres among acute and recovered

252 patients (19). However, differences in the quality of the response are observed, particularly in terms

253 of opsonization, complement activity, and neutralizing capability (20). This is attributed to the

254 significant reduction in the percentage and total numbers of T and B lymphocyte populations during

255 severe acute COVID-19. This reduction reflects a noteworthy decrease in the development of

256 germinal centres in lymph nodes, potentially impairing the affinity and repertoire of antibodies to

257 various epitopes of the total spike protein (21,22). The increase of antibody titres in recovered

258 patients suggests a gradual increase in the number of epitopes recognized by the immune system

259 during the recovery phase from COVID-19.

260 In the design of vaccines or platforms, the evaluation of T-cell activation response has been a key

261 aspect. For instance, in cases like the respiratory syncytial virus in infants or the pandemic influenza

262 H1N1 virus, assays involving lymphoproliferation and IFN-γ secretion have been utilized as

263 indicators of T-cell effector activity, providing valuable insights into the vaccine's effectiveness

264 (23,24). We analysed three pivotal scenarios during the pandemic: the cellular response in COVID-

265 19 patients, emphasizing the effector response; the vaccination schemes, specifically 6 months after

266 the second dose to ensure memory T-cell stimulation in peripheral blood; and 3 months after the third

267 dose, exemplifying restimulation with a heterologous immunization schedule. The analysis revealed

268 that, 72 h after stimulation, proliferation was induced with the vaccine in the case of patients (Fig. 3

269 and Fig. 4). This was expected, as despite a low percentage of T-cells in patients, they exhibited a

270 robust effector response primarily targeting spike and nucleocapsid protein epitopes. Consequently,

271 these T-cells were activated by AVX/COVID-12 and produced IFN-γ. Regarding vaccinated

272 volunteers, notable differences were observed in both cases. Surprisingly, when comparing

273 proliferation and interferon responses, they appeared to be similar between both doses (data not

274 reported). This contradicts expectations, since with a vaccine booster, one would anticipate a

275 substantial increase in both antibody and cellular responses, ranging from 10 to 25 times greater

276 (9,10). However, since we are examining antigenicity, we can only illustrate the epitopes present in

277 the vaccine. Therefore, upon immunization with the candidate, we anticipate the response to escalate.

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278 These observations are consistent with the identification of a substantial number of conserved
279 epitopes within the spike protein, contributing to both humoral and cellular responses across ancestral
280 SARS-CoV-2 and its variants of concern, as well as SARS-CoV, MERS-CoV, and seasonal
281 coronaviruses (25,26). This may even extend to other coronaviruses within the *Orthocoronavirinae*
282 genus (27).

283 Furthermore, considering the inoculation route (potential nasal administration in the case of
284 AVX/COVID-12), it is expected to stimulate the development of both mucosal and systemic
285 responses. These aspects will be further elucidated during the upcoming phases of the clinical trials
286 for the AVX/COVID-12 vaccine candidate.

287 In conclusion, the AVX/COVID-12 vectored vaccine demonstrates the ability to stimulate robust
288 cellular responses and is recognized by antibodies primed by the spike protein present in SARS-CoV-
289 2 viruses that infected patients, as well as in the mRNA BNT162b2 and AZ/ChAdOx-1-S vaccines.
290 These findings endorse the integration of the AVX/COVID-12 vaccine as a booster in vaccination
291 initiatives designed to combat COVID-19 caused by SARS-CoV-2 and its VOCs.

