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1 **Variant SARS-CoV-2 mRNA vaccines confer broad neutralization as primary or booster**
2 **series in mice**

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16 **Abbreviations:**

17 ACE2, angiotensin converting enzyme 2; ELISA, enzyme-linked immunosorbent assay; GMT,
18 geometric mean titer; ID50, inhibitory dilution factor; IgG, immunoglobulin G; LNP, lipid
19 nanoparticle; Nab, neutralizing antibody; NHPs, non-human primates; ns, not significant; NTD,
20 N-terminal domain; PBS, phosphate-buffered saline; PsVN, pseudovirus neutralization titer;
21 RBD, receptor binding domain; RLUs, relative luminescence units; S, spike; SARS-CoV-2,
22 severe acute respiratory syndrome coronavirus 2; TMB, tetramethylbenzidine; UTR, untranslated
23 region; VOC, variant of concern; VOI, variant of interest; VSV, vesicular stomatitis virus.

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24 **Abstract**

25 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of a global
26 pandemic. Safe and effective COVID-19 vaccines are now available, including mRNA-1273,
27 which has shown 94% efficacy in prevention of symptomatic COVID-19 disease. However, the
28 emergence of SARS-CoV-2 variants has led to concerns of viral escape from vaccine-induced
29 immunity. Several variants have shown decreased susceptibility to neutralization by vaccine-
30 induced immunity, most notably B.1.351 (Beta), although the overall impact on vaccine efficacy
31 remains to be determined. Here, we present the initial evaluation in mice of 2 updated mRNA
32 vaccines designed to target SARS-CoV-2 variants: (1) monovalent mRNA-1273.351 encodes for
33 the spike protein found in B.1.351 and (2) mRNA-1273.211 comprising a 1:1 mix of mRNA-1273
34 and mRNA-1273.351. Both vaccines were evaluated as a 2-dose primary series in mice; mRNA-
35 1273.351 was also evaluated as a booster dose in animals previously vaccinated with mRNA-1273.
36 The results demonstrated that a primary vaccination series of mRNA-1273.351 was effective at
37 increasing neutralizing antibody titers against B.1.351, while mRNA-1273.211 was effective at
38 providing broad cross-variant neutralization. A third (booster) dose of mRNA-1273.351
39 significantly increased both wild-type and B.1.351-specific neutralization titers. Both mRNA-
40 1273.351 and mRNA-1273.211 are being evaluated in pre-clinical challenge and clinical studies.

41 **Keywords:** COVID-19, SARS-CoV-2 variants of concern, mRNA-1273, primary series, booster
42 dose, neutralization

43

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44 **1. Introduction**

45 Since the declaration of a global pandemic by the World Health Organization on March 11,
46 2020, infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led
47 to approximately 4.7 million deaths worldwide [1, 2]. Shortly after the SARS-CoV-2 genetic
48 sequence was determined, mRNA-1273—a novel lipid nanoparticle (LNP) encapsulated
49 messenger RNA-based vaccine encoding for a prefusion-stabilized, full-length spike (S)
50 glycoprotein of the Wuhan-Hu-1 isolate of SARS-CoV-2—was developed [3, 4]. Vaccination with
51 two 100 μg doses of mRNA-1273 four weeks apart was 94% efficacious against symptomatic
52 COVID-19 disease; mRNA-1273 was granted Emergency Use Authorization by the Food and
53 Drug Administration in December 2020 [5, 6].

54 The emergence of SARS-CoV-2 variants with substitutions in the receptor binding domain
55 (RBD) and N-terminal domain (NTD) of the viral S protein has raised concerns among scientists
56 and health officials [7-10]. The entry of coronaviruses into host cells is mediated by interaction
57 between the RBD of the viral S protein and the host receptor, angiotensin-converting enzyme 2
58 (ACE2) [3, 11-14]. Several studies have shown that the RBD is the main target of neutralizing
59 antibodies against SARS-CoV-2 [4, 15-17]. A neutralization "supersite" has also been identified
60 in the NTD [18]. A decrease in vaccine-mediated viral neutralization has been correlated with
61 amino acid substitutions in the RBD (eg, K417T/N, E484K, and N501Y) and NTD (eg, L18F,
62 D80A, D215G, and Δ242-244) of the S protein. Some of the most recently circulating variants of
63 concern (VOCs) and variants of interest (VOIs) with key mutations in the RBD and NTD—
64 including B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.526 (Iota), and B.1.427/B.1.429
65 (Epsilon or CAL.20C) lineages—have shown reduced susceptibility to neutralization from
66 convalescent serum and resistance to monoclonal antibodies [18-25]. Note that mutations in the

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67 NTD domain, specifically the neutralization supersite, are extensive in the B.1.351 lineage virus
68 [18].

