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

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11   **Abstract**

12   Rapid emergence of SARS-CoV-2 variants is a constant threat and a major hurdle to reach heard

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 unmodifed 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.

Keywords

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

Abbreviations

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

Introduction

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

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

51   recently in India (B.1.617 – "Delta"). In late August the Mu variant first emerged in Columbia,

52   joining this list of Variants of Interest (VOI) together with Lambda while the incidence of Alpha was

53   decreasing.

54   The CoV spike (S) protein contains a receptor binding domain (RBD) critical for binding to

55   and infection of host cells, and is a major target for mutational changes which enhance adaptation

56   to the host (Berrio et al., 2020; Boni et al., 2020). Each of the VOC are characterized by a number

57   of shared mutations expressed on S, primarily located in the RBD and N-terminal domain (NTD),

58   that serve to increase inter-individual transmission, escape neutralizing antibodies acquired by

59   vaccination or prior natural SARS-CoV-2 infection, or both (Harvey et al., 2021; Zhang et al.,

60   2020). For instance, the first identified VOC, Alpha, is characterized by a D614G mutation, among

61   other mutations, that is now fixed in all globally circulating variants of the virus. D614G is

62   associated with increased transmissibility (Plante et al., 2021; Zhang et al., 2020) but does not

63   have a major impact on neutralization by serum from either vaccinated or COVID-19 convalescent

64   individuals. The following VOC that emerged in South Africa was rapidly identified as a vaccine-

65   escape mutant. This Beta variant bears several mutations in its RBD, including E484K and N501Y,

66   which significantly inhibit neutralizing activity elicited against the Ancestral Wu-1 strain of virus

67   whether acquired by vaccination or infection (Tegally et al., 2020; Cele et al., 2021). Emergence of

68   escape mutants is a major concern because most of the licensed vaccines are based on

69   expression of various forms of S using the Ancestral sequence of the S protein (Hoffmann et al.,

70   2021 ; Kyriakidis et al., 2021; Lamb, 2021). More recently, the Delta variant spread from India to

71   many countries with great speed in spite of significant proportions of fully vaccinated invididuals in

72   many countries. Delta shows the RBD mutation L452R which appeared independently in several

73   areas of the globe, including in variants Lambda, Kappa, Epsilon, Iota, and contributes to escape

74   neutralization from Abs induced by previously acquired immunity (Deng et al.2021). Additionally,

75   mutation P681R in the furin cleavage site of Delta could increase the rate of S1-S2 cleavage,

76   resulting in better transmissibility (Cherian et al., 2021).

77   Recently, we developed a SARS-CoV-2 candidate vaccine, VBI-2902a, comprised of

78   enveloped virus-like particles (eVLPs) expressing a modified prefusion form of the ancestral S

79   sequence, adjuvanted with aluminum phosphate (Alum). We recently demonstrated that VBI-2902a

80   induced strong neutralizing activity in mouse immunogenicity studies, and protected hamsters from

81   SARS-CoV-2 challenge using a virus related to the ancestral isolate (Fluckiger et al. 2021). Interim

82   results from a Phase I clinical study in healthy, seronegative individuals (ClinicalTrials.gov

83   Identifier: NCT04773665) demonstrated robust (4.3-fold greater) neutralizing activity 28 days after

84   a second, 5µg dose of VBI-2902a, relative to a panel of COVID-19 convalescent sera.

85   Employing the same strategy, we produced a new vaccine candidate, VBI-2905a, that

86   expresses a modified prefusion S based on the Beta variant sequence. Consistent with previous

87   studies, VBI-2905a elicited neutralizing antibody responses against the Beta variant which were

88   significantly greater than those induced by VBI-2902a, and responses against the ancestral strain

89   which were comparable to VBI-2902a. Consistent with the role of neutralizing antibody responses

90   as a presumed correlate of protection, greater efficacy was observed in hamsters vaccinated with

91   VBI-2905a relative to VBI-2902a when challenged with the Beta variant. Noteworthy was the

92   observation in an alternative vaccination regimen that priming with VBI-2902a followed by a single

93   booster dose of VBI-2905a induced strong neutralizing antibody responses against the ancestral

94   strain as well as both Beta and Delta VOC.

95   We also evaluated immunity elicited with a distinct eVLP-based candidate, VBI-2901a,

96   which expresses a modified prefusion S based on the ancestral sequence in addition to the related

97   S proteins from SARS CoV-1 and MERS. Immunization with VBI-2901a induced neutralizing

98   antibody titers against the Beta variant significantly greater than VBI-2902a and comparable to

99   those induced with VBI-2905a, effectively broadening immunity to VOC not contained within the

100 vaccine. Antibody binding and neutralizing titers against an extended panel of variants

101 demonstrated responses typically 3-fold greater than that observed with VBI-2902a. Collectively,

102 these results demonstrate multiple ways to broaden immunity to SARS-CoV-2 VOC.