292 **Conflict of Interest**

293 The vaccine candidate used in this study was developed by faculty members at the Icahn School of
294 Medicine at Mount Sinai including P.P., F.K. and A.G.-S. Mount Sinai is seeking to commercialize
295 this vaccine; therefore, the institution and its faculty inventors could benefit financially. The Icahn
296 School of Medicine at Mount Sinai has filed patent applications relating to SARS-849 CoV-2
297 serological assays (USA Provisional Application Numbers: 62/994,252, 63/018,457, 63/020,503 and
298 63/024,436) and NDV-based SARS-CoV-2 vaccines (USA Provisional Application Number:
299 63/251,020) which list F.K. as co-inventor. A.G.-S. and P.P. are a co-inventor in the NDV-based
300 SARS-CoV-2 vaccine patent application. Patent applications were submitted by the Icahn School of
301 Medicine at Mount Sinai. Mount Sinai has spun out a company, Kantaro, to market serological tests
302 for SARS-CoV-2 and another company, CastleVax, to commercialize SARS-CoV-2 vaccines. F.K.,
303 P.P. and A.G.-S. serve on the scientific advisory board of CastleVax and are listed as co-founders of
304 the company. F.K. has consulted for Merck, Seqirus, Curevac, and Pfizer, and is currently consulting
305 for Gritstone, Third Rock Ventures, GSK, and Avimex. The F.K. laboratory has been collaborating
306 with Pfizer on animal models of SARS-CoV-2. The A.G.-S. laboratory has received research support
307 from GSK, Pfizer, Senhwa Biosciences, Kenall Manufacturing, Blade Therapeutics, Avimex,
308 Johnson & Johnson, Dynavax, 7Hills Pharma, Pharmamar, ImmunityBio, Accurius, Nanocomposix,

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309 Hexamer, N-fold LLC, Model Medicines, Atea Pharma, Applied Biological Laboratories and Merck.

310 A.G.-S. has consulting agreements for the following companies involving cash and/or stock: Amovir,

311 Vivaldi Biosciences, Contrafect, 7Hills Pharma, Avimex, Pagoda, Accurius, Esperovax, Farmak,

312 Applied Biological Laboratories, Pharmamar, CureLab Oncology, CureLab Veterinary, Synairgen,

313 Paratus, Pfizer and Prosetta. A.G.-S. has been an invited speaker in meeting events organized by

314 Seqirus, Janssen, Abbott, and AstraZeneca. P.P. has a consulting agreement with Avimex.

315 The live vaccine used in the study was developed by members of Avimex. Avimex filed patent

316 applications with Mount Sinai and CONAHCYT. M.T., D.S.-M., C.L.-M., H.E.C.-C., F.C.-P.,

317 G.P.D.L., and B.L.-D. are named as inventors on at least one of those patent applications. The rest of

318 the participants are employees of their corresponding institutions and declare no competing interests.

319 **Author Contributions**

320 A.T.-F. Data curation, Formal Analysis, Investigation, Visualization, Writing – original draft,

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331 Project administration, Writing – review & editing; D.S.-M. Funding acquisition, Resources, Writing

332 – review & editing; W.S. Formal Analysis, Resources, Writing – review & editing; H.E.C.-C.

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334 Writing – review & editing; F.C.-P. Investigation, Writing – review & editing; P.P. Formal Analysis,

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336 editing; A.G.-S. Formal Analysis, Resources, Writing – review & editing; B.L.-D. Conceptualization,

337 Funding acquisition, Resources, Supervision, Validation, Writing – review & editing; C.L.-M.

338 Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Project

339   administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing –
340   review & editing.

Ethical Responsibilities

342   The protocol was approved by the ethics, research, and biosafety committees of the Mexican Institute
343   of Social Security (IMSS) National Scientific Research and Ethics Commissions under project
344   numbers R-2020-785-095 and R-2021-785-048. Informed consent was obtained from both patients
345   and vaccinated individuals. This project was conducted in accordance with good clinical practice.

Protection of Humans and Animals

347   No animals were used in this protocol, and the protocol was designed to comply with the ethical
348   principles of the Helsinki Declaration, Good Clinical Practices, and the applicable Mexican law.

Data Availability Statement

365   Individual de-identified participant data will not be shared beyond the limits permitted by the
366   informed consent and Mexican law. Specifically, this includes the sharing of the study protocol,

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367 statistical analysis plan, informed consent form, and approved clinical study report. Additionally,

368 other de-identified data allowed under the informed consent and Mexican law may be shared. The

369 data will be made available immediately upon publication and for a duration of 12 months thereafter.

