



HAL
open science

Spike protein genetic evolution in patients at high-risk of severe COVID-19 treated by monoclonal antibodies

Valentin Leducq, Karen Zafilaza, Antoine Fauchois, Emna Ghidaoui, Sophie Sayon, Céline Dorival, Marie-Laure Meledje, Clovis Lusivika-Nzinga, Youri Yordanov, Guillaume Martin-Blondel, et al.

► To cite this version:

Valentin Leducq, Karen Zafilaza, Antoine Fauchois, Emna Ghidaoui, Sophie Sayon, et al.. Spike protein genetic evolution in patients at high-risk of severe COVID-19 treated by monoclonal antibodies. *Journal of Infectious Diseases*, 2023, 10.1093/infdis/jiad523 . hal-04309349

HAL Id: hal-04309349

<https://hal.sorbonne-universite.fr/hal-04309349>

Submitted on 27 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

1 **Spike protein genetic evolution in patients at high-risk of severe COVID-19 treated by**
2 **monoclonal antibodies**

3 **Running title :** Antibody therapies drive Spike evolution

4 Valentin Leducq ^{1*}, Karen Zafilaza ^{1*}, Antoine Fauchois ¹, Emna Ghidaoui ¹, Sophie Sayon ¹,
5 Céline Dorival ², Marie-Laure Meledje ², Clovis Lusivika-Nzinga ², Youri Yordanov ³, Guillaume
6 Martin-Blondel ⁴, Fabrice Carrat ⁵, Anne-Geneviève Marcelin ^{1*} and Cathia Soulie ^{1*}.

7 On behalf of the COCOPREV Study Group.

8 ¹ Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, AP-HP, Hôpitaux
9 Universitaires Pitié-Salpêtrière - Charles Foix, Laboratoire de Virologie, Paris, France

10 ² Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, Paris, France.

11 ³ Sorbonne Université, Hôpital Saint Antoine, Service d'Accueil des Urgences, INSERM, Institut Pierre Louis
12 d'Epidémiologie et de Santé Publique, Paris, France

13 ⁴ Service des Maladies Infectieuses et Tropicales, CHU de Toulouse, France, Institut Toulousain des Maladies
14 Infectieuses et Inflammatoires (Infinity) INSERM, Université Toulouse III., Toulouse, France

15 ⁵ Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, Unité de Santé
16 Publique, AP-HP, Hôpital Saint-Antoine, Paris, France.

17 * These authors contributed equally to this work.

18 **Corresponding author**

19 Valentin Leducq ; 47-83 Boulevard de l'Hôpital 75013 PARIS, FRANCE, Hôpital Pitié-Salpêtrière, Service de
20 Virologie, Bâtiment CERVI ; Tel : +33142177558 ; email : valentin.leducq@sorbonne-universite.fr.

21 **Word count**

22 Abstract : 200 words ; Text : 3063 words.

23 **ABSTRACT**

24 **Background**

25 High-risk patients, often immunocompromised and not responding to vaccine, continue to
26 experience severe COVID-19 and death. Monoclonal antibodies (mAbs) were shown
27 effective to prevent severe COVID-19 for these patients. Nevertheless, concerns about the
28 emergence of resistance mutations were raised.

29 **Methods**

30 We conducted a multicentric prospective cohort study, including 264 patients with mild-to
31 moderate COVID-19 at high risk for progression to severe COVID-19 and treated early with
32 Casirivimab/Imdevimab, Sotrovimab or Tixagevimab/Cilgavimab. We sequenced the SARS-
33 CoV-2 genome during follow-up and searched for emerging Spike mutations.

34 **Results**

35 Immunocompromised patients have a 6-fold increased risk of developing mutations, which
36 are associated with a prolonged duration of viral clearance but no clinical worsening.
37 Emerging P337S/R/L/H, E340D/K/A/Q/V/G and K356T/R substitutions in patients treated
38 with Sotrovimab are associated with higher viral RNA loads for up to 14 days post-treatment
39 initiation. Tixagevimab/Cilgavimab is associated with a 5-fold increased risk of developing
40 mutations. R346K/I/T/S and K444R/N/M substitutions associated with
41 Tixagevimab/Cilgavimab have been identified in multiple SARS-CoV-2 lineages, including
42 BQ.1 and XBB.

43 **Conclusions**

44 In conclusion, the probability of emerging mutations arising in response to mAbs is
45 significant, emphasizing the crucial need to investigate these mutations thoroughly and
46 assess their impact on patients and the evolutionary trajectory of the SARS-CoV-2.

47 **Key words**

48 SARS-CoV-2, Monoclonal antibodies, COVID-19, Immunocompromised, Resistance
49 mutations.

50 **BACKGROUND**

51 Neutralizing mAbs target the Spike (S) glycoprotein on the surface of SARS-CoV-2, which
52 mediate its entry into host cells via the hACE2 receptor (1). Several mAb therapies have been
53 approved by the European Medicines Agency (EMA) since March 2021, including
54 Casirivimab/Imdevimab, Sotrovimab, and Tixagevimab/Cilgavimab, for prevention and/or
55 therapy of patients at high risk for progression to severe COVID-19, particularly
56 immunocompromised patients who failed to respond to vaccine (2).

57 These antibodies target the receptor-binding domain (RBD) of the Spike protein in order to
58 inhibit viral entry and can be classified into 4 distinct groups according to their structures
59 and target epitopes (3,4). Casirivimab is a class 1 antibody whose epitope overlaps with the
60 receptor binding motif (RBM) within the RBD and compete with binding of hACE2 host
61 receptor (5). Imdevimab is a class 3 antibody that binds outside the RBM but close enough to
62 hinder hACE2 interaction. Casirivimab/Imdevimab combination has demonstrated in vitro
63 efficacy against Alpha and Delta variants, and also significantly reduces viral load in patients,
64 as well as the risk of hospitalization and death (6,7). However, due to the large number of
65 mutations in its Spike protein, these two antibodies became ineffective against Omicron
66 variant, notably due to substitutions K417N, S477N, E484A, Q493R and G446S (8).
67 Sotrovimab is a class 3 antibody that binds outside the RBM to a highly conserved epitope in
68 sarbecoviruses and does not compete with binding of hACE2 (5). Sotrovimab has shown in
69 vitro efficacy against Delta and, unlike Casirivimab/Imdevimab, retains activity, although
70 reduced, against Omicron (9). In high-risk patients infected with Omicron, Sotrovimab
71 effectively protects against progression to severe COVID-19 (10). Sotrovimab became
72 ineffective with the emergence of BA.2 sub-variant displaying additional mutations in the

73 Spike protein (11). However, recent studies suggest that Sotrovimab may retain activity
74 against Omicron sub-variants, notably BQ.1.1 and XBB.1.5, due to the antibody-dependent
75 cell-mediated cytotoxicity (ADCC) of its crystallizable fragment (Fc) region (12). Tixagevimab
76 and Cilgavimab are class 1 and 2 antibodies, respectively, whose epitopes overlap the RBM
77 and compete with binding of hACE2 (9). This combination has been shown to be effective in
78 preventing severe COVID-19, especially as pre-exposure prophylaxis for high-risk individuals
79 (13). Tixagevimab/Cilgavimab was one of the last antibody therapies to maintain significant
80 efficacy against BA.2 and BA.5, essentially due to Cilgavimab, until the emergence of the
81 BQ.1 and XBB sub-variants (14–16).

