1 Use of eVLP-based vaccine candidates to broaden immunity against SARS-CoV-2 variants

2 Jasminka Bozic^{a1}, Tanvir Ahmed^{a1}, Barthelemy Ontsouka^a, Anne-Catherine Fluckiger^{b*}, Abebaw
3 Diress^a, Tamara Berthoud^a, Xiaoyang Yuan^a, Lanjian Yang^a, Francisco Diaz-Mitoma^c, David E.
4 Anderson^c, Catalina Soare^{a1}

5 ^aVBI Vaccines, 201-310 Hunt Club Road, Ottawa ON K1V 1C1, Canada; ^bBionaria, 11Bis Rue
6 de la Garenne, 69290 Saint-Genis-les-Ollières, France; ^cVBI Vaccines, Cambridge, 222
7 Third Street, Cambridge, 02142 Massachusetts, USA.

8 *Corresponding author:

9 Email: afluckiger@vbivaccines.com (A.-C. Fluckiger)

10 1: These authors contributed equally to this work.

11 Abstract

12 Rapid emergence of SARS-CoV-2 variants is a constant threat and a major hurdle to reach herd
13 immunity. We produced VBI-2905a, an enveloped virus-like particle (eVLP)-based vaccine
14 candidate expressing prefusion spike protein from the Beta variant that contains several escape
15 mutations. VBI-2905a protected hamsters against infection with a Beta variant virus and induced
16 high levels of neutralizing antibodies against Beta RBD. In a heterologous vaccination regimen, a
17 single injection of VBI-2905a in animals previously immunized with VBI-2902, a vaccine candidate
18 expressing S from ancestral SARS-CoV-2, hamsters were equally protected against Beta variant
19 infection. As an alternate strategy to broaden immunity, we produced a trivalent vaccine expressing
20 the prefusion spike protein from SARS-CoV-2 together with unmodified S from SARS-CoV-1 and
21 MERS-CoV. Relative to immunity induced against the ancestral strain, the trivalent vaccine VBI-
22 2901a induced higher and more consistent antibody binding and neutralizing responses against a
23 panel of variants including Beta, Delta, Kappa, and Lambda, with evidence for broadening of
24 immunity rather than just boosting cross-reactive antibodies.

25 **Keywords**

26 SARS-COV-2 variants; Vaccine; Virus-like-particles; Immunogenicity; cross-neutralizing antibodies

27 **Abbreviations**

28 eVLP, enveloped virus-like particules; CoV, coronavirus; VOC, Variant of concern; VOI, varaint of
 29 interest; RBD, receptor binding domain; NTD, N-terminal domain; Ab, antibody; nAb, neutralizing
 30 antibody; MLV, murine leukemia virus; ELISA, enzyme-linked-immuno-sorbent-assay; PRNT,
 31 plaque reduction neutralization test; EPT, end-point titer; Alum, aluminum phosphate; IP,
 32 IntraPeritoneal; IM, IntraMuscular; NRC, National Research Council Canada; VIDO,
 33 Vaccine and Infectious Disease Organization

34 **Introduction**

35 The outbreak of a severe respiratory disease in Wuhan, China in December 2019 led to the
 36 identification of a new betacoronavirus related to the severe acute respiratory syndrome (SARS)
 37 coronavirus that was named SARS-CoV-2 (Wu et al., 2020). SARS-CoV-2 rapidly spread
 38 worldwide in a global pandemic in 2019 (COVID-19) and was declared a public health emergency
 39 of international concern (World Health Organization, 2020). Unprecedented effort and innovation in
 40 vaccine development resulted in vaccines, deployed under emergency use authorization, against
 41 SARS-CoV-2 in less than a year (FDA, 2020).

42 Coronaviruses are large single-strand RNA viruses with replication that is error-prone
 43 despite some proofreading mechanisms (Smith and Denison, 2012). Resulting mutational changes
 44 can either be detrimental and lead to viral extinction or confer advantage to the virus and result in
 45 better adaptation to the host. Massive replication of SARS-CoV-2 on a global scale contributes to
 46 increasing numbers of mutations and emergence of variants. Variants of concern (VOC) are
 47 defined by clear evidence indicating a significant impact on transmissibility, severity, and/or
 48 immunity that is likely to have an impact on the disease epidemiology (EDCC, 2021). In July 2021,
 49 four VOC that first emerged as locally dominant variants before spreading globally were discovered
 50 in the U.K. (B.1.1.7 – “Alpha”), South Africa (B.1.351 – “Beta”), Brazil (P1 – “Gamma”), and more

recently in India (B.1.617 – “Delta”). In late August the Mu variant first emerged in Columbia, joining this list of Variants of Interest (VOI) together with Lambda while the incidence of Alpha was decreasing.