370 Access to the data will be granted solely to investigators with methodologically sound proposals,

371 subject to authorization by an independent review committee and the ethics committees involved in

372 approving the protocol.

373 **Privacy Rights and Informed Consent**

374 Informed Consent formats were provided and signed by all participants under Good Clinical

375 Practices and Mexican law. Personal data is confidential, and subjects are not identified personally.

376 Their data was analysed and duly de-identified.

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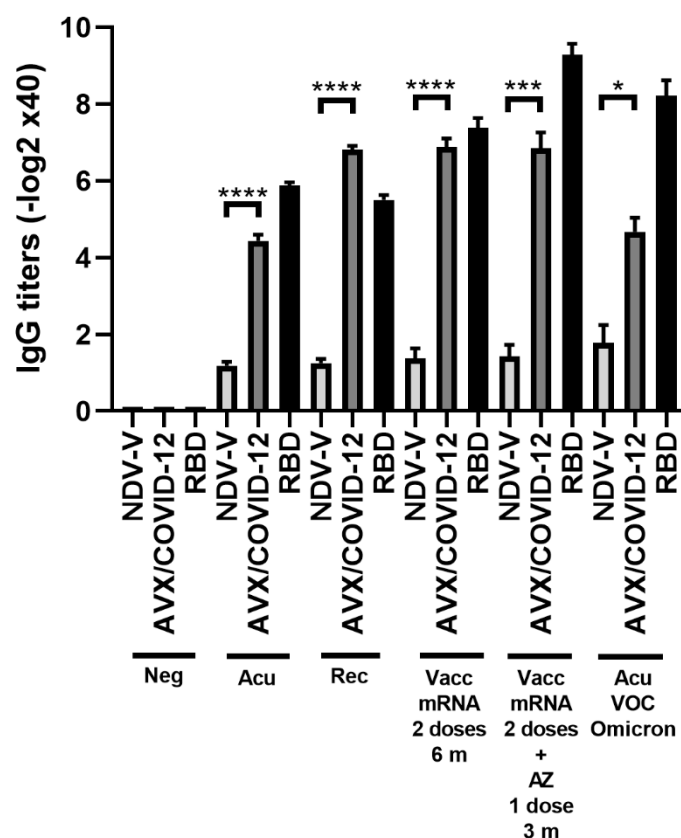
1   **Figures**