69 Using 2 orthogonal pseudovirus neutralization (PsVN) assays based on vesicular stomatitis
70 virus (VSV) and lentivirus expressing S variants, neutralizing capacity of sera from phase 1
71 participants and non-human primates (NHPs) that received 2 doses of mRNA-1273 was reported
72 [26]. No significant impact on neutralization against the B.1.1.7 variant was observed. However,
73 reduced neutralization was measured against the B.1.351 variant, and to a lesser extent, in the P.1
74 B.1.427/B.1.429 and B.1.1.7+E484K variants [26]. Clinical studies in South Africa demonstrated
75 reduced efficacy against symptomatic COVID-19 disease for the NVX-CoV2373 (Novavax),
76 AZD1222 (University of Oxford/AstraZeneca), and Ad26.COV2.S (Janssen/Johnson & Johnson)
77 vaccines [27-30]. Pfizer/BioNTech has recently reported high efficacy of the mRNA BNT162b2
78 vaccine against B.1.351 among a small number of recipients from South Africa in the phase 2–3
79 portion of a global phase 1–2–3 trial; however, a report from Israel suggests increased
80 breakthrough infection rates by B.1.351 in BNT162b2 vaccinated individuals at 7–14 days after
81 the second dose [31, 32]. Studies have demonstrated reduced neutralization titers against the full
82 B.1.351 variant following mRNA-1273 vaccination, although levels are still significant and
83 expected to be protective based on challenge studies in NHPs [26, 33, 34]. Despite this prediction
84 of continued efficacy of mRNA-1273 against this key variant of concern (VOC), the magnitude
85 and duration of vaccine-mediated protection is still unknown. Moreover, a key related question is
86 whether development of new mRNA vaccines to match the B.1.351 variant will enable enhanced
87 neutralization responses and durability.

88 Herein, we present the design and pre-clinical evaluation of updated mRNA-1273 vaccines
89 against SARS-CoV-2 variants, which include monovalent mRNA-1273.351 and a multivalent

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90 mRNA-1273.211. Like mRNA-1273, mRNA-1273.351 encodes the prefusion stabilized S protein
91 of SARS-CoV-2; however, in contrast to mRNA-1273, mRNA-1273.351 incorporates key
92 mutations present in the B.1.351 variant, including L18F, D80A, D215G, Δ242-244, R246I,
93 K417N, E484K, N501Y, D614G, and A701V (Fig. 1). To expand the breadth of coverage to
94 multiple circulating variants as well as the ancestral wild-type virus that is still circulating globally,
95 mRNA-1273.211 is a 1:1 mix of mRNA-1273 and mRNA-1273.351. Two initial studies were
96 performed in BALB/c mice to evaluate the immunogenicity of mRNA-1273.351 or mRNA-
97 1273.211 as a primary series and/or as a booster. The first study assessed the immunogenicity of
98 a primary series (day 0, 21) of mRNA-1273.351 or mRNA-1273.211 versus mRNA-1273. A
99 second study evaluated the immunogenicity of a third (booster) dose of mRNA-1273.351 213 days
100 after mice were vaccinated with a primary series of mRNA-1273, and immunogenicity was
101 assessed before and after the booster dose.

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102 ## 2. Methods

103 ### *2.1 Data reporting*

104 No statistical methods were used to predetermine sample size. The experiments were not
105 randomized and the investigators were not blinded to allocation during experiments and outcome
106 assessment.

107 ### *2.2 Pre-clinical vaccine mRNA and LNP production process*

108 A sequence-optimized mRNA encoding prefusion-stabilized Wuhan-Hu-1 or B.1.351-variant
109 SARS-CoV-2 S-2P protein was synthesized in vitro using an optimized T7 RNA polymerase-
110 mediated transcription reaction with complete replacement of uridine by N1m-pseudouridine [35].
111 The reaction included a DNA template containing the immunogen open-reading frame flanked by
112 5' untranslated region (UTR) and 3' UTR sequences and was terminated by an encoded polyA tail.
113 After transcription, the cap-1 structure was added to the 5' end using the *Vaccinia* capping enzyme
114 (New England Biolabs) and *Vaccinia* 2'-O-methyltransferase (New England Biolabs). The mRNA
115 was purified by oligo-dT affinity purification, buffer exchanged by tangential flow filtration into
116 sodium acetate, pH 5.0, sterile filtered, and kept frozen at –20°C until further use.

117 The mRNA was encapsulated in an LNP through a modified ethanol-drop nanoprecipitation
118 process described previously [36]. Ionizable, structural, helper, and polyethylene glycol lipids
119 were briefly mixed with mRNA in an acetate buffer, pH 5.0, at a ratio of 2.5:1 (lipids:mRNA).
120 The mixture was neutralized with Tris-HCl, pH 7.5, sucrose was added as a cryoprotectant, and
121 the final solution was sterile-filtered. Vials were filled with formulated LNP and stored frozen at
122 –70°C until further use. The drug product underwent analytical characterization—which included
123 the determination of particle size and polydispersity, encapsulation, mRNA purity, double-

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124 stranded RNA content, osmolality, pH, endotoxin, and bioburden—and the material was deemed
125 acceptable for in vivo study.