82 While their efficacy and susceptibility to the various emerging variants of SARS-CoV-2 over
83 time have been widely documented, concerns remain over the use of such antibody
84 therapies in the development of resistance mutations and their impact on the genetic
85 evolution of SARS-CoV-2. Our study aims to analyse the impact of these three mAb therapies
86 on the genetic evolution of the S gene in high-risk patients by employing next-generation
87 sequencing to detect viral population changes and minority variants emergence.

88 **METHODS**

89 **Study design**

90 Our study is based on the ongoing ANRS 0003S COCOPREV Study (NCT04885452) (10), a
91 multicentric prospective cohort enrolling PCR-confirmed mild-to-moderate COVID-19
92 patients at high risk of severe progression. Treatment was administered within the initial five
93 days of symptom onset under emergency use authorization or early access at one of 32
94 participating centers. Patients received either 600/600 mg or 300/300 mg of

95 Casirivimab/Imdevimab IV, 500 mg of Sotrovimab IV, or 300/300 mg of
96 Tixagevimab/Cilgavimab IV, following French health authority guidelines and physician
97 discretion. Nasopharyngeal swabs were collected on Day 0 and 7, with additional tests on
98 Day 3 and 5 for hospitalized patients, and subsequently weekly while viral RNA loads
99 remained positive.

100 **SARS-CoV-2 viral RNA load**

101 Viral RNA was isolated from nasopharyngeal swab stored in universal transport medium on
102 Nuclisens® Easymag™ (Biomérieux), with a starting volume of 300µl eluted in 70µl. Cycle
103 threshold (Ct) values were estimated using the TaqPath™ COVID-19 RT-PCR kit
104 (ThermoFisher). The SARS-CoV-2 ORF1ab, N and S genes were simultaneously amplified to
105 generate cycle thresholds (Cts), which were then converted into viral copies per milliliter of
106 sample (cp/ml) using a standard curve developed in our laboratory with standard samples
107 quantified by droplet digital PCR (ddPCR).

108 **Whole genome sequencing**

109 Patients with both an initial sample (Day 0) and at least one follow-up sample with Ct<31
110 were included. Whole SARS-CoV-2 genome sequencing was performed according to the
111 Oxford Nanopore “PCR tiling of SARS-CoV-2 virus Eco protocol”. Viral RNA was reverse
112 transcribed and amplified by PCR with the ARTIC primer pool v4.1 (Integrated DNA
113 Technologies). Samples were basecalled with super-accurate option and demultiplexed with
114 GUPPY (v6+). Reads were mapped to the Wuhan-hu-1 (MN908947.3) reference genome with
115 minimap2 (v2.24), and consensus were generated with BCFTools (v1.16). Clades and lineages

116 were assigned with Nextclade (v2+) and Pangolin (v4+). Single nucleotide variations (SNVs)
117 calling was performed with PEPPER-Margin-DeepVariant pipeline (v0.8) (17).

118 **Statistical analysis**

119 We used multivariable logistic regression to identify variables associated with S protein
120 substitution emergence. Initial univariate analyses were conducted, selecting variables with
121 a P-Value <0.3 for subsequent multivariate analysis. To address interrelated categorical
122 variables, we developed four distinct models, prioritizing the most robust ones. A
123 significance threshold of 5% was applied for establishing associations. We assessed the
124 impact of variant, treatment, and mutation emergence on SARS-CoV-2 viral RNA loads during
125 follow-up. We employed the Kruskal-Wallis test and Wilcoxon rank sum tests for pairwise
126 comparisons to identify significant categories influencing viral RNA load. All statistical
127 analyses were carried out using R, and univariate comparison tests were two-tailed.

128 **General population**

129 We extracted 65,448 high-coverage SARS-CoV-2 genomes from France between August 2021
130 and December 2022 from GISAID database. Genomes were aligned using MAFFT (v7.475),
131 and we estimated intra-population frequencies of substitutions correlated with
132 Casirivimab/Imdevimab, Sotrovimab, or Tixagevimab/Cilgavimab treatments over this
133 period.

134 **RESULTS**

135 **Patients description**

136 Among the 264 patients analysed, 74 (28%) received Casirivimab/Imdevimab, 166 (63%)
137 received Sotrovimab and 24 (9%) received Tixagevimab/Cilgavimab. All had mild-to-
138 moderate COVID-19, and risk factors for severe COVID-19, including being ≥ 65 years-old
139 (121/264, 46%) and/or being immunocompromised (207/264, 78%). Differences were
140 observed between patients who received Casirivimab/Imdevimab, Sotrovimab or
141 Tixagevimab/Cilgavimab as regards sex, hospitalisation, vaccination, certain types of
142 immunodepression or comorbidities and treatment by pre-exposure prophylaxis with mAbs
143 (Table 1). Patients treated with Casirivimab/Imdevimab had lower IgG anti-S levels at
144 baseline (median 7 BAU/ml, 0-96 IQR) than patients treated by Sotrovimab (median 48
145 BAU/ml, 0-809 IQR, $p=0.017$) or Tixagevimab/Cilgavimab (median 359 BAU/ml, 56-638 IQR
146 $p<0.001$). Delta infected patients received exclusively Casirivimab/Imdevimab (42/42, 100%),
147 whereas BA.1/BA.2 and BA.5/BQ.1 infected patients received mainly Sotrovimab (166/182,
148 91%) and Tixagevimab/Cilgavimab (21/21, 100%), respectively. At baseline, no significant
149 differences in SARS-CoV-2 viral RNA loads could be found between patients treated with
150 these three mAbs therapies ($p=0.974$). Patients treated with Casirivimab/Imdevimab had
151 lower viral RNA loads at day 7 (median 5.58, 4.96-6.41 IQR) than patients who received
152 Sotrovimab (median 6.81, 6.07-7.64 IQR, $p<0.001$) or Tixagevimab/Cilgavimab (median 6.52,
153 5.81-7.66 IQR, $p=0.022$), and day 14 (median 3.53, 2.97-4.11 IQR) than patients treated with
154 Sotrovimab (median 5.50, 3.88-6.85 IQR, $p<0.001$).

