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1 # Immunogenicity of low dose prime-boost vaccination of mRNA vaccine
2 # CV07050101 in non-human primates

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14 **Abstract**

15 Many different vaccine candidates against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-
16 2), the etiological agent of COVID-19, are currently approved and under development. Vaccine platforms
17 vary from mRNA vaccines to viral-vectored vaccines, and several candidates have been shown to produce
18 humoral and cellular responses in small animal models, non-human primates and human volunteers. In
19 this study, six non-human primates received a prime-boost intramuscular vaccination with 4 µg of mRNA
20 vaccine candidate CV07050101, which encodes a pre-fusion stabilized spike (S) protein of SARS-CoV-2.
21 Boost vaccination was performed 28 days post prime vaccination. As a control, six animals were similarly
22 injected with PBS. Humoral and cellular immune responses were investigated at time of vaccination, and
23 two weeks afterwards. No antibodies could be detected two and four weeks after prime vaccination. Two
24 weeks after boost vaccination, binding but no neutralizing antibodies were detected in 4 out of 6 non-
25 human primates. SARS-CoV-2 S protein specific T cell responses were detected in these 4 animals. In
26 conclusion, prime-boost vaccination with 4 µg of vaccine candidate CV07050101 resulted in limited
27 immune responses in 4 out of 6 non-human primates.

28

29 **Introduction**

30 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent
31 responsible for COVID-19. SARS-CoV-2 has spread worldwide and over 185 million cases have been
32 detected as of July 2021. The pandemic has resulted in an unprecedented research effort towards the
33 development of a SARS-CoV-2 vaccine and several vaccines against SARS-CoV-2 have now been
34 approved. Interestingly, whilst traditional approaches such as subunit protein vaccines[1] and inactivated
35 virus vaccines[2] are still pursued, a large number of vaccines are based on novel platforms, such as virus-
36 vectored vaccines[3-5] and nucleic acid (DNA or RNA) vaccines[6,7]. Promising results have been published
37 for these platforms, both preclinical[8-13] and clinical[3-7], showing the induction of a humoral and cellular
38 response.

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39 Preclinical assessment of SARS-CoV-2 vaccines in non-human primate models is advantageous
40 due to the close relatedness of non-human primates to humans, thereby resulting in a higher degree of
41 clinical translation than smaller animal models. Indeed, rhesus macaques have been successfully used to
42 study vaccines[14]. Inoculation of rhesus macaques with SARS-CoV-2 results in respiratory disease which
43 includes virus replication in upper and lower respiratory tract[15]. Two reports on the immune response of
44 SARS-CoV-2 mRNA vaccine candidates in non-human primates describe the induction of binding and
45 neutralizing antibodies, as well as antigen-specific T cell responses[9,10].

46 SARS-CoV-2 messenger RNA (mRNA) vaccines encoding the SARS-CoV-2 spike (S) protein
47 have a good safety and immunogenicity profile, both in non-human primates[9,10] and in humans[6,7,16]. Here,
48 we investigate the immunogenicity of another SARS-CoV-2 S mRNA vaccine, CV07050101, in non-
49 human primates. CV07050101 is based on mRNA technology, RNActive®, developed by CureVac for the
50 accelerated development of human vaccines[17-21]. The efficaciousness of this platform has been
51 demonstrated for a rabies vaccine in mice and humans[18,22]. Moreover, mRNA vaccines have been
52 discussed as particular well suited to combat outbreak pathogens[23].

53

54 **Results**

55 In order to investigate the immunogenicity of mRNA vaccine CV07050101, we vaccinate six
56 rhesus macaques (all male) at 0 and 28 days via intramuscular injection, using 4 µg per dose. As a
57 control, six rhesus macaques were injected with an equal volume of sterile PBS (Figure 1A). No adverse
58 events were observed upon vaccination, and overall hematology and clinical chemistries were
59 unremarkable. No differences between the control and vaccinated groups were noted. No binding
60 antibodies could be detected 14 or 28 days post prime vaccination (Figure 1B). 14 days post boost
61 vaccination, low titers of spike-specific binding antibodies (reciprocal endpoint IgG titers of 400-800)
62 could be detected in 4 out of 6 animals (Figure 1B). Virus-specific neutralizing antibodies were not

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63 detected in animals at any time post boost vaccination (Figure 1C). No SARS-CoV-2 spike-specific T cell
64 responses were detected 14 days post prime vaccination but were detected in the same 4 out of 6 animals
65 at 14 days post boost vaccination (Figure 1D). The detection of specific T cell responses correlated with
66 the detection of spike-specific binding antibodies.