126 ***2.3 Mouse model***

127 Animal experiments were carried out in compliance with approval from the Animal Care and
128 Use Committee of Moderna Inc. Female BALB/c mice (6 to 8 weeks old; Charles River
129 Laboratories) were used. mRNA formulations were diluted in 50 µL of 1X phosphate-buffered
130 saline (PBS), and mice were inoculated via intramuscular injection into the same hind leg for both
131 prime, boost, and third dose. Control mice received PBS because prior studies have demonstrated
132 that tested mRNA formulations do not create significant levels of non-specific immunity beyond
133 a few days [37-39]. Sample size for animal experiments was determined on the basis of criteria set
134 by the institutional Animal Care and Use Committee. Experiments were neither randomized nor
135 blinded.

136 ***2.4 Enzyme-linked Immunosorbent Assay (ELISA)***

137 Microtiter plates (96-well; Thermo) were coated with 1 µg/mL S-2P protein (Genscript)
138 corresponding to the S protein of the Wuhan-Hu-1 virus. After overnight incubation at 4°C, plates
139 were washed four times with PBS/0.05% Tween-20 and blocked for 1.5 hours at 37°C
140 (SuperBlock-Thermo). After washing, five-fold serial dilutions of mouse serum were added (assay
141 diluent: 0.05% Tween-20 and 5% goat serum in PBS). Plates were incubated for 2 hours at 37°C,
142 washed and horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG)
143 (Southern Biotech) was added at a 1:20,000 dilution (S-2P) in assay diluent. Plates were incubated
144 for 1 hour at 37°C, washed, and bound antibody was detected with a 3,3',5,5'-tetramethylbenzidine
145 (TMB) substrate (SeraCare). After incubation for 10 minutes at room temperature, the reaction
146 was stopped by adding a TMB stop solution (SeraCare) and the absorbance was measured at 450

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147 nm. Titers were determined using a four-parameter logistic curve fit in Prism v.8 (GraphPad
148 Software, Inc.) and defined as the reciprocal dilution at approximately optical density$_{450}$ = 1.0
149 (normalized to a mouse standard on each plate).

150 ***2.5 Recombinant VSV-based PsVN assay***

151 Codon-optimized full-length S protein of the original Wuhan-Hu-1 variant with D614G
152 mutation (D614G) or the indicated S variants, listed in Table 1, were cloned into a pCAGGS vector.
153 To make SARS-CoV-2 full-length S pseudotyped recombinant VSV-ΔG-firefly luciferase virus,
154 BHK-21/WI-2 cells (Kerafast) were transfected with the S expression plasmid and subsequently
155 infected with VSVΔG-firefly-luciferase as previously described [40]. For a neutralization assay,
156 serially diluted serum samples were mixed with pseudovirus and incubated at 37°C for 45 minutes.
157 The virus-serum mix was subsequently used to infect A549-hACE2-TMPRSS2 cells [41] for 18
158 hours at 37°C before adding ONE-Glo reagent (Promega) for measurement of the luciferase signal
159 by relative luminescence units (RLUs). The percentage of neutralization was calculated based on
160 the RLUs of the virus-only control, and subsequently analyzed using four-parameter logistic curve
161 (Prism v.8).

162 ***2.6 Statistical Analysis.***

163 Animal studies were completed once with all in vitro testing completed in duplicate or
164 triplicate with 1 replicate, unless otherwise stated. Two-sided Wilcoxon matched-pairs signed rank
165 test was used to compare the same animals against different viruses or at different time points.
166 Statistical analyses were performed (Prism v.8). Geometric mean titers with 95% CIs and lower
167 limits of detection are included, where applicable.

168

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169

170 ## 3. Results

171 ### *3.1 **mRNA-1273.351 and mRNA-1273.211 were immunogenic as a primary vaccination***
172 ***series in mice***

173 The immunogenicity of mRNA-1273.351 and mRNA-1273.211 vaccines against the original
174 Wuhan-Hu-1 variant with the D614G mutation (referred to as wild-type) and the B.1.351 variant
175 was evaluated in BALB/c mice 2 weeks after the first and second injection (Fig. 1). Animals were
176 vaccinated with 1 or 10 µg of mRNA-1273, mRNA-1273.351, or mRNA-1273.211 on a 0, 21-day
177 schedule (Fig. 2a). S protein-binding antibody titers were assessed using a Wuhan-Hu-1 S-2P
178 ELISA. In addition, neutralizing antibody titers were assessed in a VSV-based PsVN assay against
179 wild-type and variant viruses (Table 1).