155 **Emerging mutational profiles**

156 Following the SARS-CoV-2 genome sequencing and SNVs calling, we have listed missense
157 mutations who were absent at baseline but emerged in the S gene in patients' follow-up
158 samples. Overall, amino acid substitutions occurred at 55 distinct residues in S protein under

159 treatment with mAbs. Among these, 5 residues in RBD and 1 residue in HR2 domain were
160 subject to amino acid substitutions with a >5% prevalence in patients (Figure 1a). Among
161 patients treated with Casirivimab/Imdevimab, 27% (20/74) had a L1186F amino acid
162 substitution in HR2 domain in S2 subunit. Among patients who received Sotrovimab, 28%
163 (47/166), 13% (21/166) and 10% (17/166) had E340D/K/A/Q/V/G, K356T/R and P337S/R/L/H
164 amino acid substitutions, respectively. These 3 substitution sites are located within the RBD
165 but outside the RBM. Among patients treated with Tixagevimab/Cilgavimab, 68% (16/24)
166 and 21% (5/24) had R346K/I/T/S and K444R/N/M amino acid substitutions, respectively.
167 These substitutions are located in the RBD, outside the RBM for R346 and within the RBM
168 loop for K444. For downstream analysis, we chose to only consider the six above-mentioned
169 amino acid substitution sites in RBD and HR2 domain with highest prevalence (Figure 1b).
170 Tixagevimab/Cilgavimab was associated with a reduced median time to, and increased
171 probability of, mutation emergence in S protein compared to Casirivimab/Imdevimab and
172 Sotrovimab ($p < 0.001$) (Figure 1c). Intra-host frequencies of missense mutations selected in
173 patients who received Casirivimab/Imdevimab appeared to be lower (median 20%, 15%-24%
174 IQR) compared to intra-host frequencies of mutation selected in patients treated with
175 Sotrovimab (median 70%, 48%-91% IQR, $p < 0.001$) or Tixagevimab/Cilgavimab (median 57%,
176 51%-71% IQR, $p < 0.001$) (Figure 1d).

177 **Factors associated with the emergence of mutation**

178 According to logistic regression models, immunodepression was associated with a 5.6-fold
179 increased risk of emergence of considered mutations in S protein, regardless of sex, age,
180 level of IgG anti-S, variant and mAbs treatment ($p = 0.019$, OR 5.64, 95%CI: 1.34-23.81)
181 (Supplementary figure 1). Patients treated with immunosuppressive therapy (including

182 Rituximab and corticosteroids) have a 3-fold increased risk of mutation emergence (OR 2.94,
183 95%CI 1.03-8.37, p=0.044) and solid organ transplant recipients also tend to have these
184 mutations emerge more frequently (OR 2.97, 95%CI 0.87-10.2, p=0.083), compared to non-
185 immunocompromised patients. Interestingly, patients who received Tixagevimab/Cilgavimab
186 had a 5.3-fold increased risk of mutation emergence in S protein compared to patients
187 treated with Casirivimab/Imdevimab (p=0.025, OR 5.28, 95%CI: 1.24-22.5), while patients
188 who received Sotrovimab had a similar risk (p=0.726, OR 1.17, 95%CI: 0.49-2.80). Among
189 patients who received Sotrovimab, the emergence of mutation in S protein was associated
190 with increased viral RNA loads at day 7 (median 7.31, 6.55-7.98 IQR) and day 14 (median
191 6.32, 4.87-7.05 IQR), compared to patients with no mutation emergence at day 7 (median
192 6.5, 5.92-7.22 IQR, p<0.001) and at day 14 (median 4.25, 3.17-5.79 IQR, p<0.001) (Figure 2b).
193 The emergence of mutations in patients who received Tixagevimab/Cilgavimab seemed to
194 be associated with higher viral RNA loads at day 14, but this difference was not significant
195 (p=0.373) due to the small number of patients in this group (Figure 2c).

196 Overall, the emergence of such mutations was correlated with a longer median time to a
197 viral RNA load $\leq 4.41 \log_{10}$ cp/ml (≥ 31 Ct) (p<0.0001), regardless of mAbs treatment (Figure 3).
198 Concerning hospitalisation at day 28 of follow-up, 4/105 (4%) patients with an emerging
199 mutation in S protein and 7/159 (4%) patients without mutation emergence were
200 hospitalised. Similarly, no significant difference was observed in the evolution of patients'
201 symptoms, whether or not they developed a mutation in S protein during their follow-up
202 (Supplementary material).

203 **Emerging mutations in the general population**

204 Amino acid substitutions of L1186, E340, K356 and P337 residues, associated with
205 Casirivimab/Imdevimab and Sotrovimab therapies in the study, had low frequency levels
206 (<1%) in the French general population, between August 2021 and December 2022 (data not
207 showed). In contrast, amino acid substitution at the R346 site in the S protein, associated
208 with Tixagevimab/Cilgavimab therapy, first emerged in the general population in December
209 2021, concomitantly with BA.1 variant (Figure 4). By the end of January 2022, R346
210 substitution has reached a frequency of 55% in the general population and eventually
211 disappeared in March when BA.1 had been replaced by BA.2 variant. Finally, the R346
212 substitution emerged a second time in August 2022, in association with the K444
213 substitution, concomitantly with BQ.1 variant. By December 2022, these two substitutions
214 have reached a frequency of 84% and 85% in the general population, respectively.

215 **DISCUSSION**

216 Overall, we reported six sites of amino acid substitutions in the Spike protein during follow-
217 up of 264 high-risk patients treated with three different monoclonal antibody therapies. We
218 assessed the impact of these substitutions on patient outcome and on the evolution of
219 SARS-CoV-2.

220 Immunodepression is associated with an almost 6-fold increased risk of developing a
221 mutation in S protein following mAb treatment, especially immunosuppression related to
222 the use of immunosuppressive therapies (including rituximab and corticosteroids) and solid
223 organ transplantation. Since the majority of the patients studied here are
224 immunocompromised, it is difficult to establish a clear association between immune status
225 and the acquisition of mutations. A non-immunocompromised control group of comparable
226 size would enable definitive conclusions to be drawn. Regardless of patients' immune status

227 and the mAbs administered, mutation emergence is associated with prolonged SARS-CoV-2
228 infection, increased viral RNA load levels, and delayed viral clearance. However, these
229 mutations do not appear to affect patients' clinical progress or overall recovery. Although
230 these mutations probably have an impact on the efficacy of mAbs, particularly those
231 affecting the RBD, the three therapies still appear to be sufficiently active to prevent severe
232 forms of COVID-19 in high-risk patients.

