

1 **Previously infected vaccinees broadly neutralize SARS-CoV-2 variants**

2 Hans C. Leier^{1,4}, Timothy A. Bates^{1,4}, Zoe L. Lyski¹, Savannah K. McBride¹, David X. Lee¹,
3 Felicity J. Coulter¹, James R. Goodman², Zhengchun Lu¹, Marcel E. Curlin^{3,*}, William B.
4 Messer^{1,3,*}, Fikadu G. Tafesse^{1,*}

6 **Affiliations**

7 ¹ Department of Molecular Microbiology & Immunology, Oregon Health & Science
8 University; Portland, OR 97239, United States.

9 ² Medical Scientist Training Program, Oregon Health & Science University; Portland,
10 OR97239, United States.

11 ³ Division of Infectious Diseases, Oregon Health & Science University; Portland, OR
12 97239, United States.

14 ⁴ These authors contributed equally to this work.

16 ***Correspondence to:** Fikadu G. Tafesse tafesse@ohsu.edu, William B. Messer
17 messer@ohsu.edu, Marcel E. Curlin, curlin@ohsu.edu

18

19

20

21

22 **Abstract**

23 We compared the serum neutralizing antibody titers before and after two doses of the
24 BNT162b2 COVID-19 vaccine in ten individuals who recovered from SARS-CoV-2 infection
25 prior to vaccination to 20 individuals with no history of infection, against clinical isolates of
26 B.1.1.7, B.1.351, P.1, and the original SARS-CoV-2 virus. Vaccination boosted pre-existing
27 levels of anti-SARS-CoV-2 spike antibodies 10-fold in previously infected individuals, but
28 not to levels significantly higher than those of uninfected vaccinees. However, neutralizing
29 antibody titers increased in previously infected vaccinees relative to uninfected vaccinees
30 against every variant tested: 5.2-fold against B.1.1.7, 6.5-fold against B.1.351, 4.3-fold
31 against P.1, and 3.4-fold against original SARS-CoV-2. Our study indicates that a first-
32 generation COVID-19 vaccine provides broad protection from SARS-CoV-2 variants in
33 individuals with previous infection.

34

35

36

37

38

39

40

41

42

43 **Main**

44 In recent months, multiple coronavirus disease 2019 (COVID-19) vaccine candidates have
45 successfully concluded phase 3 trials¹, with the three candidates authorized for emergency
46 use by the U.S. Food and Drug Administration reporting efficacies of 95% (BNT162b2
47 [Pfizer-BioNTech]), 94% (mRNA-1273 [Moderna]), and 66% (Ad26.COV2.S [Janssen])²⁻⁴.
48 When combined with the substantial portion of many communities estimated to have
49 gained natural immunity through infection with severe acute respiratory syndrome
50 coronavirus 2 (SARS-CoV-2)⁵, the rollout of safe and effective vaccines has raised the
51 possibility that high levels of population immunity could soon be reached. Clouding this
52 prospect is the emergence and global spread of SARS-CoV-2 variants of concern (VOCs),
53 such as those first identified in the United Kingdom (lineage B.1.1.7)⁶, South Africa
54 (B.1.351)⁷, Brazil (P.1)⁸, and California (B.1.429)⁹. Most VOCs possess partially
55 overlapping combinations of spike mutations that enhance binding to the SARS-CoV-2
56 cellular receptor angiotensin-converting enzyme 2 (ACE2), increasing transmissibility¹⁰
57 (Supplementary Table 1). More concerning has been the emergence of spike mutations
58 with the potential to escape neutralizing antibodies raised against earlier lineages of
59 SARS-CoV-2 through infection with an original lineage or by first-generation COVID-19
60 vaccines¹¹⁻¹³. Recent population studies have validated these findings, showing surges of
61 reinfections in regions with extensive transmission of B.1.35^{17,14} and P.1⁸, and large
62 declines in vaccine efficacy against B.1.351¹⁴⁻¹⁶.