67

68 **Discussion**

69 Here, we show that prime-boost vaccination of rhesus macaques with 4 µg of CV07050101
70 results in the induction of binding antibodies in some, but not all vaccinated animals. This contrasts with
71 other studies with mRNA vaccines, in which a prime-boost vaccination elicits a robust humoral and
72 cellular response in all animals. Using a prime-boost regimen of 10 µg of mRNA-1273, which encodes a
73 prefusion-stabilized S protein utilizing modified mRNA, S-specific binding antibodies were detected in
74 all animals, whereas neutralizing antibodies were detected in 7 out of 8 animals[9]. Likewise, a prime-boost
75 vaccination using 30 µg of vaccine candidate BNT162b2, which also encodes a prefusion-stabilized S
76 protein, elicits binding and neutralizing antibodies in 6 out of 6 animals. Moreover, binding and
77 neutralizing antibodies were detected in all animals 21 days post prime only vaccination with 30 µg of
78 BNT162b2[10]. A recently released preprint investigating CV07050101 showed that vaccination of rhesus
79 macaques with 8 µg of mRNA elicits binding and neutralizing antibodies, whereas a dose of 0.5 µg of
80 mRNA did not[23].

81 One important difference between these studies is the amount of mRNA used to vaccinate
82 animals. We used 4 µg of mRNA per vaccination, whereas the other doses which elicited an immune
83 response used between 8 µg and 100 µg per vaccination[9,10,24]. Using a dose of 10 µg mRNA-1273 vaccine
84 resulted in no detectable neutralizing antibodies in 1 out of 8 animals on the day of challenge, but 100 µg
85 of mRNA-1273 resulted in neutralizing antibodies in all animals[9], suggesting a dose-dependent response
86 to the vaccine. Likewise, whereas 8 µg of CV07050101 induced an immune response, 0.5 µg did not[23].

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87 Compared to the limited immunogenicity in non-human primates we observed here, robust
88 SARS-CoV-2 neutralizing titers were observed in Balb/c mice immunized with the CV07050101 vaccine
89 after prime-boost regimen. Challenge studies in hamsters, which were performed at a later stage, utilized
90 a 10 µg prime-boost regimen of CV07050101 vaccine and a challenge dose of 10^2 $TCID_{50}$ SARS-CoV-2
91 and provided protection of the lower respiratory tract[24].

92 As the elicited immune response was low or absent in the vaccinated rhesus macaques, we
93 decided not to challenge the animals. In rhesus macaques, neutralizing antibodies are a correlate of
94 protection[25]. The presence of neutralizing antibodies in humans correlates with immunity against SARS-
95 CoV-2[26]. Since we did not detect neutralizing antibodies, we hypothesize that these animals would not
96 have been protected.

97 Since CV07050101 is now assessed in clinical trial studies, we compared the available
98 immunogenicity results. The 12 µg high dose vaccine prime-boost regime was able to induce neutralizing
99 antibody titers comparable to non-hospitalized individuals, whereas the 2-8 µg doses induced neutralizing
100 titers that were lower in clinical trial participants. However, virus neutralizing antibodies could be
101 detected in 66% of human volunteers given 4 µg of CV07050101[16], in contrast to no neutralizing
102 antibodies in serum from NHPs vaccinated with the same dose. It has been hypothesized that a difference
103 in the lubricant used in syringes can decrease integrity of the mRNA vaccine, which may explain the low
104 immunogenicity detected in this NHP study (B.P. personal communication).

105 In conclusion, we show that prime-boost vaccination of rhesus macaques with 4 µg of
106 CV07050101 does not elicit a uniform nor robust immune response. However, vaccination using 8 µg of
107 the same vaccine was protective in a NHP challenge study demonstrating protection against SARS-CoV-2
108 infection by CV07050101 vaccination[23].