233 Casirivimab/Imdevimab is associated with the emergence of L1186F substitution in Delta-
234 infected patients. This residue is conserved across coronaviruses within an E-L-L motif in the
235 HR2 domain in S2 subunit that play a crucial role in fusion-mediated viral entry (18). There
236 are no existing data showing that L1186F substitution may emerge with
237 Casirivimab/Imdevimab or that it could confer resistance to these mAbs. Besides,
238 Casirivimab (Class 1) binds to the RBM loop region (residues 471–491) and Imdevimab (Class
239 3) binds outside the RBM in RBD lower left edge (19). Together with low intra-host
240 frequencies observed in patients and its absence in the general population, this information
241 suggested that selection of L1186F substitution at individual and populational levels is
242 unlikely, and provided no evolutionary or immune escape benefits. Thus, we had no
243 explanation for the L1186F substitution emergence found exclusively in a subset of patients
244 who received Casirivimab/Imdevimab. The rapid drop in viral RNA loads in these patients
245 tended to confirm that L1186F substitution confers no resistance to Casirivimab/Imdevimab.
246 Overall, our results suggest that Casirivimab/Imdevimab combination is highly effective
247 against Delta, sufficiently to prevent the emergence of resistance mutations. Two studies
248 also showed that the Casirivimab/Imdevimab combination effectively prevented mutation
249 selection, both *in vitro* on VSV-based pseudotyped viruses displaying the original Wuhan-hu-

250 1 Spike and *in vivo* in patients infected with Alpha, Delta and other pre-Omicron variants of
251 concerns (VOCs) (20,21). Nevertheless, a third recent study reveals the selection of several
252 mutations, all within the RBM, at residues E406, G446, Y453 and L455 in a small number of
253 Delta-infected patients treated with Casirivimab/Imdevimab (22). In our study, we found
254 only one patient with emerging G446V and one patient with emerging G446V+Y453F, both
255 Delta-infected.

256 Emergence of P337 and E340 substitutions in RBD has been demonstrated several times in
257 Delta- and Omicron-infected patients who received Sotrovimab and is associated with a 27-
258 to 279-fold reduction in SARS-CoV-2 susceptibility to this mAb (23–25). In a recent study, the
259 emergence of the K356T mutation was also observed in two Omicron-infected patients
260 treated with Sotrovimab (26). A report from September 06, 2021 from the Japanese Ministry
261 of Health alerts us that a 5.9-fold decrease in neutralization activity by Sotrovimab was
262 estimated for pseudovirus particles carrying K356T and D614G mutations. In our study,
263 68/166 (41%) of patients treated with Sotrovimab acquired at least one of these three
264 substitutions. Higher viral RNA loads during the follow-up of these patients are consistent
265 with a decreased susceptibility to Sotrovimab induced by P337, E340 and K356 substitutions.
266 These three substitutions are located at the Sotrovimab epitope covering amino acids 337-
267 344 and 356-361 outside the RBM (27). Interestingly, K356 substitution could be associated
268 with the acquisition of an additional glycosylation site that could mask the antigenic epitope
269 (28). Other than this resistance to Sotrovimab, there are no data to suggest that these
270 substitutions are associated with increased transmissibility or any phenotypic change.
271 Furthermore, we did not find any of these three mutations in the general population or in
272 any of the SARS-CoV-2 VOCs.

273 Tixagevimab (Class 1) and Cilgavimab (Class 2) bind to two non-overlapping epitopes
274 covering the RBM. More precisely, Tixagevimab epitope overlaps the RBM ridge (residues
275 471-491), while Cilgavimab epitope overlaps the RBM loop (residues 443-450). Tixagevimab
276 lost all efficacy with the advent of Omicron, partly due to the emergence of the Q493R and
277 Q498R mutations, while Casirivimab demonstrated greater resilience, retaining reduced
278 activity on the BA.2 and BA.5 sub-variants (29). Emergence of R346 or K444 substitutions
279 were previously demonstrated both *in vitro* and in patients infected with BA.2 who received
280 Tixagevimab/Cilgavimab (30,31). Interestingly, both substitutions have already been found in
281 several SARS-CoV-2 lineages. First, R346K substitution (previously seen only in B.1.621 (32))
282 occurred in the BA.1.1 sub-lineage of Omicron and is associated with a higher affinity for
283 hACE2 and a 5- to 10-fold reduction in Cilgavimab activity (33–36). While these two mAbs
284 were ineffective against BA.1, with BA.2 and the disappearance of R346K, Cilgavimab regains
285 efficacy while Tixagevimab remains ineffective (37,38). Shortly thereafter, a multitude of
286 Omicron sub-lineages display substitutions of the R346 residue, such as BA.4.6, BF.7,
287 BA.5.2.6, BA.4.1.9, and BE.1.2 harbouring R346T ; BA.4.7 and BA.5.2.1 harbouring R346S ;
288 and BA.5.9 with R346I. These sub-lineages with R346X mutations are completely resistant to
289 Cilgavimab and induce a 2.4 to 2.6 fold reduction in plasma neutralizing activity from BA.5
290 infection (39,40). Spike K444 substitutions have been found in several Omicron sub-lineages,
291 such as BA.4.6.3 (K444N), BA.2.3.20 (K444R) or BA.5.2.7 (K444M). Furthermore, Ortega et al.
292 showed that K444N/R mutations within the RBM loop were identified as stabilizing hACE2-
293 Spike interaction and increasing the affinity for hACE2 (36). Together, R346 and K444
294 substitutions are part of a convergent evolution of RBD in a multitude of Omicron sub-
295 lineages with growth advantages over BA.5 (41). Mutations R346T and K444T have now

296 become widespread in the general population as they are found in the BQ.1.1 and XBB.1.5
297 variants which became dominant in late 2022.

298 Interestingly, the risk of emergence of mutations was 5-fold higher in patients treated with
299 Tixagevimab/Cilgavimab. Within this bi-therapy, only Cilgavimab retained activity on post-
300 BA.1 variants and was finally used as a monotherapy (38). However, bi-therapy with
301 Casirivimab/Imdevimab has shown greater efficacy against the Delta variant, while recent
302 studies have shown that Sotrovimab may retain efficacy thanks to its Fc-effector functions,
303 even with virus partially resistant to neutralization, unlike the other two mAb combinations
304 (12,42,43). Together, these data suggest that mAb monotherapy, without Fc-effector
305 functions, is highly sensitive to the emergence of mutations located in the targeted epitope
306 reducing neutralizing activity and may explain the higher risk of mutation emergence with
307 Tixagevimab/Cilgavimab.

308 In conclusion, our analysis highlights how using mAbs to treat high-risk COVID-19 patients
309 can drive genetic evolution of SARS-CoV-2, potentially leading to treatment resistance
310 through the rapid and frequent acquisition of mutations in Spike protein in
311 immunocompromised patients. To mitigate this risk, our findings suggest that employing bi-
312 therapies and mAbs featuring Fc-effector functions may be beneficial. Moreover, we have
313 identified these resistance mutations across multiple SARS-CoV-2 lineages, including various
314 VOCs, emphasizing the need to assess the impact of mAb treatments on SARS-CoV-2
315 evolution more broadly within the population.