The CoV spike (S) protein contains a receptor binding domain (RBD) critical for binding to and infection of host cells, and is a major target for mutational changes which enhance adaptation to the host (Berrio et al., 2020; Boni et al., 2020). Each of the VOC are characterized by a number of shared mutations expressed on S, primarily located in the RBD and N-terminal domain (NTD), that serve to increase inter-individual transmission, escape neutralizing antibodies acquired by vaccination or prior natural SARS-CoV-2 infection, or both (Harvey et al., 2021; Zhang et al., 2020). For instance, the first identified VOC, Alpha, is characterized by a D614G mutation, among other mutations, that is now fixed in all globally circulating variants of the virus. D614G is associated with increased transmissibility (Plante et al., 2021; Zhang et al., 2020) but does not have a major impact on neutralization by serum from either vaccinated or COVID-19 convalescent individuals. The following VOC that emerged in South Africa was rapidly identified as a vaccine-escape mutant. This Beta variant bears several mutations in its RBD, including E484K and N501Y, which significantly inhibit neutralizing activity elicited against the Ancestral Wu-1 strain of virus whether acquired by vaccination or infection (Tegally et al., 2020; Cele et al., 2021). Emergence of escape mutants is a major concern because most of the licensed vaccines are based on expression of various forms of S using the Ancestral sequence of the S protein (Hoffmann et al., 2021 ; Kyriakidis et al., 2021; Lamb, 2021). More recently, the Delta variant spread from India to many countries with great speed in spite of significant proportions of fully vaccinated individuals in many countries. Delta shows the RBD mutation L452R which appeared independently in several areas of the globe, including in variants Lambda, Kappa, Epsilon, Iota, and contributes to escape neutralization from Abs induced by previously acquired immunity (Deng et al.2021). Additionally, mutation P681R in the furin cleavage site of Delta could increase the rate of S1-S2 cleavage, resulting in better transmissibility (Cherian et al., 2021).

Recently, we developed a SARS-CoV-2 candidate vaccine, VBI-2902a, comprised of enveloped virus-like particles (eVLPs) expressing a modified prefusion form of the ancestral S sequence, adjuvanted with aluminum phosphate (Alum). We recently demonstrated that VBI-2902a

induced strong neutralizing activity in mouse immunogenicity studies, and protected hamsters from SARS-CoV-2 challenge using a virus related to the ancestral isolate (Fluckiger et al. 2021). Interim results from a Phase I clinical study in healthy, seronegative individuals (ClinicalTrials.gov Identifier: NCT04773665) demonstrated robust (4.3-fold greater) neutralizing activity 28 days after a second, 5µg dose of VBI-2902a, relative to a panel of COVID-19 convalescent sera.

Employing the same strategy, we produced a new vaccine candidate, VBI-2905a, that expresses a modified prefusion S based on the Beta variant sequence. Consistent with previous studies, VBI-2905a elicited neutralizing antibody responses against the Beta variant which were significantly greater than those induced by VBI-2902a, and responses against the ancestral strain which were comparable to VBI-2902a. Consistent with the role of neutralizing antibody responses as a presumed correlate of protection, greater efficacy was observed in hamsters vaccinated with VBI-2905a relative to VBI-2902a when challenged with the Beta variant. Noteworthy was the observation in an alternative vaccination regimen that priming with VBI-2902a followed by a single booster dose of VBI-2905a induced strong neutralizing antibody responses against the ancestral strain as well as both Beta and Delta VOC.

We also evaluated immunity elicited with a distinct eVLP-based candidate, VBI-2901a, which expresses a modified prefusion S based on the ancestral sequence in addition to the related S proteins from SARS CoV-1 and MERS. Immunization with VBI-2901a induced neutralizing antibody titers against the Beta variant significantly greater than VBI-2902a and comparable to those induced with VBI-2905a, effectively broadening immunity to VOC not contained within the vaccine. Antibody binding and neutralizing titers against an extended panel of variants demonstrated responses typically 3-fold greater than that observed with VBI-2902a. Collectively, these results demonstrate multiple ways to broaden immunity to SARS-CoV-2 VOC.

Material and Methods

Plasmids, eVLP production, and adjuvant formulation

105 Expression plasmid for the production of eVLPs expressing SARS-CoV-2 S proteins have been
 106 described previously (Fluckiger et al. 2021). Briefly, the prefusion modified form of S was obtained
 107 by introducing a mutation at the furin cleavage site (RRAR → GSAS) and two Proline at position
 108 K986-V987 of the Wuhan reference and swapping the transmembrane cytoplasmic domain with
 109 that of the VSV-G protein. VBI-2902a was produced using the Wuhan-Hu-1 spike sequence
 110 (Genbank accession number MN908947), and VBI-2905a was produced using the same strategy
 111 with S sequence from Beta variant B.1.351 isolate EPI_ISL_911433 (GISAID). Production and
 112 purification of eVLPs were conducted as described elsewhere (Fluckiger et al. 2021). The
 113 preparation of eVLPs expressing either Wuhan reference Spike or Beta variant Spike were
 114 formulated in Aluminum phosphate (Alum, Adjuphos® , Invitrogen) to obtain vaccine candidate
 115 VBI-2902a and VBI-2905a, respectively. To produce VBI-2901, Two additionnal plasmids were
 116 produced that expressed the optimized sequences for full-lenght unmodified S protein from SARS-
 117 CoV-1 and MERS-CoV. To produce trivalent eVLPs, HEK-293SF-3F6 were cotransfected with
 118 these 2 plasmids together with the plasmid coding for prefusion ancestral SARS-CoV-2 S used for
 119 VBI-2902a production, and the MLVGAG plasmid as described. Expression of SARS-CoV-2 S,
 120 SARS-CoV-1 S, MERS-CoV S and GAG were determined by Western blot analysis
 121 (Supplementary material Fig.S1).