180 High levels of binding antibody were elicited by vaccination in all animals 2 weeks after the
181 first and second injection, with 4.5 to 9.4-fold increased S-2P binding titers measured after the
182 second dose (Fig. 2b). Slightly lower antibody levels were observed for mRNA-1273.351
183 compared with mRNA-1273, potentially due to the coating S-2P protein used in the ELISA being
184 homologous to mRNA-1273. These results demonstrate that both mRNA-1273.351 and mRNA-
185 1273.211 are immunogenic in mice. mRNA-1273 elicited higher neutralization titers against the
186 D614G than B.1.351 pseudovirus (Fig. 2c,d), although the approximate 2-fold difference was
187 smaller than previously measured with phase 1 clinical trial sera [26]. mRNA-1273.351 elicited
188 higher neutralization titers against B.1.351 compared with the D614G pseudovirus (Fig. 2c,d),
189 with an approximate 4-fold difference in measured titers. When mRNA-1273.211 was used to
190 vaccinate mice, similar neutralization titers were elicited against both the D614G and B.1.351
191 pseudoviruses (Fig. 2c,d), with no significant difference in neutralization titers. Thus, vaccination

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192 of mice with mRNA-1273.351 elicited high levels of neutralizing antibody against the B.1.351
193 pseudovirus and comparably lower levels versus D614G, whereas the multivalent mRNA-
194 1273.211 vaccine stimulated robust neutralization responses against both D614G and B.1.351
195 pseudoviruses.

196 Sera from mice collected 2 weeks after the second injection was also assessed against SARS-
197 CoV-2 variants that emerged in Brazil (P.1) and California (B.1.427/B.1.429 or CAL.20C). As
198 described in Table 1, some of the mutations in these variants were different from both the Wuhan-
199 Hu-1 and B.1.351 lineages, although the RBD mutations (K417T/N, E484K, N501Y) are common
200 to both the P.1 and B.1.351 viruses. As observed in previous assessments of NHPs and clinical trial
201 sera [26], mice vaccinated with mRNA-1273 showed an approximate 2-fold reduction in
202 neutralizing antibody levels against both the CAL.20C and P.1 variants (Fig. 2e). These reductions
203 in neutralization titers against CAL.20C and P.1 variants were more pronounced in mice vaccinated
204 with mRNA-1273.351 (3.7-fold and 2.6-fold reductions in geometric mean titers [GMTs] for
205 mRNA-1273.351 compared with mRNA-1273, respectively). However, the multivalent mRNA-
206 1273.211 vaccine neutralized these variants similarly to mRNA-1273.

207 *3.2 **mRNA-1273.351 was an effective third (booster) dose in animals previously vaccinated** 208 **with a primary vaccination series of mRNA-1273***

209 To evaluate the ability of the mRNA-1273.351 to boost pre-existing immunity and increase
210 neutralization against both the wild-type and the B.1.351 virus, BALB/c mice were immunized
211 with 1 or 0.1 µg mRNA-1273 on day 1 and 22, and the level and durability of the antibody
212 responses were evaluated over the course of 7 months. Sera collection occurred on day 212, and a
213 third dose of 1 or 0.1 µg mRNA-1273.351 was administered on day 213 (Fig. 3a).

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214 High levels of binding antibody were elicited by vaccination with mRNA-1273, with peak
215 titers measured 2 weeks after the second dose (Fig. 3b). After an initial drop in antibody levels,
216 titers were stable over the 7-month monitoring period. Following the mRNA-1273.351 booster
217 injection, antibody levels dramatically rose, exceeding the previously measured peak for both the
218 1 and 0.1 µg dose levels.

219 Neutralization titers were measured in the D614G PsVN assay on day 36 and 212, 1 day prior
220 to the third dose. Titers remained high, with an ~1.5-fold drop measured over that period. Note
221 that neutralizing titers at day 212 were measured in both the D614G and B.1.351 PsVN assays
222 (Fig. 3c-e), with 6.6-fold higher titers measured in the D614G PsVN assay; this difference is
223 similar to neutralization reductions observed with sera of NHPs and humans who received 2 doses
224 of mRNA-1273 and increases the potential correlation of this animal model to what may be
225 observed in humans.

226 The third dose of mRNA-1273.351 increased neutralization titers 4.5- and 15-fold in the
227 D614G and B.1.351 PsVN assays, respectively (Fig. 3c,d). The difference between the 2 assays
228 narrowed to 2-fold following the booster dose (Fig. 3c,e); the GMT of 15,524 against B.1.351 was
229 ~1.5 fold higher than the peak titer against D614G 2 weeks after the second dose (Fig. 3c). Animals
230 vaccinated at the 0.1 µg dose level had lower titers, but similar trends for binding antibody and
231 neutralization titers were observed (data not shown).