316

317 **AUTHOR CONTRIBUTIONS**

318 Conceptualization of the study: G.M-B, A-G.M, C.S, F.C, Y.Y, C.D ; Data curation: V.L, K.Z, M-
319 L.M, C.L-N, G.M-B, A-G.M, C.S, F.C, C.D, Y.Y ; Data analysis: V.L, K.Z, A.F, C.S, G.M-B, A-G.M,
320 F.C ; Technical experimentation : V.L, K.Z, E.G, S.S, C.S ; Statistical analysis : A.F, V.L ; Writing
321 – original draft: V.L, K.Z, C.S, A.F, A-G.M, G.M-B ; Writing – review & editing: F.C, Y.Y.

322 All authors have read the final version of the manuscript.

323 **FUNDING**

324 The ANRS0003S COCOPREV cohort is conducted with the support of ANRS|MIE and funded
325 by French Ministries « Ministère des Solidarités et de la Santé and Ministère de
326 l'Enseignement Supérieur, de la Recherche et de l'Innovation ».

327 **ETHICAL APPROVAL**

328 This study received the ethical approval of the « Comité de Protection des Personnes (SUD-
329 EST IV) ».

330 **ACKNOWLEDGMENTS**

331 This study has been labelled as a National Research Priority by the National Orientation
332 Committee for Therapeutic Trials and other researches on Covid-19 (CAPNET). The
333 investigators would like to acknowledge ANRS | Emerging infectious diseases for their
334 scientific support, the French Ministry of Health and Prevention and the French Ministry of
335 Higher Education, Research and Innovation for their funding and support.

336 This study would have not been possible without the teams involved in the COCOPREV Study
337 and designated as the CO- COPREV Study Group: Magali Garcia, Valentin Giraud, Agathe
338 Metais, France Cazenave-Roblot, Jean-Philippe Martellosio (CHU de Poitiers); Anne-Marie
339 Ronchetti, Thomas Gabas, Naima Had- jadj, Célia Salanoubat, Amélie Chabrol, Pierre
340 Housset, Agathe Par- don, Anne-Laure Faucon, Valérie Caudwell, Latifa Hanafi (CHU Sud
341 Francilien, Corbeil-Essonnes); Laurent Alric, Grégory Pugnet, Mor- gane Mourguet, Eva Bories,
342 Delphine Bonnet, Sandrine Charpentier, Pierre Delobel, Alexa Debard, Colleen Beck, Xavier
343 Boumaza, Stella Rousset (CHU de Toulouse); Fanny Lanternier, Claire Delage, Elisabete
344 Gomes Pires, Morgane Cheminant, Nathalie Chavarot (Hôpital Necker, Paris); Anthony
345 Chauvin, Xavier Eyer; Véronique Delcey (Hôpital Lariboisière, Paris); Simon Bessis, Romain
346 Gueneau (Hôpital du Kremlin Bicêtre); Pelagie Thibaut, Marine Nadal, Mar- tin Siguier,
347 Marwa Bachir, Christia Palacios (Hôpital Tenon, Paris); Valérie Pourcher, Antoine Faycal,
348 Vincent Berot, Cécile Brin, Siham Djebara, Karen Zafilaza, Stephane Marot, Sophie Sayon,
349 Valentin Leducq (Hôpital de la Pitié Salpêtrière, Paris); Karine Lacombe, Yasmine Abi Aad,
350 Thibault Chiarabini, Raynald Feliho, Nadia Valin, Fabien Brigant, Julien Boize, Pierre-Clément
351 Thiébaud, Marie Moreau, Charlotte Billard (Hôpital St Antoine, Paris), Nathalie De Castro,
352 Geoffroy Liégeon, Blandine Denis, Jean-Michel Molina, Lucia Etheve (Hôpital Saint Louis,
353 Paris); André Cabié, Sylvie Abel, Ornella Cabras, Karine Guitteaud, Sandrine Pierre-François
354 (CHU de Martinique); Vincent Dubee, Diama Ndiaye, Jonathan Pehlivan, Michael Phelippeau,
355 Rafael Mahieu (CHU d'Angers); Charles Cazanave (CHU de Bordeaux); Alexandre Duvignaud,
356 Thierry Pistone, Arnaud Desclaux, Didier Neau, Jean-François Faucher, Benjamin Festou,
357 Magali Dupuy-Grasset, Véronique Loustaud-Ratti, Delphine Chainier (CHU de Limoges);
358 Nathan Peiffer-Smadja, Olivia Da Conceicao, Michael Thy, Lio Collas, Cindy Godard, Donia
359 Bouzid, Vittiaroat Ing, Laurent Pereira, Thomas Pavlowsky, Camille Ravaut (Hôpital Bichat,

360 Paris); Antoine Asquier-Khati, David Boutoille, Marie Chauveau, Colin Deschanvres, François
361 Raffi (CHU de Nantes); Audrey Le Bot, Marine Cailleaux, François Benezit, Anne Maillard,
362 Benoit Hue, Pierre Tattevin (CHU de Rennes); François Coustilleres, Claudia Carvalho-
363 Schneider, Simon Jamard, Laetitia Petit, Karl Stefic (CHU de Tours); Natacha Mrozek,
364 Clement Theis, Magali Vidal, Leo Sauvat, Delphine Martineau (CHU de Clermond-Ferrand);
365 Benjamin Lefèvre, Guillaume Baronnet, Agnès Didier (CHRU de Nancy); Florence Ader,
366 Thomas Perpoint, Anne Conrad, Paul Chabert, Pierre Chauvelot (CHU de Lyon); Aurélie
367 Martin, Paul Loubet, Julien Mazet, Romaric Larcher, Didier Laureillard (CHU de Nîmes);
368 Mathilde De-vaux (Hôpital de Poissy); Jérôme Frey, Amos Woerlen, Aline Remillon, Laure
369 Absensur-Vuillaume, Pauline Bouquet (CHU de Metz); Albert Trinh-Duc, Patrick Rispal
370 (Hôpital d'Agen); Philippe Petua, Julien Carillo (Hôpital de Tarbes); Aurore Perrot, Karen
371 Delavigne, Pierre Cougoul, Jérémie Dion, Odile Rauzy (Oncopole, Toulouse) Yazdan
372 Yazdanpanah, Ventzislava Petrov-Sanchez, Alpha Diallo, Soizic Le Mestre, Guillaume Le Meut
373 (ANRS-MIE); Isabelle Goderel, Frédéric Chau, Brahim Soltana, Jessica Chane Tang (IPLESP),
374 Jeremie Guedj (Université de Paris, IAME, INSERM, Paris), Yvanie Caille (Renaloo).

375 We would like to thank the ANRS-MIE Biological Resource Centre (Bordeaux, FRANCE) for
376 centralising all the biological samples of the study and making them available to the
377 virological analysis centre (CHU Pitié-Salpêtrière, Paris, FRANCE) for further analysis.