63 To investigate whether vaccination of individuals previously infected by SARS-CoV-2
64 confers greater protection from VOCs than vaccination of individuals with no evidence of

65 previous infection, in a cohort of BNT162b2 vaccinees (Supplementary Table 2) we
66 identified a group of 10 study participants who had received a positive COVID-19 PCR test
67 result prior to vaccination, along with an age- and sex-balanced group of 20 participants
68 who had not. While vaccination of previously infected individuals boosted the 50% maximal
69 effective concentration (EC₅₀) of antibodies against the immunodominant SARS-CoV-2
70 spike receptor-binding domain (RBD) ten-fold (pre-vaccination geometric mean titer [GMT],
71 82.15; post-vaccination GMT, 823.3), the vaccine-elicited antibody titers of uninfected
72 individuals (GMT, 699.5) were not significantly lower (Fig. 1A). Similarly, post-vaccination
73 levels of RBD-binding IgG (Fig. 1B) and IgA (Fig. 1C) did not differ significantly between
74 the two groups.

75 We then measured the pre- and post-vaccination neutralizing activity of the two groups of
76 sera against an early SARS-CoV-2 isolate (USA-WA1/2020) and isolates of B.1.1.7,
77 B.1.351, and P.1 (Fig. 2). Pre-vaccination sera from previously infected participants
78 provided higher levels of neutralization against USA-WA1/2020 (GMT, 39.0) than against
79 the three VOCs (GMT, 25.7 for B.1.1.7; GMT<20 for B.1.351; GMT, 31.2 for P.1),
80 consistent with previous reports of convalescent sera^{12,17}. Similarly, post-vaccination sera
81 from uninfected participants showed greater neutralization of USA-WA1/2020 than of the
82 VOCs (GMT, 578.6 for USA-WA1/2020; 223.0 for B.1.1.7; 47.5 for B.1.351; 171.9 for P.1).
83 However, post-vaccination serum from previously infected individuals possessed
84 significantly higher neutralizing activity against every SARS-CoV-2 lineage relative to post-
85 vaccination serum from uninfected participants: neutralizing antibody titers increased by a
86 factor of 3.5 against USA-WA1/2020 (95% confidence interval [CI], 2.8 to 4.0); by a factor
87 of 5.2 against B.1.1.7 (95% CI, 2.37 to 9.8); by a factor of 6.5 against B.1.351 (95% CI, 3.4
88 to 12.3); and by a factor of 4.3 against P.1 (95% CI, 2.8 to 6.5). Notably, there was no

89 significant difference ($P=0.2736$, Wilcoxon rank-sum test) between the post-vaccination
90 neutralizing antibody titers of previously infected participants against B.1.351 (GMT, 307.3;
91 95% CI, 91.0 to 1038) and those of uninfected participants against USA-WA1/2020 (GMT,
92 578.6; GMT, 332.5 to 1007), suggesting that first-generation COVID-19 vaccines could
93 retain near-complete efficacy against even the most resistant VOCs when administered
94 following natural infection.

95 Overall, our findings provide important evidence for broad and potent neutralizing antibody
96 responses against emerging SARS-CoV-2 variants, even with exposure to only wildtype
97 SARS- CoV-2 antigen. This reinforces a recent report that natural infection with B.1.351
98 elicits a similar cross-reactive neutralizing antibody response against B.1.351, P.1, and
99 original SARS-CoV-2¹⁸. While these and other laboratory results must be validated by
100 ongoing population-level studies, they indicate a novel role for COVID-19 vaccines in
101 protecting hard-hit populations from future waves of the pandemic.

102

103

104

105

106

107

108

109

110 **References**

- 111 1. G. Forni, A. Mantovani, COVID-19 vaccines: where we stand and challenges ahead.
112 *Cell Death Differ.* **28**, 626-639 (2021).
- 113 2. L. R. Baden *et al.*, Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N.*
114 *Eng. J. Med.* **384**, 403-416 (2021).
- 115 3. F. P. Polack *et al.*, Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N.*
116 *Eng. J. Med.* **383**, 2603-2615 (2020).
- 117 4. J. Sadoff *et al.*, Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against
118 Covid-19. *N. Eng. J. Med.*, <https://doi.org/10.1056/NEJMoa2101544> (2021).
- 119 5. X. Chen *et al.*, Serological evidence of human infection with SARS-CoV-2: a
120 systematic review and meta-analysis. *Lancet Glob. Health* **9**, E598-E609 (2021).
- 121 6. N. G. Davies *et al.*, Estimated transmissibility and impact of SARS-CoV-2 lineage
122 B.1.1.7 in England. *Science*, <https://doi.org/10.1126/science.abg3055> (2021).
- 123 7. H. Tegally *et al.*, Emergence and rapid spread of a new severe acute respiratory
124 syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations
125 in South Africa. medRxiv, 2020.12.21.20248640 (2020).
- 126 8. E. C. Sabino *et al.*, Resurgence of COVID-19 in Manaus, Brazil, despite high
127 seroprevalence. *Lancet* **397**, 452-455 (2021).
- 128 9. W. Zhang *et al.*, Emergence of a Novel SARS-CoV-2 Variant in Southern California.
129 *JAMA* **325**, 1324-1326 (2021).