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232 **4. Discussion**

233 In this study, mRNA-1273.351 and mRNA-1273.211 were evaluated in mice as both a primary
234 vaccination series and as a third booster dose in animals previously vaccinated with 2 injections
235 of mRNA-1273. As a primary vaccination series, both vaccines were potently immunogenic after
236 the first injection, with both S-2P binding and neutralizing antibody titers significantly increasing
237 after the second injection. Neutralizing activity of mRNA-1273.351 against the D614G variant
238 was 4-fold lower than that against the B.1.351 variant and 6.3-fold lower against D614G variant
239 compared to mRNA-1273. In contrast, the multivalent mRNA-1273.211 vaccine elicited robust
240 and comparable neutralizing titers against both D614G and B.1.351, which closely match those
241 observed against D614G after mRNA-1273 vaccination. Thus, as a primary vaccination series, a
242 multivalent approach appears most effective in broadening immune responses—as neutralization
243 potency was enhanced against both B.1.351 and P.1 versus mRNA-1273 and remained significant
244 against B.1.427/B.1.429/CAL.20C.

245 mRNA-1273.351 was evaluated as a booster injection in mice vaccinated with mRNA-1273
246 approximately 7 months previously. The third dose of mRNA-1273.351 dramatically boosted both
247 S-2P binding antibody titers (Fig. 3a,b) and D614G and B.1.351 PsV neutralization titers (Fig. 3c-
248 e). Neutralizing titers against B.1.351 PsV increased to levels well above the peak neutralizing
249 titer against D614G after the second dose of mRNA-1273, the latter of which was fully protective
250 in mice challenged with the mouse-adapted USA-WA1-F6/2020 variant [41]. In addition, the
251 booster dose also increased neutralizing titers against D614G, although the fold-increase was less
252 than that against B.1.351, as expected. Overall, the third injection of mRNA-1273.351
253 dramatically increased both D614G and B.1.351 neutralization titers, with titers much higher than
254 the day 36 peak. Further, the difference in titers measured in the D614G and B.1.351 PsVN assays

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255 decreased from a 6.6-fold difference prior to the boosting dose, to a 2-fold difference 2 weeks after
256 the third dose.

257 The number of animals available for boosting in this study allowed evaluation of only 1
258 boosting scenario (ie, mRNA-1273.351). Ongoing studies will evaluate the ability of mRNA-1273,
259 mRNA-1273.351, and mRNA-1273.211 to effectively boost immunity driven by a primary
260 vaccination series of mRNA-1273. Studies will also evaluate mRNA-1273.351 and mRNA-
261 1273.211 in additional primary vaccination and boosting studies in mice, golden Syrian hamsters,
262 and rhesus macaques, with either Wuhan-Hu-1 or B.1.351 challenge planned. These studies are
263 designed to assess the level of neutralization of sera derived from vaccinated animals against
264 pseudoviruses with either the Wuhan-Hu-1 D614G or the B.1.351 S proteins and the level of
265 protection provided against Wuhan-Hu-1 and B.1.351 challenge. Global surveillance for the
266 emergence of additional SARS-CoV-2 VOCs and the neutralization of VOCs by mRNA-1273
267 vaccinee sera are also ongoing. If additional variants emerge that reduce the neutralization capacity
268 of mRNA-1273 further, additional mRNA vaccine designs may be developed and evaluated. In
269 this study, the multivalent mRNA-1273.211 vaccine has already demonstrated to be an
270 immunogenic strategy against multiple variants, and ongoing preclinical and clinical studies will
271 provide further evidence of the utility of using a booster dose of mRNA-1273.211. This approach
272 also supports seasonal adjustment, allowing for changes in response to the evolution of the SARS-
273 CoV-2 virus.

274 Several potential limitations to the current study should be highlighted. Sera from mRNA-1273
275 vaccinated NHP or human sera was shown to have 6-8 fold reduced neutralizing activity against
276 the B.1.351 variant SARS-CoV-2 in several assessments, although the level of neutralization
277 remained at levels that are predicted to be protective [26]. Results in this study, however, showed