2

3   **Figure 1. Binding of serum IgG from COVID-19 patients and vaccinated individuals to the**
4   **AVX/COVID-12 vaccine and the Receptor-Binding Domain (RBD) of the spike protein of SARS-**
5   **CoV-2.** ELISAs were conducted to assess the binding of the AVX/COVID-12 vaccine and the receptor
6   binding domain (RBD) to serum IgG antibodies from patients in the acute phase of COVID-19 (Acu)
7   (n=12) and a subgroup with the Omicron variant (Acu VOC Omicron) (n=10), as well as individuals
8   who had recovered from the disease (Rec) (n=6). Additionally, samples were collected from individuals
9   vaccinated with two doses of the BNT162b2 Pfizer vaccine after 6 months of the second dose (Vacc
10   mRNA) (n=6) and those who received two doses of Pfizer and were boosted with the AZ (AZ/ChAdOx-
11   1-S) vaccine after 3 months of the third dose (n=7). Control groups included NDV-V (NDV LaSota
12   virus empty vector) and AVX/COVID-12 (NDV expressing SARS-CoV-2 S protein and RBD). Kruskal
13   Wallis. $p<0.05$*, $p<0.01$**, $p<0.001$*** and $p<0.0001$****.
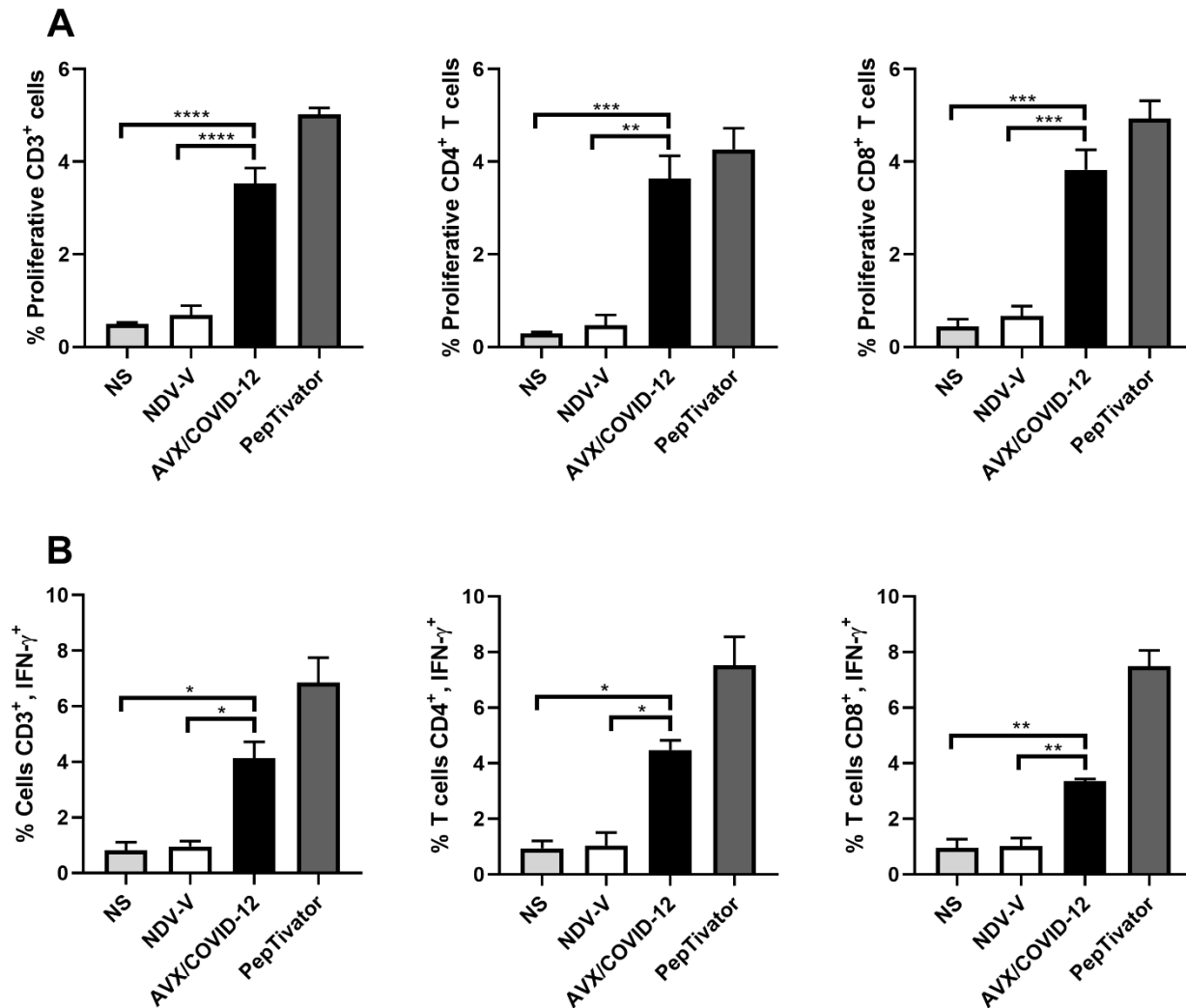
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Figure 2. AVX/COVID-12 Vaccine Induces Proliferation and IFN-γ Response in T Cells from Acute COVID-19 Patients. Proliferation response (A) or the percentage of IFN-γ+ (B) in T cells from acute COVID-19 patients (ancestral first wave) was assessed following stimulation with NDV-V (empty vector of NDV LaSota virus), AVX/COVID-12, or PepTivator (S1 and S2 peptides) for 72 hours. Flow cytometry analysis encompassed individual evaluations of CD4+ and CD8+ responses in single, FSClow SSClow CD3+ cells. NS (Not Stimulated). n=3, ANOVA, Tukey. p<0.05*, p<0.01**, p<0.001*** and p<0.0001****.
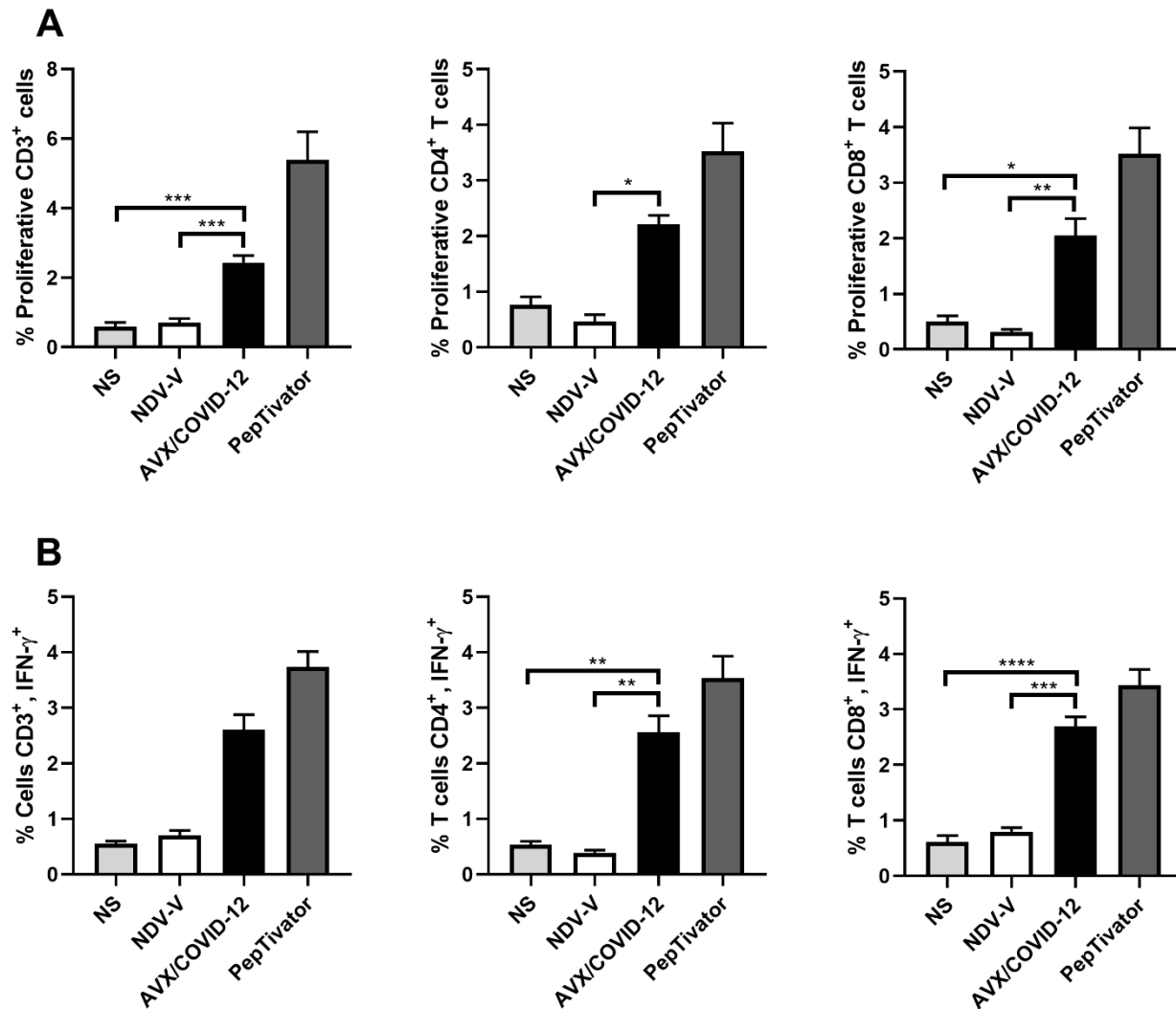
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Figure 3. AVX/COVID-12 Vaccine Elicits Proliferation and IFN-γ Response in T Cells from Individuals Vaccinated with mRNA BNT162b2. Proliferation response (A) or