- 130 10. J. A. Plante *et al.*, The Variant Gambit: COVID's Next Move. *Cell Host Microbe* **29**,
131 508-515 (2021).
- 132 11. R. E. Chen *et al.*, Resistance of SARS-CoV-2 variants to neutralization by monoclonal
133 and serum-derived polyclonal antibodies. *Nat. Med.* **27**, 717-726 (2021).
- 134 12. M. Hoffmann *et al.*, SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing
135 antibodies. *Cell*, <https://doi.org/10.1016/j.cell.2021.03.036> (2021).
- 136 13. P. Wang *et al.*, Antibody Resistance of SARS-CoV-2 Variants B.1.351 and B.1.1.7.
137 *Nature*, <https://doi.org/10.1038/s41586-021-03398-2> (2021).
- 138 14. E. Mahase, Covid-19: Novavax vaccine efficacy is 86% against UK variant and 60%
139 against South African variant. *BMJ*, n296 (2021).
- 140 15. T. Kustin *et al.*, Evidence for increased breakthrough rates of SARS-CoV-2 variants of
141 concern in BNT162b2 mRNA vaccinated individuals. medRxiv, 2021.04.06.21254882
142 (2021).
- 143 16. S. A. Madhi *et al.*, Efficacy of the ChAdOx1 nCoV-19 Covid-19 Vaccine against the
144 B.1.351 Variant. *N. Eng. J. Med.*, <https://doi.org/10.1056/NEJMoa2102214> (2021).
- 145 17. T. A. Bates *et al.*, Neutralization of SARS-CoV-2 variants by convalescent and
146 vaccinated serum. medRxiv, 2021.04.04.21254881 (2021).
- 147 18. T. Moyo-Gwete *et al.*, Cross-Reactive Neutralizing Antibody Responses Elicited by
148 SARS-CoV-2 501Y.V2 (B.1.351). *N. Eng. J. Med.*,
149 <https://doi.org/10.1056/NEJMc2104192> (2021).
- 150 19. T.A. Bates *et al.*, Cross-reactivity of SARS-CoV structural protein antibodies against

151 SARS-CoV-2. *Cell Rep.* **34**, 108737 (2021).

152 20. J.B. Case *et al.*, Growth, detection, quantification, and inactivation of SARS-CoV-2.

153 *Virology* **548**, 39-48 (2020)

154

155

156

157

158

159

160

161

162

163

164

165

166

167

168

169 **Acknowledgments**

170 The authors thank the clinical staff at Oregon Health & Science University who assisted in
171 enrolling study participants and collecting the serum samples used in this report.