109

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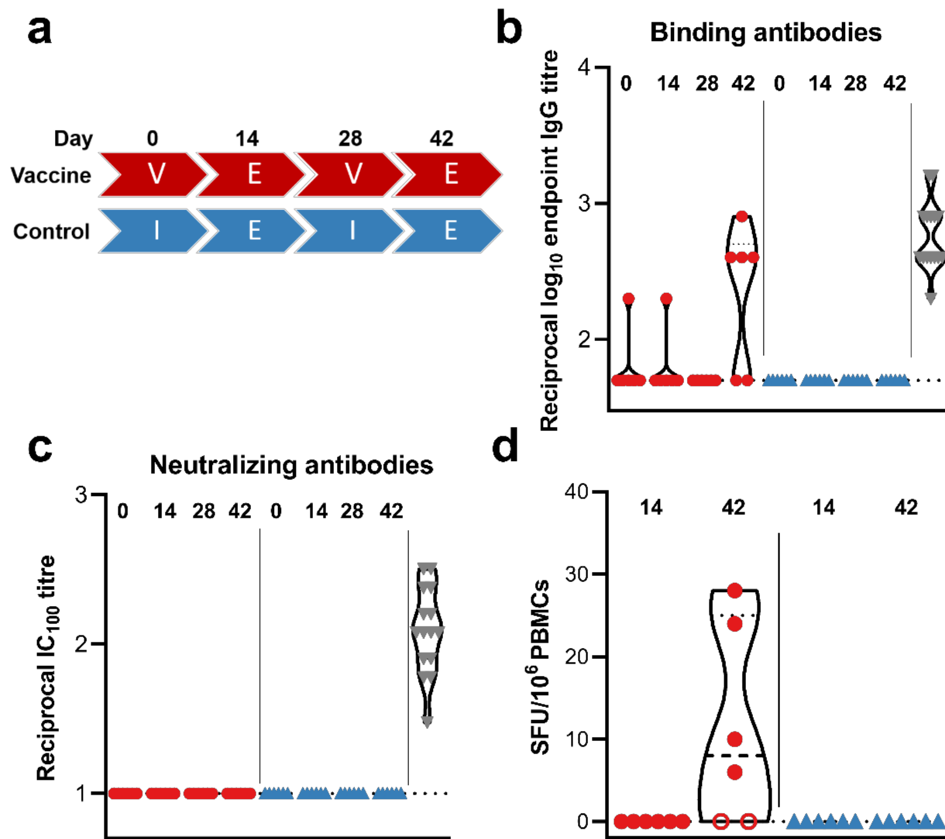

110

111 Figure. Humoral and cellular response after vaccination with CV07050101. a) Study schedule. Two
112 groups (N=6) were vaccinated (V) or administered PBS (I) twice, four weeks apart. Fourteen days post
113 each vaccination, exams (E) were performed. The presence of SARS-CoV-2 spike-specific binding (b)
114 and neutralizing (c) antibodies in serum obtained from rhesus macaques at time of vaccination and 14
115 days afterwards where measured using ELISA and infectious virus neutralization assays. d) SARS-CoV-2
116 S-specific T cell responses were measured via ELIspot. Closed red circles = animal positive in ELISA
117 assay; open red circle = animal negative in ELISA assay; blue triangles = control animals; grey triangles
118 = convalescent human sera; dotted line = lower limit of detection.

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119 **Methods**

120 *Ethics statement*

121 Animal study approval was provided by the Institutional Animal Care and Use Committee (IACUC) at
122 Rocky Mountain Laboratories. Animal experiments were conducted in an AAALAC-approved facility,
123 following the basic principles and guidelines in The Guide for the Care and Use of Laboratory Animals,
124 the Animal Welfare Act, United States Department of Agriculture and the United States Public Health
125 Service Policy on Humane Care and Use of Laboratory Animals. Rhesus macaques were housed in
126 individual primate cages allowing social interactions, in a climate-controlled room with a fixed light/dark
127 cycle (12-hours/12-hours). Animals were monitored a minimum of twice daily and commercial monkey
128 chow, treats, vegetables, and fruit were provided. Water was available ad libitum. A variety of human
129 interaction, commercial toys, videos, and music was used as environmental enrichment.

130 *Vaccine mRNA and lipid nanoparticle production*

131 CV07050101 is an lipid nanoparticle-formulated RNActive® SARS-CoV-2 vaccine composed of the
132 active pharmaceutical ingredient, an mRNA that encodes a pre-fusion conformation stabilized version of
133 the full-length spike (S) protein of SARS-CoV-2 virus (GenBank YP_009724390.1) including the K986P
134 and V987P prefusion stabilizing mutations, and four lipid components: cholesterol, 1,2-distearoyl-sn-
135 glycero-3-phosphocholine (DSPC), PEGylated lipid and a cationic lipid[24].