percentage of IFN-γ+ (B) in T cells stimulated with NDV-V (empty vector of NDV LaSota virus), AVX/COVID-19 vaccine, or PepTivator (S1 and S2 peptides) for 72 hours. Flow cytometry analysis includes single, FSClow SSClow CD3+ cells, and individual responses of CD4+ and CD8+, respectively. NS (Not Stimulated). n=6, Kruskal Wallis or ANOVA, Tukey. $p<0.05*$, $p<0.01**$, $p<0.001***$ and $p<0.0001****$.
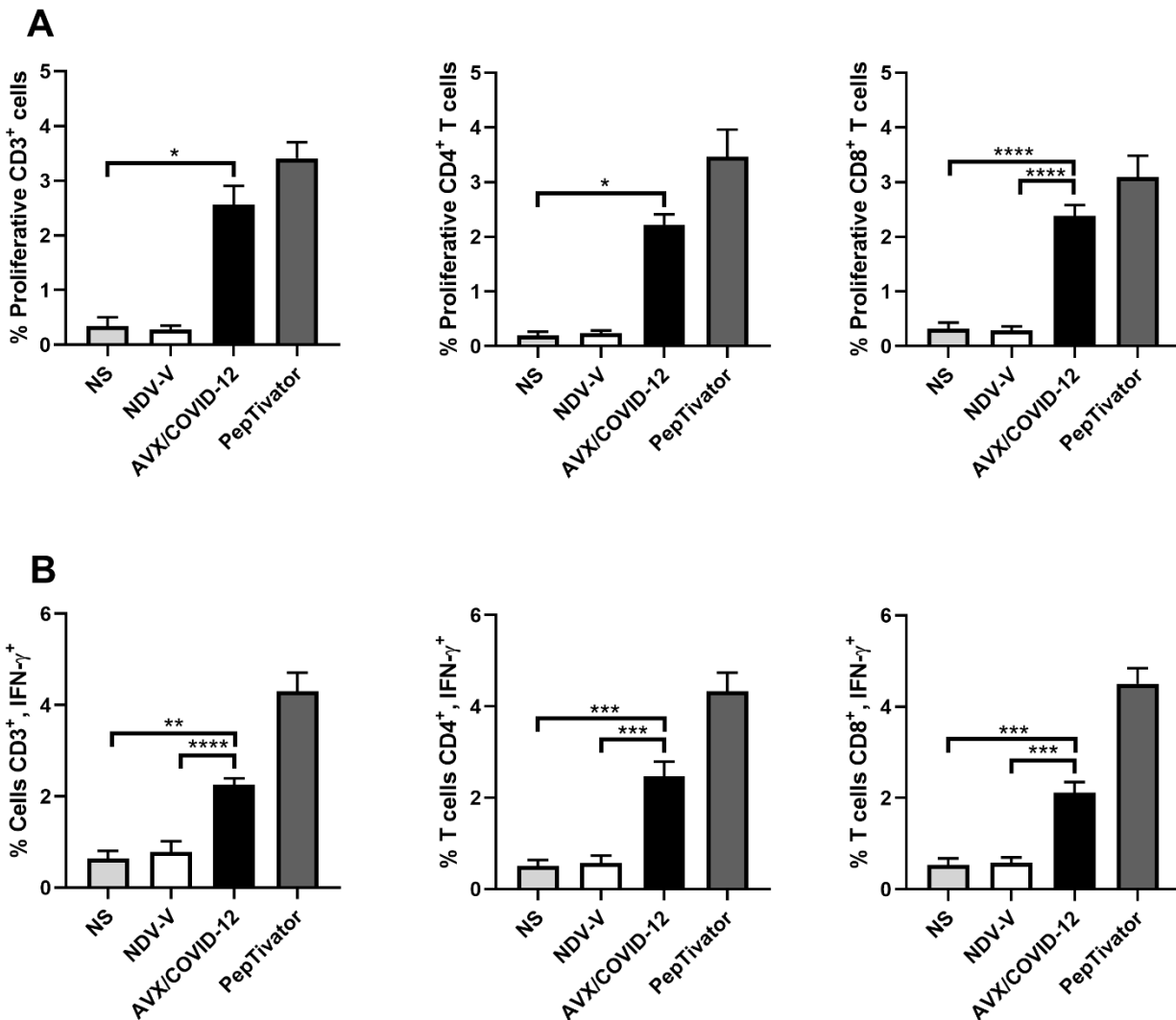
Figure 4. AVX/COVID-12 Vaccine Induces Proliferation and IFN-γ Response in T Cells from Individuals Vaccinated with mRNA BNT162b2 and Boosted with AZ/ChAdOx-1-S Vaccine. Proliferation response (A) or percentage of IFN-γ+ (B) in T cells stimulated with NDV-V (empty vector of NDV LaSota virus), AVX/COVID-12, or PepTivator (S1 and S2 peptides) for 72 hours. Flow cytometry analysis includes single, FSClow SSClow CD3+ cells and individual response of CD4+ and CD8+ respectively. NS (no stimulated). n=6, Kruskal Wallis. p<0.05*, p<0.01**, p<0.001*** and p<0.0001****.