103 **Material and Methods**

104 *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 specificty 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 previsouly. 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 pjm155, 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 Kruskall-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 974x10^3) of Ab

211 binding titers against the ancestral S RBD with significantly reduced cross-reactivity against the

212 Beta variant RBD (GMT 74x10^3), though there was good cross-reactivity against the Delta variant

213 RBD (GMT 616x10^3). Antisera from immunization with VBI-2905a showed similar crossreactivty

214 against Ancestral, Delta and Beta RBD (respectively GMT 322x10^3, 192x10^3 and 217x10^3). 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 1x10^5 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

261 but has been replaced by variants containing mutations which are enabling escape from nAbs

262 induced against the ancestral strain (Berio et al. 2020; Boni et al, 2020). In the present study, we

263 compared several strategies to broaden antibody-based immunity which is presumed to be a

264 correlate of protection against SARS-CoV-2.

265   In addition to our eVLP vaccine expressing the prefusion S from the ancestral SARS-CoV-2

266 virus, we produced an eVLP-based vaccine expressing the prefusion S from Beta variant. Beta

267 was chosen for its deleterious mutations E484K and K417N, which enable escape neutralization

268 from ancestral virus mAbs (Hoffman et al, 2021). We have previously demonstrated that 2 doses

269 of VBI-2902a protected hamsters against infection by the ancestral Wuhan SARS-CoV-2 virus and

270 we confirmed here that VBI-2905a also protected hamsters from infection with the SARS-CoV-2

271 Beta variant. We have also demonstrated that a heterologous boost with Beta variant vaccine VBI-

272 2905a given to animals that had received a single priming dose of ancestral strain vaccine VBI-

273 2902a protected against the new Beta variant while also maintaining cross-reactivity against the

274 ancestral strain Moreover, heterologous eVLP boosting with VBI-2905a also induced high levels of

275 antibody reactivity against the globally dominant Delta VOC. Additional challenge studies are in

276 progress to evaluate if a heterologous boosting strategy can confer protection in Syrian golden

277 hamsters against infection with the Delta variant.

278   Building upon the flexibility of the eVLP vaccine technology, we produced VBI-2901a, a

279 multivalent coronavirus candidate containing S proteins from SARS-CoV-2, SARS-CoV, and

280 MERS-CoV with the intent to broaden immunity to emerging VOC as well as novel, related

281 betacoronaviruses that may infect humans in the future. Vaccines currently in use or in clinical

282 evaluation that are based on the ancestral strain induce neutralizing antibody responses that are

283 less reactive against the Beta VOC, with titers typically 5-10 lower than against the ancestral strain

284 (Wibmer et al., 2021; Wang et al., 2021). In marked contrast, VBI-2901a elicited robust nAb

285 responses not only against the ancestral SARS-CoV-2 strain, but also against the Beta variant,

286 providing evidence of the vaccine candidate's ability to broaden immunity and "anticipate" an

287 emerging variant not contained within the vaccine. High levels of cross-neutralizing activity elicited

288 by VBI-2901a were also observed against the Delta and Kappa variants. Other studies have shown

289 that plasma from individuals previously infected with SARS-CoV-1 who received the BNT162b2

290   mRNA vaccine, which is based on the ancestral SARS-CoV-2 virus, contained a broad spectrum of

291   neutralizing antibodies against 10 sarbecoviruses tested, including SARS-COV-2 variants, several

292   strains of SARS-CoV-1, and Bat and Pangolin CoV (Tan et al., 2021). Further studies are

293   underway to better understand how VBI-2901a, which similarly exposes the B cell repertoire to

294   spike proteins from both SARS-CoV-1 and SARS-CoV-2, broadens neutralizing activity against

295   SARS-CoV-2 variants as well as to assess neutralizing responses to phylogenetically more distant

296   coronaviruses.

297   Broadening of the neutralizing antibody response has also been shown using nanoparticles

298   of mosaïc RBD from various betacoronavirus species (Cohen et al., 2021; Walls et al., 2021).

299   However, the N terminal domain of the S protein is another important target for neutralizing

300   antibodies and the site of many mutations that could potentially contribute to antibody

301   neutralization escape (Andreoni et al. 2021). Given that VBI-2901a expresses the full-length

302   ectodomain of the Coronaviruses spike, it will be critical to determine the respective roles and

303   importance of the RBD, NTD, and the highly conserved S2 domains in broadening immunity.