378 **CONFLICT OF INTEREST STATEMENT**

379 All authors declare no conflicts of interest.

380 **REFERENCES**

- 381 1. Huang Q, Han X, Yan J. Structure-based neutralizing mechanisms for SARS-CoV-2
382 antibodies. *Emerg Microbes Infect.* 11(1):2412–22.
- 383 2. EMA. European Medicines Agency. 2023 [cited 2023 Sep 27]. COVID-19 medicines.
384 Available from: [https://www.ema.europa.eu/en/human-regulatory/overview/public-](https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/covid-19-medicines)
385 [health-threats/coronavirus-disease-covid-19/covid-19-medicines](https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/covid-19-medicines)
- 386 3. Chen Y, Zhao X, Zhou H, Zhu H, Jiang S, Wang P. Broadly neutralizing antibodies to SARS-
387 CoV-2 and other human coronaviruses. *Nat Rev Immunol.* 2023 Mar;23(3):189–99.
- 388 4. Barnes CO, Jette CA, Abernathy ME, Dam KMA, Esswein SR, Gristick HB, et al. SARS-CoV-
389 2 neutralizing antibody structures inform therapeutic strategies. *Nature.* 2020
390 Dec;588(7839):682–7.
- 391 5. Mader AL, Tydykov L, Glück V, Bertok M, Weidlich T, Gottwald C, et al. Omicron’s binding
392 to sotrovimab, casirivimab, imdevimab, CR3022, and sera from previously infected or
393 vaccinated individuals. *iScience.* 2022 Mar 14;25(4):104076.
- 394 6. Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhore R, et al. REGN-COV2, a
395 Neutralizing Antibody Cocktail, in Outpatients with Covid-19. *N Engl J Med.* 2020 Dec
396 17;NEJMoa2035002.
- 397 7. Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhore R, et al. REGEN-COV
398 Antibody Combination and Outcomes in Outpatients with Covid-19. *N Engl J Med.* 2021
399 Dec 2;385(23):e81.
- 400 8. McCallum M, Czudnochowski N, Rosen LE, Zepeda SK, Bowen JE, Walls AC, et al.
401 Structural basis of SARS-CoV-2 Omicron immune evasion and receptor engagement.
402 *Science.* 2022 Feb 25;375(6583):864–8.
- 403 9. Planas D, Saunders N, Maes P, Guivel-Benhassine F, Planchais C, Buchrieser J, et al.
404 Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. *Nature.* 2022
405 Feb;602(7898):671–5.
- 406 10. Martin-Blondel G, Marcelin AG, Soulié C, Kaisaridi S, Lusivika-Nzinga C, Dorival C, et al.
407 Outcome of very high-risk patients treated by Sotrovimab for mild-to-moderate COVID-
408 19 Omicron, a prospective cohort study (the ANRS 0003S COCOPREV study). *J Infect.*
409 2022 Jun;84(6):e101–4.
- 410 11. Iketani S, Liu L, Guo Y, Liu L, Chan JFW, Huang Y, et al. Antibody evasion properties of
411 SARS-CoV-2 Omicron sublineages. *Nature.* 2022 Apr;604(7906):553–6.
- 412 12. Bruel T, Vrignaud LL, Porrot F, Staropoli I, Planas D, Guivel-Benhassine F, et al. Antiviral
413 activities of sotrovimab against BQ.1.1 and XBB.1.5 in sera of treated patients [Internet].
414 medRxiv; 2023 [cited 2023 Jun 22]. p. 2023.05.25.23290512. Available from:
415 <https://www.medrxiv.org/content/10.1101/2023.05.25.23290512v1>

- 416 13. Soeroto AY, Yanto TA, Kurniawan A, Hariyanto TI. Efficacy and safety of tixagevimab-
417 cilgavimab as pre-exposure prophylaxis for COVID-19: A systematic review and meta-
418 analysis. *Rev Med Virol*. 2023 Mar;33(2):e2420.
- 419 14. Bruel T, Stéfic K, Nguyen Y, Toniutti D, Staropoli I, Porrot F, et al. Longitudinal analysis of
420 serum neutralization of SARS-CoV-2 Omicron BA.2, BA.4, and BA.5 in patients receiving
421 monoclonal antibodies. *Cell Rep Med*. 2022 Dec 20;3(12):100850.
- 422 15. Takashita E, Yamayoshi S, Simon V, van Bakel H, Sordillo EM, Pekosz A, et al. Efficacy of
423 Antibodies and Antiviral Drugs against Omicron BA.2.12.1, BA.4, and BA.5 Subvariants. *N*
424 *Engl J Med*. 2022 Aug 4;387(5):468–70.
- 425 16. Cao Y, Jian F, Wang J, Yu Y, Song W, Yisimayi A, et al. Imprinted SARS-CoV-2 humoral
426 immunity induces convergent Omicron RBD evolution. *Nature*. 2023 Feb;614(7948):521–
427 9.
- 428 17. Shafin K, Pesout T, Chang PC, Nattestad M, Kolesnikov A, Goel S, et al. Haplotype-aware
429 variant calling with PEPPER-Margin-DeepVariant enables high accuracy in nanopore long-
430 reads. *Nat Methods*. 2021 Nov;18(11):1322–32.
- 431 18. Jana ID, Bhattacharya P, Mayilsamy K, Banerjee S, Bhattacharje G, Das S, et al. Targeting
432 an evolutionarily conserved ‘E-L-L’ motif in the spike protein to develop a small molecule
433 fusion inhibitor against SARS-CoV-2. *BioRxiv Prepr Serv Biol*. 2022 Mar
434 21;2022.03.16.484554.
- 435 19. Ghotloo S, Maghsood F, Golsaz-Shirazi F, Amiri MM, Moog C, Shokri F. Epitope mapping
436 of neutralising anti-SARS-CoV-2 monoclonal antibodies: Implications for immunotherapy
437 and vaccine design. *Rev Med Virol*. 2022 Sep;32(5):e2347.
- 438 20. Copin R, Baum A, Wloga E, Pascal KE, Giordano S, Fulton BO, et al. The monoclonal
439 antibody combination REGEN-COV protects against SARS-CoV-2 mutational escape in
440 preclinical and human studies. *Cell*. 2021 Jul 22;184(15):3949-3961.e11.
- 441 21. Baum A, Fulton BO, Wloga E, Copin R, Pascal KE, Russo V, et al. Antibody cocktail to
442 SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual
443 antibodies. *Science*. 2020 Aug 21;369(6506):1014–8.
- 444 22. Ragonnet-Cronin M, Nutalai R, Huo J, Dijokaite-Guraliuc A, Das R, Tuekprakhon A, et al.
445 Generation of SARS-CoV-2 escape mutations by monoclonal antibody therapy. *Nat*
446 *Commun*. 2023 Jun 7;14:3334.
- 447 23. Rockett R, Basile K, Maddocks S, Fong W, Agius JE, Johnson-Mackinnon J, et al.
448 Resistance Mutations in SARS-CoV-2 Delta Variant after Sotrovimab Use. *N Engl J Med*.
449 2022 Apr 14;386(15):1477–9.
- 450 24. Cameroni E, Bowen JE, Rosen LE, Saliba C, Zepeda SK, Culap K, et al. Broadly neutralizing
451 antibodies overcome SARS-CoV-2 Omicron antigenic shift. *Nature*. 2022
452 Feb;602(7898):664–70.