122 *Mouse immunization study*

123 Six- to 8-week-old female C57BL/6 mice were purchased from Charles River (St Constant,
 124 Quebec Canada). The animals were acclimatized for a period of at least 7 days before any
 125 procedures were performed. The animal studies were conducted under ethics protocols approved
 126 by the NRC Animal Care Committee. Mice were maintained in a controlled environment in
 127 accordance with the “Guide for the Care and Use of Laboratory Animals” at the Animal Research
 128 facility of the NRC’s Human Health Therapeutics Research Centre (Montreal). Mice were randomly
 129 assigned to experimental groups of 10 to 15 mice and received intraperitoneal (IP) injections with
 130 0.5 mL of adjuvanted SARS-CoV-2 eVLPs as described elsewhere (Fluckiger, 2021). Blood was

131 collected on day -1 before injection and day 14 after each injection for humoral immunity
132 assessment at time of euthanasia.

133 *Hamster challenge study*

134

135 Syrian golden hamsters (males, 5-6 weeks old) were purchased from Charles River Laboratories
136 (Saint-Constant, Quebec, Canada). The study was conducted under approval of the CCAC
137 committee at the Vaccine and Infectious Disease Organization (VIDO) International Vaccine Centre
138 (Saskatchewan, Canada). Animals were randomly assigned to each experimental group (A, B)
139 (n=10/group). Animals received 2 intramuscular (IM) injection of either 0.9%-saline buffer (saline
140 control group) or VBI-2902a (VBI-2902a group), or VBI-2905a (VBI-2905a group), or a first dose of
141 VBI-2902a followed by a second injection of VBI-2905a (Heterologous boost group). Each dose of
142 eVLP-based vaccine contained 1µg of Spike protein formulated with 125 µg of Alum. Injection was
143 performed by intramuscular (IM) route at one side of the thighs in a 100 µL volume. The schedule
144 for immunization, challenge and sample collection is depicted on Fig. 2a. All animals were
145 challenged intranasally via both nares with 50 µL/nare containing 1×10^5 TCID₅₀ of hCoV-19/South
146 Africa/KRISP-EC-K005321/2020 (Seq. available at GISAID: EPI_ISL_678470) strain per animal.
147 Body weights and body temperature were measured at immunization for 3 days and daily from the
148 challenge day. General health conditions were observed daily through the entire study period.
149 Blood samples were collected as indicated on Fig. 2a.

150 *Antibody binding titers*

151 Anti-SARS-CoV-2 specific IgG binding titers in sera were measured by standard ELISA procedure
152 described elsewhere (Kirchmeier et al., 2014), using recombinant SARS-CoV-2 S RBD proteins
153 (Sinobiological). For total IgG binding titers, detection was performed using a goat anti-mouse IgG-
154 Fc HRP (Bethyl), or Goat anti-Hamster IgG HRP (ThermoFisher), or goat anti-human IgG heavy
155 and light chain HRP-conjugated (Bethyl). HRP-conjugated Goat anti-mouse IgG1 and HRP-
156 conjugated goat anti-mouse IgG2b HRP (Bethyl) were used for the detection of isotype subtype.

157 Determination of Ab binding titers to Spike RBDs was performed using SARS-COV-2 RDB
158 recombinant protein for the specificity of choice as described in Suppl. Table 1. The detection was
159 completed by adding 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution, and the reaction
160 stopped by adding liquid stop solution for TMB substrate. Absorbance was read at 450 nm in an
161 ELISA microwell plate reader. Data fitting and analysis were performed with SoftMaxPro 5, using a
162 four-parameter fitting algorithm.

163 *Virus neutralization assays*

164 Neutralizing activity in mouse serum samples was measured by standard plaque reduction
165 neutralization test (PRNT) on Vero cells at the NRC using 100 PFU of
166 SARS-CoV-2/Canada/ON/VIDO-01/2020 (Wu-1 virus) or hCoV-19/South Africa/KRISP-EC-
167 K005321/2020 (Beta virus). Results were represented as PRNT90 end point titer (EPT),
168 corresponding to the lowest dilution inhibiting respectively 90% of plaque formation in Vero cell
169 culture.

170

171 *Neutralization assay with pseudoparticles*

172 Production of pseudoparticles (pp) pseudotyped with various spike proteins and neutralization
173 assay was adapted from Dreux et al, 2009. Expression plasmid were designed using full length S
174 protein sequences as described previously. Accession number and mutations are listed in Suppl.
175 Material Table 1. We produced infectious SARS-CoV-2pp carrying a GFP-firefly luciferase double
176 reporter gene (plasmid pj155, Garrone et al., 2011) instead of green fluorescent protein (GFP).
177 Luciferase activity in infected hACE2-HEK293 cells was measured with a Bright-Glo Luciferase
178 assay system (Promega) and a Beckman Coulter DTX880 plate reader. Data were expressed in
179 relative luminescence units (RLUs). The percentage of neutralization was calculated by comparing
180 the luciferase activity in cells infected with SARS-CoV-2pp in the presence of serum from
181 immunized animals with luciferase activity in cells infected with SARS-CoV-2pp in the absence of
182 serum.

183 *Statistics*

184 All statistical analyses were performed using GraphPad Prism 9 software (La Jolla, CA). Unless
185 indicated, multiple comparison was done with Dunn's corrected Kruskal-Wallis test on unpaired
186 samples and Friedman test on paired samples. The data were considered significant if $p < 0.05$.
187 Geometric mean titers (GMT) with standard deviation are represented on graphs. No samples or
188 animals were excluded from the analysis. Randomization was performed for the animal studies.