304   Whereas vaccines based on the ancestral strain of SARS-CoV-2 protect against severe

305   disease caused by variants of concern, variants such as Beta are less sensitive to vaccine-induced

306   immunity and efficacy rates are accordingly lower. This is likely to become more apparent as

307   vaccine-induced immunity wanes and as variants continue to emerge with even greater numbers of

308   mutations. One strategy to address these concerns is to administer booster doses to increase

309   neutralizing antibody titers against the ancestral strain, a subset of which may cross-neutralize

310   variants of concern. We have described three alternate strategies that have the potential to

311   broaden immunity to a greater extent. An eVLP-based candidate based on the Beta variant S

312   protein, VBI-2905a, induces potent immunity against not just the Beta virus, but also against the

313   ancestral strain, though it is less potent against the Delta variant. However, building upon immunity

314   induced against the ancestral strain with a priming dose of VBI-2902a, a single booster dose of

315   VBI-2905a resulted in potent and more balanced neutralizing antibody responses against the

316   ancestral virus, and Beta and Delta variants. Finally, we have described a novel trivalent eVLP

317   candidate, VBI-2901a, which elicited potent and broad immunity against all variants tested,

318 including Beta, Delta, Lambda, and Kappa, with the testing for the potential to neutralize more

319 distantly related viruses currently underway.

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450   **Legend to figures**

451   **Figure 1: Immunogenicity of VBI-2902a and VBI-2905a in mice.** C57BL/6 mice, 8 per group,

452 received 2 IP injections 3 weeks apart , of VBI-2902a or VBI-2905a or a first injection of VBI-2902a

453 followed by a second injection of VBI-2905a (Heterologous boost), each containing 0.1 µg of S.

454 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 preceeded 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.

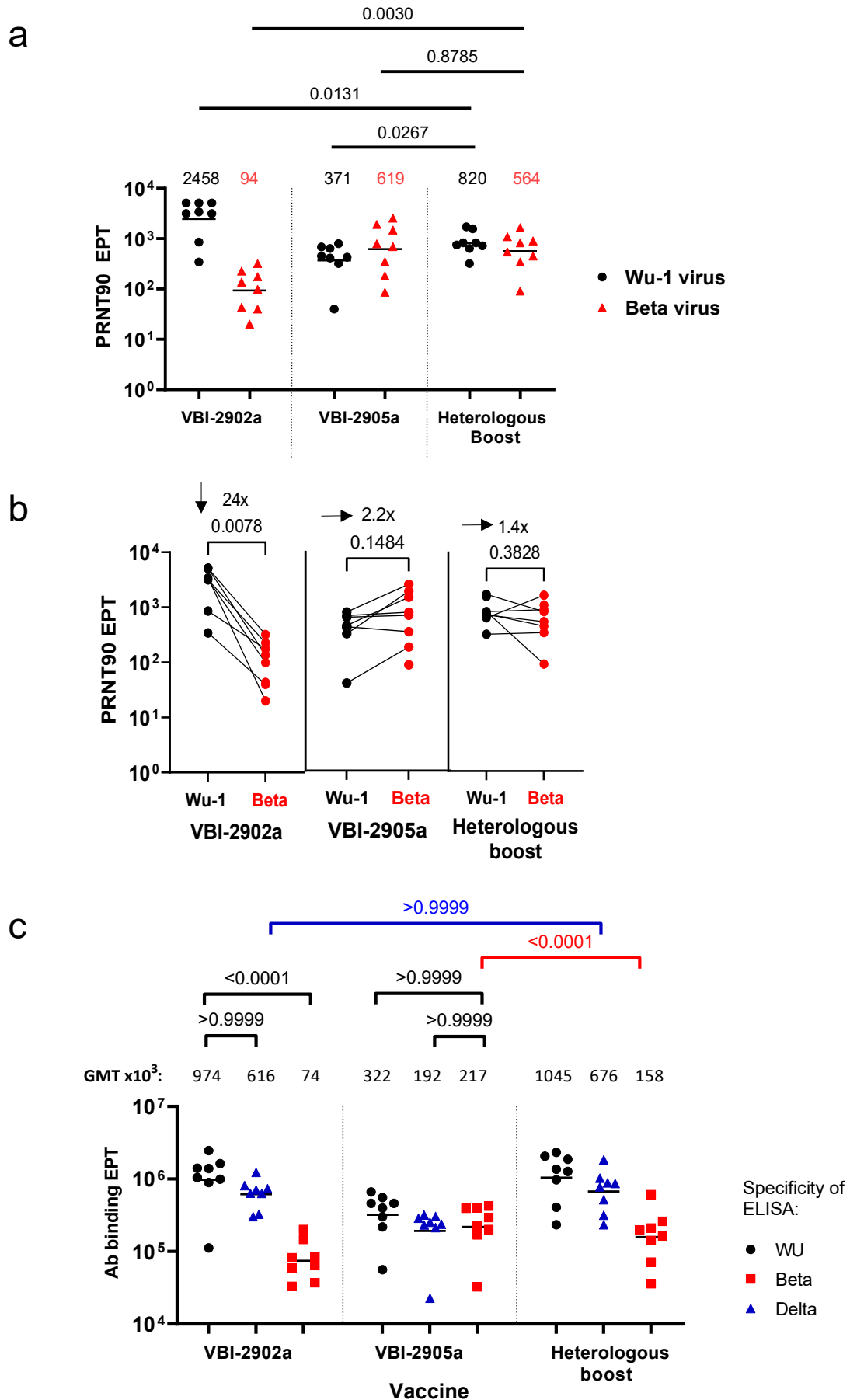462 Statistical significance was determined by Kruskall-Wallis test.