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278 that after the primary series of mRNA-1273, only a 2-fold reduced neutralization against the
279 B.1.351 virus was evident 2 weeks after the second dose. A 6.6-fold reduction was seen at day 212,
280 more relevant to what has been measured from human sera. Further studies in animal species more
281 predictive of responses in humans such as NHPs as well as clinical studies in humans are ongoing.
282 Further, only mRNA-1273.351 was evaluated as a third dose. The ability of the multivalent
283 mRNA-1273.211 vaccine to boost immunity against both the D614G and B.1.351 viruses has not
284 yet been assessed in preclinical models, although a recent exploratory analysis among a subset of
285 patients in a clinical trial showed a mRNA-1273.211 booster dose increased neutralization against
286 D614G and several VOCs, including B.1.351 [42]. Finally, very little antibody waning was
287 measured in the 7-month evaluation of antibody levels in mice prior to the delivery of the boosting
288 dose. This level of durability may not be reflective of that measured in NHPs or humans [42,43].
289 The emergence of SARS-CoV-2 variants and the ability of the virus to partially overcome
290 natural or vaccine-induced immunity served as a call to action. Not only are continued vaccination
291 efforts needed to prevent the emergence of future VOCs, but strategies are needed for new SARS-
292 CoV-2 vaccine research and development that can enhance the level of protection against key
293 VOCs should they arise. The mRNA vaccine platform approach against SARS-CoV-2 VOCs has
294 now been demonstrated in mice to be effective at broadening neutralization across variants and to
295 boost antibody levels when applied as a third dose, with mitigation of the significant reduction in
296 neutralization seen against the B.1.351 lineage. The mRNA platform allows for rapid design of
297 vaccine antigens that incorporate key mutations, allowing for rapid future development of
298 alternative variant-matched vaccines should they be needed. The designs evaluated in this study
299 demonstrated potent cross-variant neutralization as a primary series and the ability to evolve the

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300 immune response through boosting; additional VOC designs can be rapidly developed and
301 deployed in the future if needed to address the evolving SARS-CoV-2 virus.
302

303 **Declaration of Competing Interests**

304 K.W., A.C., M.C., S.E., L.M., D.L., A.W., C.H., C.P., A.H., H.J., J.Q., N.N., S.F., E.N., T.C.,
305 H.B., G.B.E.S.-J., A.C., and D.K.E. are employees of and shareholders in Moderna Inc.
306 S.O.C., A.B.M., K.S.C., R.S., and B.S.G. report no conflict of interest.

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316 reviewing and editing, all authors. All authors have read and agreed to the published version of the
317 manuscript.

318 **Data Availability**

319 The authors declare that the data supporting the findings of this study are available within this
320 article.

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442 **Tables**

443 **Table 1. S-protein substitutions in SARS-CoV-2 variants evaluated in this study.**

Variant Name	Amino Acid Changes in S Protein Relative to Wuhan-Hu-1
D614G	D614G
B.1.351 (Beta) (501Y.V2)	L18F, D80A, D215G, Δ242-244, R246I, K417N, E484K, N501Y, D614G, A701V
P.1 (Gamma) (501Y.V3)	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F
B.1.427/B.1.429 (Epsilon) (452R.V1, CAL.20C)	S13I, W152C, L452R, D614G

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446 **Figure Legends**

447 **Fig. 1. Model of S protein.** mRNA-1273.351 encodes the B.1.351 lineage S variant. Surface
448 representation of the trimeric S protein in the vertical view with the locations of surface-exposed
449 mutated residues highlighted in red spheres and labelled on the gray monomer. The inset shows
450 superimposition of ACE2 receptor domain and the RBD. S protein structure, 6VSB [3]. ACE2-
451 RBD structure, 6M0J [44].

452 ACE2 = angiotensin converting enzyme 2; NTD = N-terminal domain; RBD = receptor binding
453 domain.

454

455 **Fig. 2. S protein-binding antibody and neutralization of variant SARS-CoV-2 pseudoviruses**
456 **by serum from vaccinated BALB/c mice. a,** BALB/c mice were immunized on a two-dose
457 schedule with 1 or 10 µg mRNA-1273, mRNA-1273.351, mRNA-1273.211 (1:1 mix of mRNA-
458 1273 and mRNA-1273.351), or PBS. **b,** Results from individual mouse sera (n = 10) following
459 dose 1 (day 15) and after dose 2 (day 36) are represented as dots on each figure; the horizontal line
460 indicates the GMT. **c,** BALB/c mice were immunized with 1 µg mRNA-1273, mRNA-1273.351,
461 mRNA-1273.211. Each bar indicates the GMT value after dose 2 (day 36), which is listed as text
462 above each plot. **d**, GMT values from individual mouse sera (n = 8 per antigen [randomly selected])
463 after dose 2 (day 36) of 1 µg mRNA-1273, mRNA-1273.351, or mRNA-1273.211 are represented
464 as dots on each figure, with lines connecting matched pairs for the D614G and B.1.351
465 neutralization titers. Fold difference in neutralization against each virus was shown in text above
466 each plot. Wilcoxon matched-pairs signed-rank test. Two-tailed p values: $*<0.1$; $**<0.01$. **e,**
467 BALB/c mice were immunized with 1 µg mRNA-1273, mRNA-1273.351, or the multivalent
468 mRNA-1273.211 vaccine (n = 8). Each bar indicates the GMT value after dose 2 (day 36), which