189 **Results**

190

191 **Heterologous boosting with eVLPs bearing S protein from the Beta variant broadens** 192 **immunity**

193 VBI-2902a is an eVLP-based vaccine candidate that expresses a modified prefusion SARS-CoV-2
194 S protein from the ancestral Wu-1 strain, adjuvanted with Alum (Fluckiger et al., 2021). VBI-2905a
195 expresses a modified prefusion SARS-CoV-2 S protein from the Beta variant and is also
196 adjuvanted with Alum. We immunized mice with 2 injections of VBI-2902a, 2 injections of VBI-
197 2905a, or a first injection of VBI-2902a followed by a second injection of VBI-2905a (heterologous
198 boost). As previously described (Fluckiger et al., 2021), 2 doses of VBI-2902a induced high levels
199 of neutralizing Ab response against the ancestral Wu-1 strain (GMT = 2,458) which were
200 significantly reduced against the Beta variant (GMT = 94) (Fig.1a-b). By contrast, VBI-2905a
201 induced Abs that neutralized Beta and ancestral viruses at similar levels in mice, yielding only a
202 2.2-fold difference with non significant $p = 0.1484$ (Fig. 1a-b). Sera from mice in the heterologous
203 boost group cross-neutralized both the Beta variant and the ancestral strain with similar potencies
204 (1,4 fold difference with $p = 0.3828$). Heterologous boosting with VBI-2905a significantly increased
205 the PRNT90 against the ancestral strain compared to 2 doses of VBI-2905a alone (from GMT of
206 371 to 820, $p = 0.0267$) to levels that were closer to those reached after two doses of VBI-2902a

207 ($p = 0.0131$), while PRNT90 GMTs against the Beta variant were comparable to 2 doses of VBI-
208 2905a (respectively GMT = 564 vs GMT = 619, $p = 0.8785$).

209 Analysis of Ab binding titers to S protein RBDs was consistent with the neutralization data
210 (Fig.1c). VBI-2902a induced high levels (most of the sera $>10^6$ EPT with GMT 974×10^3) of Ab
211 binding titers against the ancestral S RBD with significantly reduced cross-reactivity against the
212 Beta variant RBD (GMT 74×10^3), though there was good cross-reactivity against the Delta variant
213 RBD (GMT 616×10^3). Antisera from immunization with VBI-2905a showed similar crossreactivity
214 against Ancestral, Delta and Beta RBD (respectively GMT 322×10^3 , 192×10^3 and 217×10^3). Animals
215 receiving the heterologous prime boost regimen had similar reactivity to ancestral and Delta RBD
216 as compared with the VBI-2902a group, and similar reactivity to Beta RBD compared to the VBI-
217 2905a group.

218 **Heterologous boosting with VBI-2905a protects hamsters against SARS-COV-2 Beta variant**

219 Golden Syrian hamsters were intramuscularly vaccinated 3 weeks apart with two doses of
220 eVLP vaccine candidates, comprised of: two doses of VBI-2902a (group VBI-2902a), two doses of
221 VBI-2905a (group VBI-2905a), or a priming dose of VBI-2902a followed by a second, booster dose
222 of VBI-2905a (group heterologous boost) (Fig. 2a).

223 Neutralizing activities titers against the ancestral virus were comparable across all groups,
224 including hamsters immunized with 2 doses of the Beta S candidate (VBI-2905a) (Fig.2b).
225 Neutralization of the Beta variant was lower after immunization with VBI-2902a, with a significant
226 9.6-fold decrease of Beta nAb compared to homotypic immunization with VBI-2905a (GMT 99 in
227 VBI-2902a and 1083 in VBI-2905a, $p = 0.0033$). In contrast, nAb titers against Beta RBD were
228 similar in groups that received either two doses of VBI-2905a or heterologous boosting.

229 Three weeks after the second immunization, hamsters were exposed to 1×10^5 TCID₅₀ of
230 the Beta variant virus in each nare. In the placebo group, hamsters began losing weight the day
231 after infection which continued until day 6-8. Vaccination with 2 doses of VBI-2902a based on the
232 ancestral S protein induced limited protection against challenge with moderate weight loss
233 recorded until day 4, and only a fraction (3/5) of the animals fully regained their initial body weight
234 after day 7. By contrast, hamsters vaccinated with 2 doses of VBI-2905a exhibited transient weight

235 loss up to day 2-3 and then rapidly regained weight. A similar pattern was observed in hamsters
236 that received VBI-2905a as a boost. As we have observed in previous hamster challenge studies of
237 VBI-2902a, there was a correlation between neutralizing antibody titers against the Beta variant
238 and protection from disease (weight loss) after challenge (data not shown).

239 **Immunization with a pan-coronavirus candidate may protect against VOC not contained** 240 **within the vaccine**

241 We hypothesized that exposing the immune system to multiple spike proteins at the same
242 time might help broaden humoral immunity that could recognize emerging variants or new
243 coronaviruses more phylogenetically distant to the vaccine candidate. To test this hypothesis we
244 produced VBI-2901a, a trivalent eVLP vaccine formulated with Alum, that expresses a prefusion
245 form of the ancestral SARS-CoV-2 S (identical to VBI-2902) with unmodified full length S from
246 SARS-CoV-1 and MERS-CoV (Suppl. Fig. S1). Mice that received 2 doses of trivalent VBI-2901a
247 had increased nAb titers (GMT 2915) against the ancestral virus relative to mice that received 2
248 doses of monovalent VBI-2902a (GMT 831) or VBI-2905a (GMT 448) (Fig. 3a). Moreover, trivalent
249 VBI-2901a induced neutralization activity against the Beta virus that was equivalent to what was
250 observed in response to homotypic VBI-2905a, and significantly higher than that observed after
251 VBI-2902a vaccination (Fig. 3a). Neutralization of both Delta and Kappa variant pseudotyped
252 particles confirmed broadened neutralizing immunity elicited by VBI-2901a, with titers
253 approximately 3-fold greater than those induced by VBI-2902a (Fig. 3b). Consistent with the
254 neutralization activity, VBI-2901a induced higher and/or more consistent levels of Ab binding to the
255 RBD among all variants evaluated, including Beta, Delta, and Lambda (Fig. 3c).