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
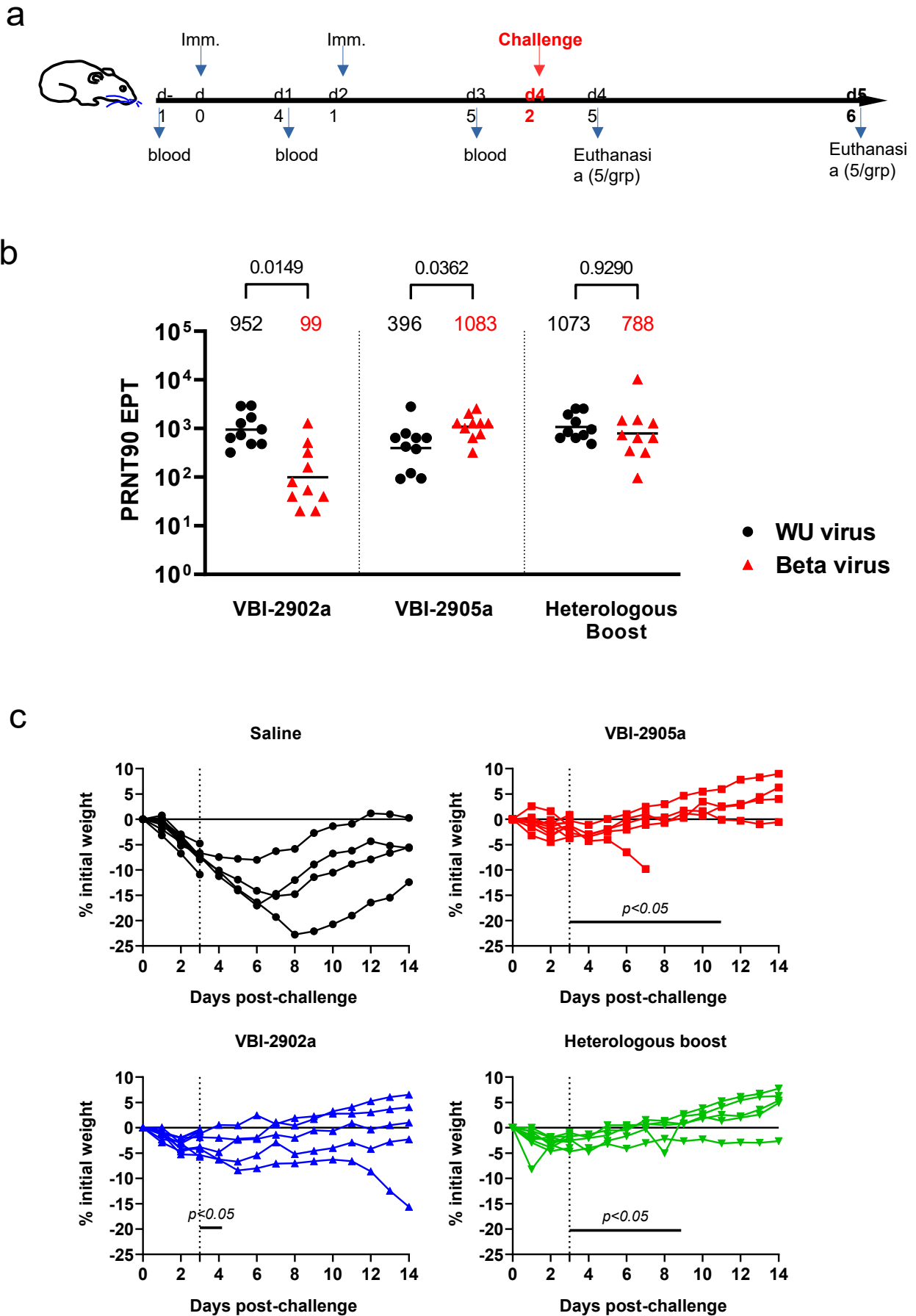
482   EPT measured by PRNT90 against Wu-1 virus and Beta variant. GMT are indicated above each

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