172 **Funding**

173 M.J. Murdock Charitable Trust

174 National Institutes of Health training grant T32AI747225

175 Oregon Health & Science University Innovative IDEA grant 1018784

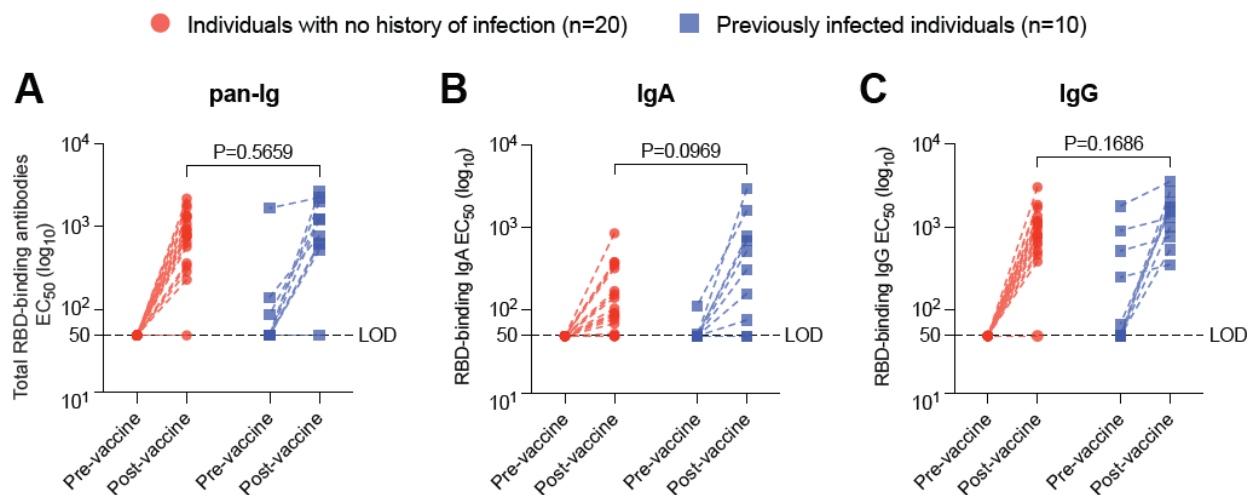
176 National Institutes of Health grant R01AI145835

177 **Competing interests**

178 Authors declare that they have no competing interests.

179 **Data and materials availability**

180 All data are available in the main text or the supplementary materials.



181

182 **Figure 1. Anti-SARS-CoV-2 antibody levels elicited by vaccination and natural**
183 **infection. (A)** Half maximal effective concentration (EC₅₀) of total pan-Ig antibodies
184 specific to the spike RBD were measured by enzyme-linked immunosorbent assay
185 (ELISA) in serum collected from donors previously infected with SARS-CoV-2 pre- and
186 post-vaccination with the BNT162b2 vaccine. **(B)** EC₅₀ of RBD-binding IgA. **(C)** EC₅₀
187 of RBD-binding IgG. Statistical comparisons were made using the Wilcoxon rank-sum
188 test. LOD denotes limit of detection.

189

190

191

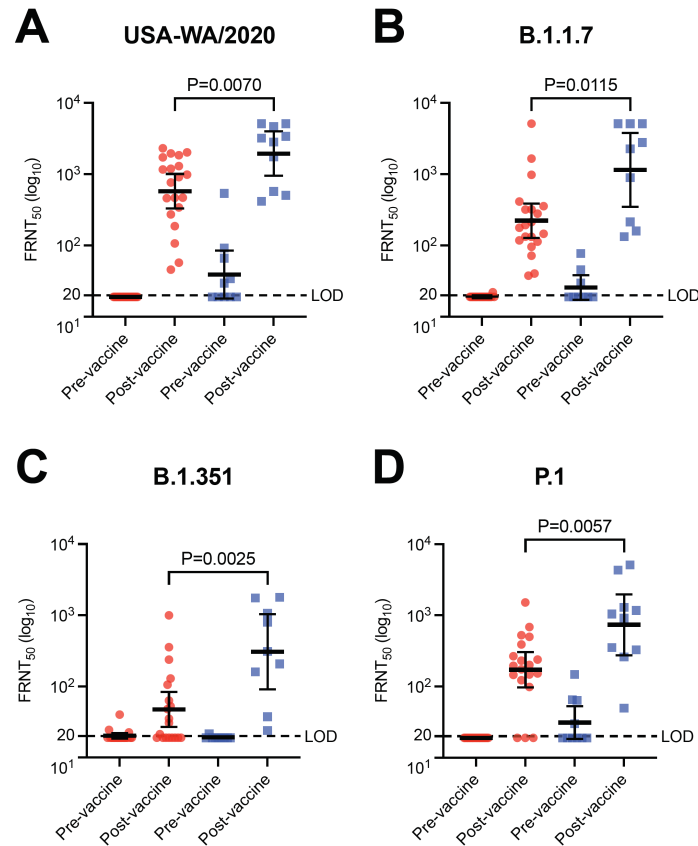
192

193

194

195

● Individuals with no history of infection (n=20) ■ Previously infected individuals (n=10)