256 **Discussion**

257 Less than a year after identification of the new SARS-CoV-2 virus, variants emerged with
258 impact on transmissibility, severity and immunity (EDCC, 2021) that challenge the development
259 and durability of vaccine strategies designed to reach herd immunity. Indeed, all approved
260 vaccines have been designed against the ancestral SARS-COV-2 virus that is no longer circulating

but has been replaced by variants containing mutations which are enabling escape from nAbs induced against the ancestral strain (Berio et al. 2020; Boni et al, 2020). In the present study, we compared several strategies to broaden antibody-based immunity which is presumed to be a correlate of protection against SARS-CoV-2.

In addition to our eVLP vaccine expressing the prefusion S from the ancestral SARS-CoV-2 virus, we produced an eVLP-based vaccine expressing the prefusion S from Beta variant. Beta was chosen for its deleterious mutations E484K and K417N, which enable escape neutralization from ancestral virus mAbs (Hoffman et al, 2021). We have previously demonstrated that 2 doses of VBI-2902a protected hamsters against infection by the ancestral Wuhan SARS-CoV-2 virus and we confirmed here that VBI-2905a also protected hamsters from infection with the SARS-CoV-2 Beta variant. We have also demonstrated that a heterologous boost with Beta variant vaccine VBI-2905a given to animals that had received a single priming dose of ancestral strain vaccine VBI-2902a protected against the new Beta variant while also maintaining cross-reactivity against the ancestral strain. Moreover, heterologous eVLP boosting with VBI-2905a also induced high levels of antibody reactivity against the globally dominant Delta VOC. Additional challenge studies are in progress to evaluate if a heterologous boosting strategy can confer protection in Syrian golden hamsters against infection with the Delta variant.

Building upon the flexibility of the eVLP vaccine technology, we produced VBI-2901a, a multivalent coronavirus candidate containing S proteins from SARS-CoV-2, SARS-CoV, and MERS-CoV with the intent to broaden immunity to emerging VOC as well as novel, related betacoronaviruses that may infect humans in the future. Vaccines currently in use or in clinical evaluation that are based on the ancestral strain induce neutralizing antibody responses that are less reactive against the Beta VOC, with titers typically 5-10 lower than against the ancestral strain (Wibmer et al., 2021; Wang et al., 2021). In marked contrast, VBI-2901a elicited robust nAb responses not only against the ancestral SARS-CoV-2 strain, but also against the Beta variant, providing evidence of the vaccine candidate's ability to broaden immunity and "anticipate" an emerging variant not contained within the vaccine. High levels of cross-neutralizing activity elicited by VBI-2901a were also observed against the Delta and Kappa variants. Other studies have shown that plasma from individuals previously infected with SARS-CoV-1 who received the BNT162b2

mRNA vaccine, which is based on the ancestral SARS-CoV-2 virus, contained a broad spectrum of neutralizing antibodies against 10 sarbecoviruses tested, including SARS-COV-2 variants, several strains of SARS-CoV-1, and Bat and Pangolin CoV (Tan et al., 2021). Further studies are underway to better understand how VBI-2901a, which similarly exposes the B cell repertoire to spike proteins from both SARS-CoV-1 and SARS-CoV-2, broadens neutralizing activity against SARS-CoV-2 variants as well as to assess neutralizing responses to phylogenetically more distant coronaviruses.

Broadening of the neutralizing antibody response has also been shown using nanoparticles of mosaic RBD from various betacoronavirus species (Cohen et al., 2021; Walls et al., 2021). However, the N terminal domain of the S protein is another important target for neutralizing antibodies and the site of many mutations that could potentially contribute to antibody neutralization escape (Andreoni et al. 2021). Given that VBI-2901a expresses the full-length ectodomain of the Coronaviruses spike, it will be critical to determine the respective roles and importance of the RBD, NTD, and the highly conserved S2 domains in broadening immunity.

Whereas vaccines based on the ancestral strain of SARS-CoV-2 protect against severe disease caused by variants of concern, variants such as Beta are less sensitive to vaccine-induced immunity and efficacy rates are accordingly lower. This is likely to become more apparent as vaccine-induced immunity wanes and as variants continue to emerge with even greater numbers of mutations. One strategy to address these concerns is to administer booster doses to increase neutralizing antibody titers against the ancestral strain, a subset of which may cross-neutralize variants of concern. We have described three alternate strategies that have the potential to broaden immunity to a greater extent. An eVLP-based candidate based on the Beta variant S protein, VBI-2905a, induces potent immunity against not just the Beta virus, but also against the ancestral strain, though it is less potent against the Delta variant. However, building upon immunity induced against the ancestral strain with a priming dose of VBI-2902a, a single booster dose of VBI-2905a resulted in potent and more balanced neutralizing antibody responses against the ancestral virus, and Beta and Delta variants. Finally, we have described a novel trivalent eVLP candidate, VBI-2901a, which elicited potent and broad immunity against all variants tested,

including Beta, Delta, Lambda, and Kappa, with the testing for the potential to neutralize more
distantly related viruses currently underway.