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469 is listed as text above each plot. The horizontal dotted line indicates the lower limit of quantitation
470 for NAb titer at 40 ID_{50}.
471 ELISA = enzyme-linked immunosorbent assay; GMT = geometric mean titer; ID_{50} = inhibitory
472 dilution factor; IgG = immunoglobulin G; Nab = neutralizing antibody; ns, not significant; PBS =
473 phosphate-buffered saline; PsVN = pseudovirus neutralization titer
474

475 **Fig. 3. S-protein binding antibody and neutralization of D614G and B.1.351 SARS-CoV-2**
476 **pseudoviruses by serum from 1 µg mRNA-1273.351 boosted BALB/c mice**. **a**, BALB/c mice
477 were immunized with 1 or 0.1 µg mRNA-1273 (dose 1 on day 1; dose 2 on day 22) and were
478 boosted with 1 or 0.1 µg mRNA-1273.351 on day 213. **b**, Results from individual mouse sera (n
479 = 5 per group) are represented as dots on each figure, and the line is the mean of each group. The
480 horizontal dotted line indicates the lower limit of quantitation for $\log_{10}$ IgG titer at 1.398. **c**,
481 BALB/c mice previously immunized with mRNA-1273 were given a third dose with 1 µg mRNA-
482 1273.351, with PsVN assessed against wild-type D614G and B.1.351 prior to dose 3 (Day 212)
483 and 3 weeks after dose 3 (Day 233). Postdose 2 peak neutralization titer (Day 36) was referenced
484 against D614G. **d**, Fold rise in neutralization against both viruses from the boosting dose of
485 mRNA-1273.351. **e**, Fold difference in neutralization prior to and after dose 3. Postdose 2 peak
486 neutralization titer reference (D614G assay). The box indicates the GMT, which is listed as text
487 above each plot. The horizontal dotted line indicates the lower limit of quantitation for NAb titer
488 at 40 ID_{50}. Results from individual mouse sera is represented as dots on each figure, with lines
489 connecting the D614G and B.1.351 neutralization titers.

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490 ELISA = enzyme-linked immunosorbent assay; IgG = immunoglobulin G; PBS = phosphate-
491 buffered saline; PsVN = pseudovirus neutralization; GMT = geometric mean titer; ID_{50} = inhibitory
492 dilution factor; NAb = neutralizing antibody.

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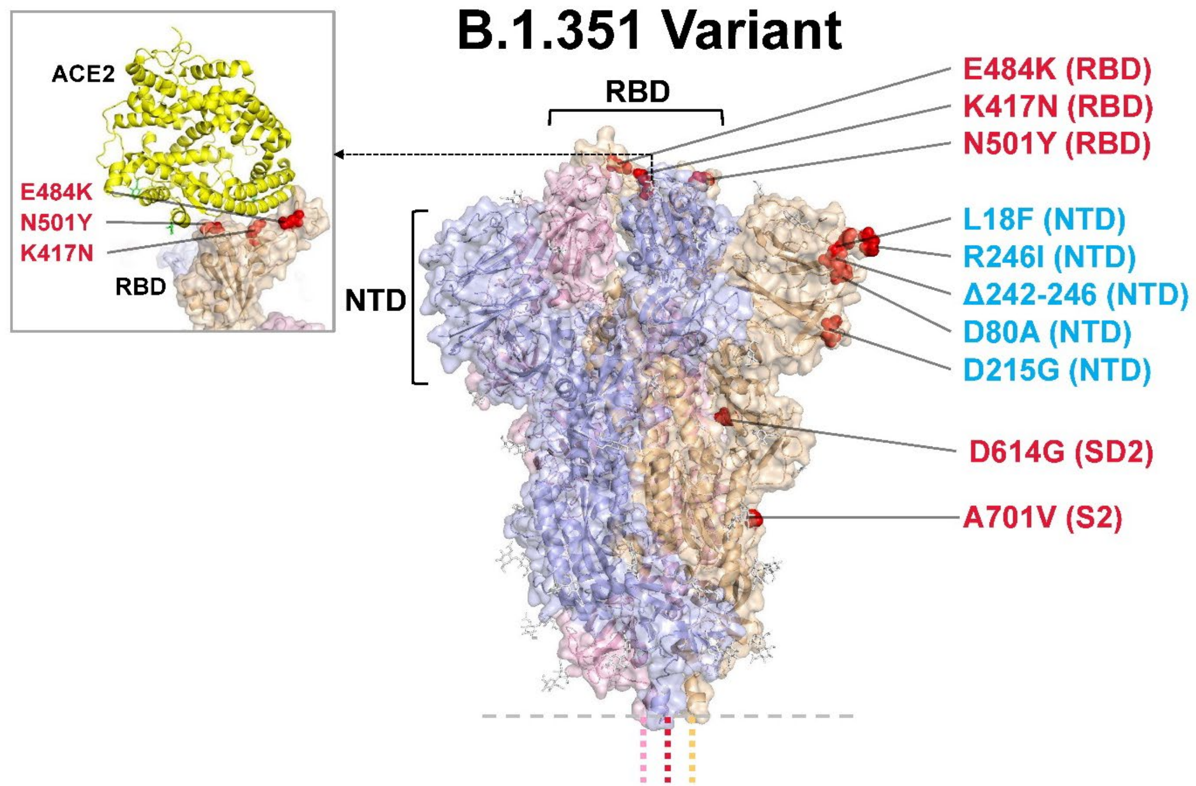
493 **Figures**