136 *Study design*

137 12 male rhesus macaques between 3-5 years old were screened for SARS-CoV-2 status by ELISA, and
138 when found to be negative for prior exposure were sorted by body weight, and then divided into two
139 groups of six animals, resulting in near equal contribution of body weights. Group 1 (vaccine) was
140 vaccinated with 4 µg of mRNA vaccine CV07050101 in sterile PBS at 0 and 28 days, group 2 (control)
141 was vaccinated with sterile PBS at 0 and 28 days via intramuscular injection, using Monoject 1 mL
142 Tuberculin syringes (Covidien, 25G x 5/8”). Blood samples were obtained before vaccination and 14 days

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143 after each vaccination. Hematology analysis was completed on a ProCyte DX (IDEXX Laboratories,
144 Westbrook, ME, USA) and the following parameters were evaluated: red blood cells (RBC), hemoglobin
145 (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean
146 corpuscular hemoglobin concentration (MCHC), red cell distribution weight (RDW), platelets, mean
147 platelet volume (MPV), white blood cells (WBC), neutrophil count (abs and %), lymphocyte count (abs
148 and %), monocyte count (abs and %), eosinophil count (abs and %), and basophil count (abs and %).
149 Serum chemistries were completed on a VetScan VS2 Chemistry Analyzer (Abaxis, Union City, CA) and
150 the following parameters were evaluated: glucose, blood urea nitrogen (BUN), creatinine, calcium,
151 albumin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline
152 phosphatase (ALP), total bilirubin, globulin, sodium, potassium, chloride, and total carbon.

153 *Enzyme-linked immunosorbent assay*

154 A plasmid encoding the prefusion stabilized SARS-CoV-2 spike protein with a T4 fibritin trimerization
155 motif was obtained from the Vaccine Research Centre, Bethesda, USA and expressed in-house. Maxisorp
156 plates (Nunc) were coated overnight at 4 °C with 100 ng/well spike protein in PBS. Plates were blocked
157 with 100 µl of casein in PBS (Thermo Fisher) for 1hr at RT. Serum serially diluted 2x in casein in PBS
158 was incubated at RT for 1hr. Antibodies were detected using affinity-purified polyclonal antibody
159 peroxidase-labeled goat-anti-monkey IgG (Seracare, 074-11-021) in casein and TMB 2-component
160 peroxidase substrate (Seracare, 5120-0047), developed for 5-10 min, and reaction was stopped using stop
161 solution (Seracare, 5150-0021) and read at 450 nm. All wells were washed 3x with PBST 0.1% tween in
162 between steps. Threshold for positivity was set at 3x OD value of negative control (serum obtained from
163 non-human primates prior to start of the experiment) or 0.2, whichever one was higher.

164 *ELISpot*

165 PBMCs were isolated from ethylene diamine tetraaceticacid (EDTA) whole blood using LeucosepTM
166 tubes (Greiner Bio-one International GmbH) and Histopaque®-1077 density gradient cell separation

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167 medium (Sigma-Aldrich) according to the manufacturers' instructions. IFN-γ ELISpot assay of PBMCs
168 was performed using the ImmunoSpot® Human IFN- γ Single-Color Enzymatic ELISpot Assay Kit
169 according to the manufacturer's protocol (Cellular Technology Limited). PBMCs were plated at a
170 concentration of 300,000 cells per well and were stimulated with two contiguous peptide pools spanning
171 the length of the SARS-CoV-2 S protein sequence at a concentration of 2 μg/mL per peptide
172 (Mimotopes). Imaging was performed using the CTL ImmunoSpot® Software (Cellular Technology
173 Limited). Spot forming units (SFU) were hand counted and calculated per 10^6 PBMCs as summed across
174 the peptide pools for each animal.

175 *SARS-CoV-2 virus neutralization*

176 VeroE6 cells were maintained in Dulbecco's modified Eagle's media (DMEM) supplemented with 10%
177 fetal bovine serum (Gibco), 1 mM L-glutamine, 50 U/mL streptomycin and 50 ug/mL penicillin. Sera
178 were heat-inactivated (30 min, 56 °C), two-fold serial dilutions were prepared in DMEM supplemented
179 with 2% fetal bovine serum (Gibco), 1 mM L-glutamine, 50 U/mL streptomycin and 50 ug/mL penicillin
180 and 100 $TCID_{50}$ of SARS-CoV-2 was added. After 1hr incubation at 37 °C and 5% CO_2, virus:serum
181 mixture was added to VeroE6 cells and incubated at 37 °C and 5% CO_2. At 6 dpi, cytopathic effect was
182 scored. The virus neutralization titer was expressed as the reciprocal value of the highest dilution of the
183 serum which still inhibited 100% of virus replication. A positive control standardized against the NIBSC
184 serum control 20/130 was used in all VN assays.

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251

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261 AG, Tuebingen Germany, a publicly listed company developing RNA-based vaccines and
262 immunotherapeutics. B.P. may hold shares or stock options in the company and is an inventor on several
263 patents on mRNA vaccination and use thereof. All other authors report no competing interests.