Acknowledgment

The authors want to thank Adam Asselin, Matthew Yorke, Teresa Daoud, Rebecca Wang, Gillian
Lampkin (VBI vaccines) for outstanding technical support; NRC Animal Resources Group and the
VIDO Saskatchewan team for remarkable care with animal experiments.

Funding

The work performed in this manuscript was supported by the Coalition for Epidemic Preparedness
Innovations (CEPI)

References

1. Andreano E, Piccini G, Licastro D, Casalino L, Johnson NV, Paciello I, Dal Monego S,
Pantano E, Manganaro N, Manenti A, Manna R, Casa E, Hyseni I, Benincasa L, Montomoli
E, Amaro RE, McLellan JS, Rappuoli R. SARS-CoV-2 escape from a highly neutralizing
COVID-19 convalescent plasma. *Proc Natl Acad Sci U S A*. 2021 Sep
7;118(36):e2103154118. doi: 10.1073/pnas.2103154118. PMID: 34417349.
2. Berrio A, Gartner V, Wray GA. Positive selection within the genomes of SARS-CoV-2 and
other Coronaviruses independent of impact on protein function. *PeerJ*. 2020 Oct
16;8:e10234. doi: 10.7717/peerj.10234. PMID: 33088633; PMCID: PMC7571416.
3. Boni MF, Lemey P, Jiang X, Lam TT, Perry BW, Castoe TA, Rambaut A, Robertson DL.
Evolutionary origins of the SARS-CoV-2 sarbecovirus lineage responsible for the COVID-19
pandemic. *Nat Microbiol*. 2020 Nov;5(11):1408-1417. doi: 10.1038/s41564-020-0771-4.
Epub 2020 Jul 28. PMID: 32724171.
4. Cele S, Gazy I, Jackson L, Hwa SH, Tegally H, Lustig G, Giandhari J, Pillay S, Wilkinson E,
Naidoo Y, Karim F, Ganga Y, Khan K, Bernstein M, Balazs AB, Gosnell BI, Hanekom W,
Moosa MS; Network for Genomic Surveillance in South Africa; COMMIT-KZN Team,

- Lessells RJ, de Oliveira T, Sigal A. Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma. *Nature*. 2021 May;593(7857):142-146. doi: 10.1038/s41586-021-03471-w. Epub 2021 Mar 29. PMID: 33780970.
5. Cherian S, Potdar V, Jadhav S, Yadav P, Gupta N, Das M, Rakshit P, Singh S, Abraham P, Panda S, Team N. SARS-CoV-2 Spike Mutations, L452R, T478K, E484Q and P681R, in the Second Wave of COVID-19 in Maharashtra, India. *Microorganisms*. 2021 Jul 20;9(7):1542. doi: 10.3390/microorganisms9071542. PMID: 34361977; PMCID: PMC8307577.
6. Cohen AA, Gnanapragasam PNP, Lee YE, Hoffman PR, Ou S, Kakutani LM, Keeffe JR, Wu HJ, Howarth M, West AP, Barnes CO, Nussenzweig MC, Bjorkman PJ. Mosaic nanoparticles elicit cross-reactive immune responses to zoonotic coronaviruses in mice. *Science*. 2021 Feb 12;371(6530):735-741. doi: 10.1126/science.abf6840. Epub 2021 Jan 12. PMID: 33436524; PMCID: PMC7928838.
7. Deng X, Garcia-Knight MA, Khalid MM, Servellita V, Wang C, Morris MK, Sotomayor-González A, Glasner DR, Reyes KR, Gliwa AS, Reddy NP, Sanchez San Martin C, Federman S, Cheng J, Balcerek J, Taylor J, Streithorst JA, Miller S, Sreekumar B, Chen PY, Schulze-Gahmen U, Taha TY, Hayashi JM, Simoneau CR, Kumar GR, McMahon S, Lidsky PV, Xiao Y, Hemarajata P, Green NM, Espinosa A, Kath C, Haw M, Bell J, Hacker JK, Hanson C, Wadford DA, Anaya C, Ferguson D, Frankino PA, Shivram H, Lareau LF, Wyman SK, Ott M, Andino R, Chiu CY. Transmission, infectivity, and neutralization of a spike L452R SARS-CoV-2 variant. *Cell*. 2021 Jun 24;184(13):3426-3437.e8. doi: 10.1016/j.cell.2021.04.025. Epub 2021 Apr 20. PMID: 33991487; PMCID: PMC8057738.
8. Dreux M, Cosset FL. Detection of neutralizing antibodies with HCV pseudoparticles (HCVpp). *Methods Mol Biol*. 2009;510:427-38. doi: 10.1007/978-1-59745-394-3_32. PMID: 19009280.
9. Fluckiger AC, Ontsouka B, Bozic J, Diress A, Ahmed T, Berthoud T, Tran A, Duque D, Liao M, McCluskie M, Diaz-Mitoma F, Anderson DE, Soare C. An enveloped virus-like particle vaccine expressing a stabilized prefusion form of the SARS-CoV-2 spike protein elicits highly potent immunity. *Vaccine*. 2021 Aug 16;39(35):4988-5001. doi:

- 10.1016/j.vaccine.2021.07.034. Epub 2021 Jul 16. PMID: 34304928; PMCID: PMC8282453.
10. Garrone P, Fluckiger AC, Mangeot PE, Gauthier E, Dupeyrot-Lacas P, Mancip J, Cangialosi A, Du Chéné I, LeGrand R, Mangeot I, Lavillette D, Bellier B, Cosset FL, Tangy F, Klatzmann D, Dalba C. A prime-boost strategy using virus-like particles pseudotyped for HCV proteins triggers broadly neutralizing antibodies in macaques. *Sci Transl Med*. 2011 Aug 3;3(94):94ra71. doi: 10.1126/scitranslmed.3002330. PMID: 21813755.
11. Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, Ludden C, Reeve R, Rambaut A; COVID-19 Genomics UK (COG-UK) Consortium, Peacock SJ, Robertson DL. SARS-CoV-2 variants, spike mutations and immune escape. *Nat Rev Microbiol*. 2021 Jul;19(7):409-424. doi: 10.1038/s41579-021-00573-0. Epub 2021 Jun 1. PMID: 34075212; PMCID: PMC8167834.
12. Hoffmann M, Arora P, Groß R, Seidel A, Hörnich BF, Hahn AS, Krüger N, Graichen L, Hofmann-Winkler H, Kempf A, Winkler MS, Schulz S, Jäck HM, Jahrsdörfer B, Schrezenmeier H, Müller M, Kleger A, Münch J, Pöhlmann S. SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies. *Cell*. 2021 Apr 29;184(9):2384-2393.e12. doi: 10.1016/j.cell.2021.03.036. Epub 2021 Mar 20. PMID: 33794143; PMCID: PMC7980144.
13. European Centre for Disease Prevention and Control, SARS-Co-V-2 variants of concern as of 23 september 2021; <https://www.ecdc.europa.eu/en/covid-19/variants-concern>
14. U. S. Food & Drug Administration, Decelber 11, 2020 press release <https://www.fda.gov/news-events/press-announcements/fda-takes-key-action-fight-against-covid-19-issuing-emergency-use-authorization-first-covid-19>
15. World Health Organization, Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV); [https://www.who.int/news/item/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-\(2005\)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-\(2019-ncov\)](https://www.who.int/news/item/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov))

16. Kirchmeier M, Fluckiger AC, Soare C, Bozic J, Ontsouka B, Ahmed T, Diress A, Pereira L, Schödel F, Plotkin S, Dalba C, Klatzmann D, Anderson DE. Enveloped virus-like particle expression of human cytomegalovirus glycoprotein B antigen induces antibodies with potent and broad neutralizing activity. *Clin Vaccine Immunol*. 2014 Feb;21(2):174-80. doi: 10.1128/CVI.00662-13. Epub 2013 Dec 11. PMID: 24334684; PMCID: PMC3910943.
17. Kyriakidis NC, López-Cortés A, González EV, Grimaldos AB, Prado EO. SARS-CoV-2 vaccines strategies: a comprehensive review of phase 3 candidates. *NPJ Vaccines*. 2021 Feb 22;6(1):28. doi: 10.1038/s41541-021-00292-w. PMID: 33619260; PMCID: PMC7900244.
18. Lamb YN. BNT162b2 mRNA COVID-19 Vaccine: First Approval. *Drugs*. 2021 Mar;81(4):495-501. doi: 10.1007/s40265-021-01480-7. PMID: 33683637; PMCID: PMC7938284.
19. Plante JA, Liu Y, Liu J, Xia H, Johnson BA, Lokugamage KG, Zhang X, Muruato AE, Zou J, Fontes-Garfias CR, Mirchandani D, Scharon D, Bilello JP, Ku Z, An Z, Kalveram B, Freiberg AN, Menachery VD, Xie X, Plante KS, Weaver SC, Shi PY. Spike mutation D614G alters SARS-CoV-2 fitness. *Nature*. 2021 Apr;592(7852):116-121. doi: 10.1038/s41586-020-2895-3. Epub 2020 Oct 26. Erratum in: *Nature*. 2021 Jul;595(7865):E1. PMID: 33106671; PMCID: PMC8158177.
20. Smith EC, Denison MR. Implications of altered replication fidelity on the evolution and pathogenesis of coronaviruses. *Curr Opin Virol*. 2012 Oct;2(5):519-24. doi: 10.1016/j.coviro.2012.07.005. Epub 2012 Aug 1. PMID: 22857992; PMCID: PMC7102773.
21. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, Doolabh D, Pillay S, San EJ, Msomi N, Mlisana K, von Gottberg A, Walaza S, Allam M, Ismail A, Mohale T, Glass AJ, Engelbrecht S, Van Zyl G, Preiser W, Petruccione F, Sigal A, Hardie D, Marais G, Hsiao NY, Korsman S, Davies MA, Tyers L, Mudau I, York D, Maslo C, Goedhals D, Abrahams S, Laguda-Akingba O, Alisoltani-Dehkordi A, Godzik A, Wibmer CK, Sewell BT, Lourenço J, Alcantara LCJ, Kosakovsky Pond SL, Weaver S, Martin D, Lessells RJ, Bhiman JN, Williamson C, de Oliveira T. Detection of a SARS-CoV-2 variant of concern in