196

197 **Figure 2. Immunity from natural SARS-CoV-2 infection boosted by subsequent**

198 **vaccination broadens protection against variants of concern.** Neutralization of

199 SARS-CoV-2 variants by pre-and post-vaccination sera collected from previously

200 infected and naïve individuals. Shown are the results of a 50% focus reduction

201 neutralization testing (FRNT₅₀) for an early SARS- CoV-2 isolate USA-WA1/2020 (A)

202 and VOC clinical isolates of B.1.1.7 (B), B.1.351 (C), and P.1 (D). Horizontal bars

203 represent geometric mean titer and I bars represent 95% confidence intervals.

204 Statistical comparisons were made using the Wilcoxon rank-sum test. LOD denotes limit

205 of detection.

206

207

Supplementary Materials for

208

Previously infected vaccinees broadly neutralize SARS-CoV-2 variants

209

Hans C. Leier, Timothy A. Bates, Zoe L. Lyski, Savannah K. McBride, David X. Lee,

210

Felicity J. Coulter, James R. Goodman, Zhengchun Lu, Marcel E. Curlin, William B.

211

Messer, Fikadu G. Tafesse

212

Contains:

213

Materials and methods

214

Supplementary Tables 1-2

215

216

217

218

219

220 **Materials and Methods**

221 **Cohort**

222 This study was conducted in accordance with the Oregon Health & Science University
223 (OHSU) Institutional Review Board (IRB#00022511). Study participants were enrolled at
224 OHSU immediately after receiving the first dose of the BNT162b2 (Pfizer-BioNTech)
225 COVID-19 vaccine. After receiving informed consent, 4-6 mL whole blood was drawn
226 and centrifuged 10 minutes at 1000 x g. A second blood sample was obtained at least
227 14 days after participants received their second vaccine dose. Serum was stored at -
228 20°C until heat inactivation in preparation for FRNTs and ELISAs. n=10 participants
229 were identified with a positive COVID-19 PCR test prior to vaccination and n=20 age-
230 and sex-balanced participants who did not have a history of positive PCR test or RBD-
231 binding antibodies in an ELISA. See Supplementary Table 1 for more details of the
232 cohort.

233 **ELISA**

234 The 96-well ELISA plates (Corning, Cat# 3590) were coated with recombinant RBD
235 protein prepared as previously described¹⁹ at a concentration of 1 ug/mL in PBS and
236 incubated overnight at 4°C. Coating antigen was removed, plates were washed once
237 with PBST containing 0.05% Tween (wash buffer) and blocked for 1 hour at RT with 5%
238 milk prepared in PBST containing 0.05% Tween (dilution buffer). 100 mL of 1:20 (pre-
239 vaccine), or 1:200 (post- vaccine) dilution of serum in dilution buffer was added to each
240 starting well. Three-fold serial dilutions were performed in dilution buffer. Plates were
241 incubated at room temperature for 1 hour. The plates were washed 3 times with wash

242 buffer and 100 mL of 1:10,000 dilution of anti-human GOXHU IGG/A/M HRP
243 (Invitrogen, Cat# A18847) detection antibody was added and incubated at room
244 temperature for 1 hour. After washing the plates 3 times with wash buffer, 100 mL of
245 colorimetric detection reagent containing 0.4 mg/ml o-phenylenediamine and 0.01%
246 hydrogen 45 peroxide in 0.05 M citrate buffer (pH 5) were added and the reaction was
247 stopped after 20 minutes by the addition of 100 mL 1 M HCl. Optical density (OD) at
248 492 nm was measured using a CLARIOstar plate reader. Plates were normalized,
249 background removed, and endpoint antibody titers determined by the highest dilution
250 with a positive signal, defined as 4-fold above background. Final endpoint titer values
251 below the detection limit of 20 were set to 19.

252 **Viruses**

253 SARS-CoV-2 isolates were obtained from BEI Resources (Supplementary Table 2):
254 Isolate USA/CA_CDC_5574/2020 [B.1.1.7] (BEI Resources NR-54011); Isolate hCoV-
255 54 19/South Africa/KRISP-K005325/2020 [B.1.351] (BEI Resources NR-54009); Isolate
256 hCoV-19/Japan/TY7-503/2021 [P.1] (BEI Resources NR-54982); and Isolate USA-
257 WA1/2020 [early isolate] (BEI Resources NR-52281). Isolates were propagated and
258 titrated in Vero E6 cells as previously described²⁰.