40   **Supplementary Material**
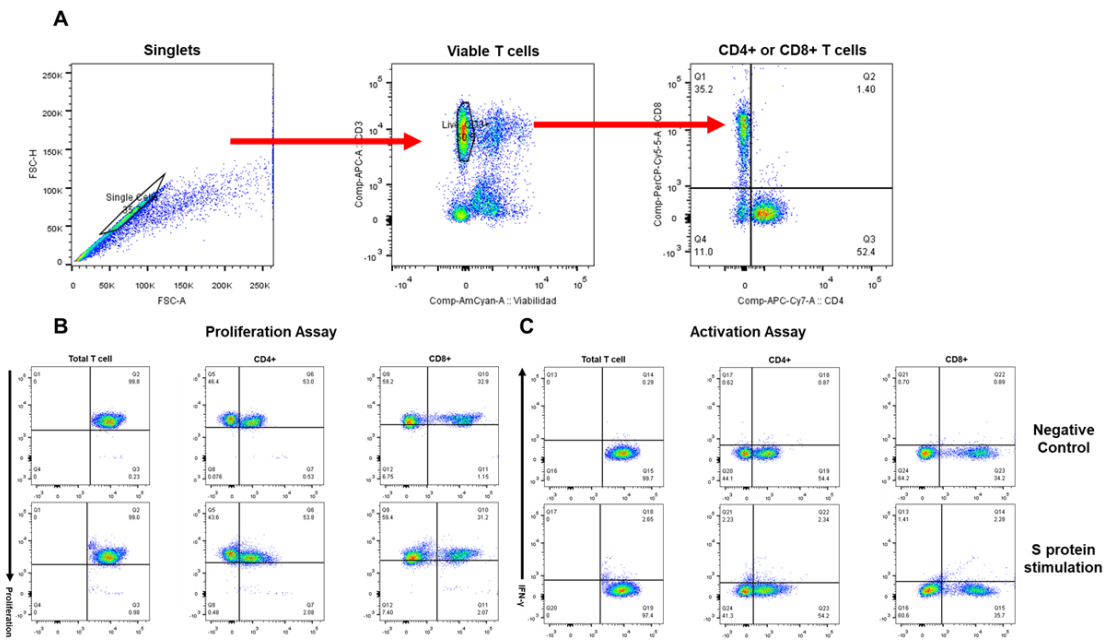
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42   **Figure 1S. Flow cytometry gating strategy.** Representative PBMC sample gating hierarchy. From left

43   to right and top to bottom: (A) FSC-A versus SSC-A for cellular viability and identification of CD4+ or

44   CD8+ T cells, (B) proliferation assay, and (C) individual T cell responses for the production of IFN-γ.

45