- South Africa. *Nature*. 2021 Apr;592(7854):438-443. doi: 10.1038/s41586-021-03402-9. Epub 2021 Mar 9. PMID: 33690265.
22. Wang R, Chen J, Gao K, Wei GW. Vaccine-escape and fast-growing mutations in the United Kingdom, the United States, Singapore, Spain, India, and other COVID-19-devastated countries. *Genomics*. 2021 Jul;113(4):2158-2170. doi: 10.1016/j.ygeno.2021.05.006. Epub 2021 May 15. PMID: 34004284; PMCID: PMC8123493.
23. Wibmer CK, Ayres F, Hermanus T, Madzivhandila M, Kgagudi P, Oosthuysen B, Lambson BE, de Oliveira T, Vermeulen M, van der Berg K, Rossouw T, Boswell M, Ueckermann V, Meiring S, von Gottberg A, Cohen C, Morris L, Bhiman JN, Moore PL. SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. *Nat Med*. 2021 Apr;27(4):622-625. doi: 10.1038/s41591-021-01285-x. Epub 2021 Mar 2. PMID: 33654292.
24. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, Hu Y, Tao ZW, Tian JH, Pei YY, Yuan ML, Zhang YL, Dai FH, Liu Y, Wang QM, Zheng JJ, Xu L, Holmes EC, Zhang YZ. A new coronavirus associated with human respiratory disease in China. *Nature*. 2020 Mar;579(7798):265-269. doi: 10.1038/s41586-020-2008-3. Epub 2020 Feb 3. Erratum in: *Nature*. 2020 Apr;580(7803):E7. PMID: 32015508; PMCID: PMC7094943.
25. Zhang L, Jackson CB, Mou H, Ojha A, Peng H, Quinlan BD, Rangarajan ES, Pan A, Vanderheiden A, Suthar MS, Li W, Izard T, Rader C, Farzan M, Choe H. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. *Nat Commun*. 2020 Nov 26;11(1):6013. doi: 10.1038/s41467-020-19808-4. PMID: 33243994; PMCID: PMC7693302.

Legend to figures

Figure 1: Immunogenicity of VBI-2902a and VBI-2905a in mice. C57BL/6 mice, 8 per group, received 2 IP injections 3 weeks apart, of VBI-2902a or VBI-2905a or a first injection of VBI-2902a followed by a second injection of VBI-2905a (Heterologous boost), each containing 0.1 µg of S. Blood was collected at day 14 after the second injection for monitoring of the humoral response.

455 **(a)** Sera from each group were analyzed in PRNT assay with a 90% threshold (PRNT90) using
 456 Wu-1 virus and Beta virus as described in Material and Methods. GMT and results from two-tailed
 457 Mann–Whitney U-test are indicated. **(b)** Change in neutralization between Wu-1 and Beta viruses.
 458 Fold change was calculated for each serum as the ratio between reactivity to Ancestral and Beta
 459 RBD, Fold change in each group is indicated as the geometric mean preceded by an arrow.
 460 Statistical analysis was determined using two tailed Wilcoxon test. **(c)** Ab binding titers were
 461 evaluated by ELISA using recombinant Delta RBD as described in Material and Methods.
 462 Statistical significance was determined by Kruskal-Wallis test.

463 **Figure 2: Beta variant challenge in Syrian golden hamsters after immunization with VBI-**
 464 **2902a and VBI-2905a. (a)** Schematic representation of the challenge experiments. Four groups of
 465 10 Syrian gold hamsters received 2 IM injections 3 weeks apart , of placebo saline buffer or VBI-
 466 2902a or VBI-2905a or a first injection of VBI-2902a followed by a second injection of VBI-2905a,
 467 with 1µg of S per dose. Animals in Placebo groups received Saline buffer. Blood was collected 2
 468 weeks after each injection. Three weeks after the last injection (day 42) hamsters were exposed to
 469 SARS-CoV-2 Beta virus at 1×10^5 TCID₅₀ per animal via both nares. At 3 days post infection (dpi),
 470 5 animals per groups were sacrificed for viral load analysis. The remaining animals were clinically
 471 evaluated daily until end of study at 14dpi. **(b)** Neutralization activity was measured by PRNT90 in
 472 immunized groups; results are represented as PRNT90 EPT. GMT and statistical significance from
 473 two tailed Friedman test are indicated **(c)** Hamsters were monitored daily for weight change.
 474 Results are represented for each animal in each groups as kinetic of weight change from day 0 to
 475 day 14 after infection. One animal from VBI-2905a group was sacrificed at day 7 because of
 476 worsening of clinical presentation after a fight in the cage. Significant days of weight loss relative to
 477 Saline group ($p < 0.005$) are indicated. Statistical analysis was performed with unpaired non
 478 parametric multiple t test using Holm-Šidák method.

479 **Figure 3: Immunogenicity of trivalent VBI-2901a.** Three groups of 10 mice were immunized with
 480 2 doses of VBI-2901a (01a) or VBI-2902a (02a) or VBI-2905a (05a) 3 weeks apart. Blood was
 481 collected at day 14 after the last injection for monitoring of the humoral response. **(a)** Neutralization

482 EPT measured by PRNT90 against Wu-1 virus and Beta variant. GMT are indicated above each
 483 group. **(b)** Neutralization of pseudoparticles expressing S from Wu-1 virus, or Delta or Kappa
 484 variants are represented as half-maximum inhibitory dilutions (Neutralization ID50). Geometric
 485 means are indicated above each panel. Due to technical limitations, only 8 sera per groups were
 486 tested against Wu-1 and Kappa pseudoparticles and 4 sera against Delta pseudoparticles. Sera
 487 were randomly picked. **(c)** Ab binding titers measured in ELISA against recombinant RBD from Wu-
 488 1 ancestral virus, or Beta, Delta, and Kappa variants.

|

489 | FIGURE 1

490 FIGURE 2

491 | FIGURE 3