259 **FRNT**

260 Serial dilutions of patient sera and virus neutralization were carried out in biological
261 duplicate in a 96-well plate format. Briefly, each sample was added in duplicate 1:10 to
262 dilution media (Opti-MEM, 2% FBS), and four-fold serial dilutions were made for a range
263 of 1:10 – 1:2560. An equal volume of dilution media containing 100 FFU of SARS-CoV-

264 2 was added to each well (final dilutions of sera, 1:20–1:5120) and incubated 1 h at
265 37°C. The virus-serum mixtures were then added to monolayers of Vero E6 cells,
266 incubated with occasional rocking 1 h at 37°C, and covered with overlay media (Opti-
267 MEM, 2% FBS, 1% methylcellulose). Overlay media was then removed, and plates
268 were fixed for 1 h in 4% paraformaldehyde in PBS. Foci were developed as described.
269 Plates were imaged with a CTL Immunospot Analyzer, then foci were counted using
270 CTL ImmunoSpot (7.0.26.0) Percent neutralization values for FRNT50 were compiled
271 and analyzed using python (v3.7.6) with numpy (v1.18.1), scipy (v1.4.1), and pandas
272 (v1.0.1) data analysis libraries. Replicate data were fit with a three-parameter logistic
273 model and final FRNT50 values below the lowest dilution of 20 were set to 19 while
274 values above the maximum dilution of 5120 were set to 5121. FRNT50 curves were
275 plotted using python with the Matplotlib (v3.1.3) data visualization library.

276 **Statistical analysis**

277 Aggregated ELISA endpoint titers and FRNT50 values were analyzed in GraphPad
278 Prism (v9.0.2). Comparisons were performed using the Wilcoxon rank-sum test.
279 Reported group averages are geometric means, with error bars representing 95%
280 confidence intervals.

281

282

283

284

285 **Supplementary Table 1.** List of mutations in B.1.1.7, B.1.351 and P.1 SARS-CoV-2

286 clinical isolates.*

287

Lineage	GISAIID Clade	GISAIID ID	Spike mutations (RBD highlighted)	Non-Spike mutations
B.1.1.7	GR	EPI_ISL_683466	H69del, V70del, Y145del, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H	N D3L, N G204R, N R203K, N S235F, NS8 Q27stop, NS8 R52I, NS8 Y73C, NSP3 A890D, NSP3 A1305V, NSP3 I1412T, NSP3 T183I, NSP6 F108del, NSP6 G107del, NSP6 S106del, NSP12 P323L, NSP13 K460R, NSP14 E347G
B.1.351	GH	EPI_ISL_678570	D80A, D215G, L242del, A243del, L244del, K417N, E484K, N501Y, D614G, A701V	E P71L, N T205I, NS3 Q57H, NS3 S171L, NSP2 T85I, NSP3 K837N, NSP5 K90R, NSP6 F108del, NSP6 G107del, NSP6 S106del, NSP12 P323L
P.1			D138Y, D614G, K417T, E484K, N501Y, G181V, H655Y, L18F, P26S, R190S, T20N, T1027I, V1176F	N G204R, N P80R, N R203K, NS3 S253P, NS8 E92K, NSP3 K977Q, NSP3 S370L, NSP3 T186A, NSP6 F108del, NSP6 G107del, NSP6 S106del, NSP12 P323L, NSP13 E341D

288 *Obtained from BEI Resources

289

290

291

292

293

294

295

296

297 **Supplementary Table 2.** Description of cohort.

298

	Previously infected (n=10)	No history of infection (n=20)
Sex – no. (%)		
Female	6 (60)	15 (75)
Male	4 (40)	5 (25)
Median age – yrs (range)	36.5 (23-61)	44 (23-60)
Median time between vaccine doses – days (range)	22 (20-25)	21 (21-32)
Median time from second vaccine dose to second serum sample – days (range)	18 (14-28)	17 (14-28)
Median time from positive COVID-19 PCR test to first vaccine dose – days (range)	52 (13-302)	NA

299