494 **Figure 1**



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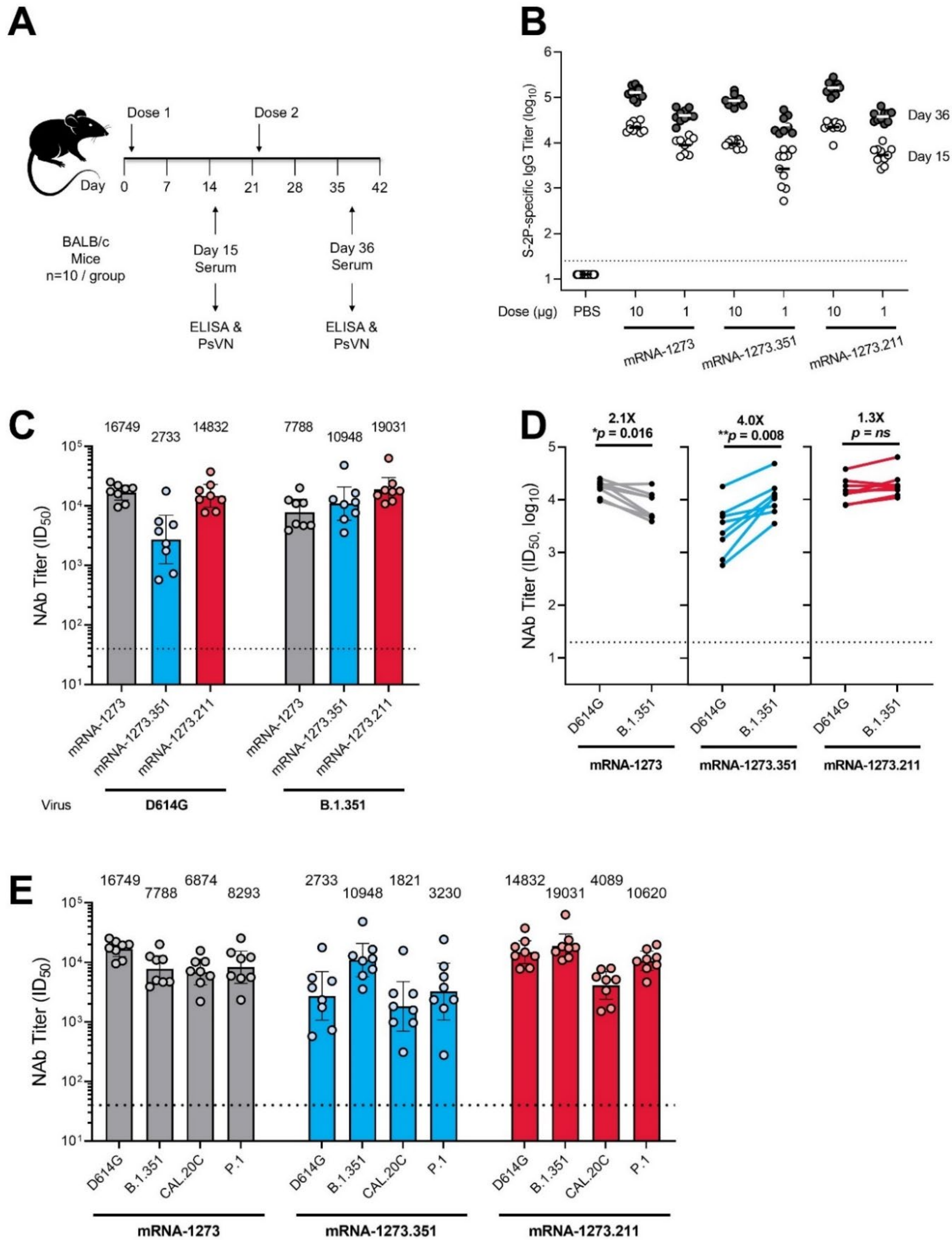
497 **Figure 2**



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500 **Figure 3**