- 453 25. Vellas C, Trémeaux P, Del Bello A, Latour J, Jeanne N, Ranger N, et al. Resistance
454 mutations in SARS-CoV-2 omicron variant in patients treated with sotrovimab. *Clin*
455 *Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2022 Sep;28(9):1297–9.
- 456 26. Andrés C, González-Sánchez A, Jiménez M, Márquez-Algaba E, Piñana M, Fernández-
457 Naval C, et al. Emergence of Delta and Omicron variants carrying resistance-associated
458 mutations in immunocompromised patients undergoing sotrovimab treatment with
459 long-term viral excretion. *Clin Microbiol Infect*. 2023 Feb 1;29(2):240–6.
- 460 27. Pinto D, Park YJ, Beltramello M, Walls AC, Tortorici MA, Bianchi S, et al. Cross-
461 neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature*. 2020
462 Jul;583(7815):290–5.
- 463 28. Lin WS, Chen IC, Chen HC, Lee YC, Wu SC. Glycan Masking of Epitopes in the NTD and
464 RBD of the Spike Protein Elicits Broadly Neutralizing Antibodies Against SARS-CoV-2
465 Variants. *Front Immunol [Internet]*. 2021 [cited 2023 Sep 28];12. Available from:
466 <https://www.frontiersin.org/articles/10.3389/fimmu.2021.795741>
- 467 29. Focosi D, Casadevall A. A Critical Analysis of the Use of Cilgavimab plus Tixagevimab
468 Monoclonal Antibody Cocktail (Evusheld™) for COVID-19 Prophylaxis and Treatment.
469 *Viruses*. 2022 Sep 9;14(9):1999.
- 470 30. Vellas C, Kamar N, Izopet J. Resistance mutations in SARS-CoV-2 omicron variant after
471 tixagevimab-cilgavimab treatment. *J Infect*. 2022 Nov;85(5):e162–3.
- 472 31. Keam SJ. Tixagevimab + Cilgavimab: First Approval. *Drugs*. 2022 Jun 1;82(9):1001–10.
- 473 32. Fratev F. R346K Mutation in the Mu Variant of SARS-CoV-2 Alters the Interactions with
474 Monoclonal Antibodies from Class 2: A Free Energy Perturbation Study. *J Chem Inf*
475 *Model*. 2022 Feb 14;62(3):627–31.
- 476 33. Liu L, Iketani S, Guo Y, Chan JFW, Wang M, Liu L, et al. Striking antibody evasion
477 manifested by the Omicron variant of SARS-CoV-2. *Nature*. 2022 Feb;602(7898):676–81.
- 478 34. Cui Z, Liu P, Wang N, Wang L, Fan K, Zhu Q, et al. Structural and functional
479 characterizations of infectivity and immune evasion of SARS-CoV-2 Omicron. *Cell*. 2022
480 Mar 3;185(5):860-871.e13.
- 481 35. Li L, Liao H, Meng Y, Li W, Han P, Liu K, et al. Structural basis of human ACE2 higher
482 binding affinity to currently circulating Omicron SARS-CoV-2 sub-variants BA.2 and
483 BA.1.1. *Cell*. 2022 Aug 4;185(16):2952-2960.e10.
- 484 36. Ortega JT, Pujol FH, Jastrzebska B, Rangel HR. Mutations in the SARS-CoV-2 spike protein
485 modulate the virus affinity to the human ACE2 receptor, an in silico analysis. *EXCLI J*.
486 2021 Mar 8;20:585–600.
- 487 37. Tao K, Tzou PL, Kosakovsky Pond SL, Ioannidis JPA, Shafer RW. Susceptibility of SARS-
488 CoV-2 Omicron Variants to Therapeutic Monoclonal Antibodies: Systematic Review and
489 Meta-analysis. *Microbiol Spectr*. 2022 Jun 14;10(4):e00926-22.

- 490 38. Bruel T, Hadjadj J, Maes P, Planas D, Seve A, Staropoli I, et al. Serum neutralization of
491 SARS-CoV-2 Omicron sublineages BA.1 and BA.2 in patients receiving monoclonal
492 antibodies. *Nat Med*. 2022 Jun;28(6):1297–302.
- 493 39. Jian F, Yu Y, Song W, Yisimayi A, Yu L, Gao Y, et al. Further humoral immunity evasion of
494 emerging SARS-CoV-2 BA.4 and BA.5 subvariants. *Lancet Infect Dis*. 2022 Nov
495 1;22(11):1535–7.
- 496 40. Liu Z, VanBlargan LA, Bloyet LM, Rothlauf PW, Chen RE, Stumpf S, et al. Identification of
497 SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody
498 neutralization. *Cell Host Microbe*. 2021 Mar 10;29(3):477-488.e4.
- 499 41. Focosi D, Quiroga R, McConnell S, Johnson MC, Casadevall A. Convergent Evolution in
500 SARS-CoV-2 Spike Creates a Variant Soup from Which New COVID-19 Waves Emerge. *Int*
501 *J Mol Sci*. 2023 Jan 23;24(3):2264.
- 502 42. Martin-Blondel G, Marcelin AG, Soulié C, Kaisaridi S, Lusivika-Nzinga C, Dorival C, et al.
503 Sotrovimab to prevent severe COVID-19 in high-risk patients infected with Omicron BA.2.
504 *J Infect*. 2022 Oct;85(4):e104–8.
- 505 43. Roe TL, Brady T, Schuko N, Nguyen A, Beloor J, Guest JD, et al. Molecular
506 Characterization of AZD7442 (Tixagevimab-Cilgavimab) Neutralization of SARS-CoV-2
507 Omicron Subvariants. *Microbiol Spectr*. 2023 Mar 6;11(2):e00333-23.

508

509

510 **Figure 1: Mutational profiles and kinetics under mAbs therapies.**

511 (a) Prevalence of emerging amino acid substitutions in the spike protein under
512 Casirivimab/Imdevimab, Sotrovimab and Tixagevimab/Cilgavimab therapies. (b) Positions of
513 emerging amino acid substitutions with >5% prevalence in the Spike protein primary
514 structure and Spike and RBD three-dimensional structures. (c) Survival curve analysis
515 showing the evolution of the probability of mutation emergence in the Spike protein over
516 time under treatment with Casirivimab/Imdevimab (grey), Sotrovimab (yellow) and
517 Tixagevimab/Cilgavimab (blue). (d) Density plot representing intra-host frequencies of
518 emergent substitutions in spike protein under treatment with Casirivimab/Imdevimab (grey),
519 Sotrovimab (yellow) and Tixagevimab/Cilgavimab (blue). Mean frequencies are represented
520 by dots for each measurement time.

521 **Figure 2: Viral load evolution over time depending on resistance mutation acquisition.**

522 Comparisons of viral loads (log₁₀ copies/ml) at day 0, day 7 and day 14 of patients treated
523 with Casirivimab/Imdevimab (grey), Sotrovimab (yellow) and Tixagevimab/Cilgavimab (blue)
524 according to whether they developed a resistance mutation during follow-up or not. Box
525 plots represent the inter quartile range (IQR: 25%-75%), the line within box plots indicates
526 the median and whiskers indicate the minimum (Q1 - 1.5IQR) and maximum (Q3 + 1.5IQR)
527 values. Outliers are show as dots.

528 **Figure 3: Viral load persistence over time depending on resistance mutation acquisition.**

529 Survival curve analysis showing the evolution of the probability to have a SARS-CoV-2 viral
530 load $\geq 4.41 \log_{10} \text{ cp/ml}$ (or $\leq 31\text{Ct}$) between patients without resistance mutation emergence
531 during follow-up in Spike protein (green) and patients with resistance mutation emergence
532 during follow-up in Spike protein (red). Patients with mutation emergence in Spike protein
533 have a shorter median time to the measurement of a viral load bellow the fixed threshold
534 ($p < 0.0001$).

535 **Figure 4: Frequencies of Tixagevimab/Cilgavimab induced substitutions in the general**
536 **population.**

537 Evolution of R346X (blue) and K444X (purple) substitutions frequencies associated with
538 Tixagevimab/Cilgavimab treatment in the French general population from August 2021 to
539 December 2022.

540 **Supplementary figure 1: Results of selected logistic regression models.**

541 Model #1 looks for associations between the emergence of resistance mutations and sex,
542 age, type of immunodepression, mAb treatment and anti-S IgG levels. Model #2 looks for
543 associations between the emergence of resistance mutations and sex, age, SARS-CoV-2
544 variant, immunodepression and anti-S IgG levels. Positions of dots indicate whether the odds
545 ratios are lower or greater than 1 for each of the variables studied. Whiskers indicate the
546 95% confidence interval.

547 **Table 1** : Baseline characteristics of patients

	All N=264	Casirivimab Imdevimab N=74	Sotrovimab N=166	Tixagevimab Cilgavimab N=24	p-value
Age (median, Q1-Q3)	62.5 [50-73]	60.5 [48-75]	62 [50-73]	67.5 [62-75]	0.351
≥65 years (%)	121 (46)	31 (42)	74 (45)	16 (67)	
≥85 years (%)	18 (7)	6 (8)	10 (6)	2 (8)	
Male sex (%)	152 (58)	53 (72)	86 (52)	13 (54)	0.015
BMI (median, Q1-Q3)	25 [22-29]	25 [21-29]	25 [23-29]	25 [22-27]	0.376
Immunocompromised patients (%)	207 (78)	58 (78)	131 (79)	18 (75)	0.909
Including :					
Immunosuppressive therapy including rituximab (%)	114 (43)	34 (46)	69 (42)	11 (46)	0.788
Corticosteroids >10 mg/day for > 2 weeks (%)	31 (12)	18 (24)	12 (7)	1 (4)	< 0.001
Solid organ transplantation (%)	95 (36)	21 (28)	63 (38)	11 (46)	0.207
Cancer (%)	21 (8)	0 (0)	18 (11)	3 (13)	0.002
Ongoing chemotherapy for cancer or haematological malignancies (%)	36 (14)	9 (12)	26 (16)	1 (4)	0.338
Allogeneic hematopoietic stem cell transplantation (%)	10 (4)	2 (3)	8 (5)	0 (0)	0.686
Kidney failure with GFR < 30 mL/min or dialysis (%)	7 (3)	7 (9)	0 (0)	0 (0)	< 0.001
Systemic lupus or vasculitis with immunosuppressive medications (%)	7 (3)	1 (1)	5 (3)	1 (4)	0.475
Other immunosuppressive conditions (%)	1 (0)	1 (1)	0 (0)	0 (0)	0.371
Other risk factors for severe COVID-19 (%)	152 (58)	39 (53)	95 (57)	18 (75)	0.156
Including :					
Diabetes (type 1 and type 2, %)	61 (23)	19 (26)	32 (19)	10 (42)	0.043
Obesity (BMI>30, %)	55 (21)	12 (16)	38 (23)	5 (21)	0.501
COPD and chronic respiratory failure (%)	11 (4)	5 (7)	6 (4)	0 (0)	0.383
Chronic kidney disease (%)	42 (16)	14 (19)	22 (13)	6 (25)	0.217
Congestive heart failure (%)	11 (4)	3 (4)	6 (4)	2 (4)	0.488
High blood pressure (%)	47 (18)	12 (16)	30 (18)	5 (21)	0.880
Other chronic disease (%)	29 (11)	0 (0)	26 (16)	3 (13)	< 0.001
Vaccination, >1 dose (%)	224/254 (88)	55/67 (82)	152/164 (93)	17/23 (74)	0.006
Vaccination, ≥3 doses (%)	186/254 (73)	35/67 (52)	136/164 (83)	16/23 (70)	< 0.001
IgG anti-Spike > 260 BAU/ml at baseline (%)	83/257 (32)	9/71 (13)	59/163 (36)	15/23 (65)	< 0.001
IgG anti-Spike (BAU/ml) at baseline - median [Q1-Q3]	39 [0 - 403]	7 [0 - 96]	48 [0 - 809]	359 [56 - 638]	0.002
Pre-exposure prophylaxis (%)	12 (5)	0 (0)	8 (5)	4 (17)	0.004
Mild / Moderate COVID-19 (%)	228 (92) / 21 (8)	51 (82) / 11 (18)	153 (94) / 10 (6)	24 (100) / 0 (0)	0.006
Delta variant (%)	61 (23)	61 (82)	0 (0)	0 (0)	
BA.1 (%)	115 (44)	13 (18)	102 (61)	0 (0)	
BA.2 (%)	67 (25)	0 (0)	64 (39)	3 (12.5)	
BA.5 (%)	18 (7)	0 (0)	0 (0)	18 (75)	
BQ.1 (%)	3 (1)	0 (0)	0 (0)	3 (12.5)	
Viral RNA load [log ₁₀ cp/ml] at baseline – median [Q1-Q3]	8.28 [7.31 - 8.89]	8.23 [7.30 - 8.85]	8.25 [7.31 - 8.97]	8.46 [7.33 - 8.79]	0.974
Viral RNA load [log ₁₀ cp/ml] at day 7 – median [Q1-Q3]	6.46 [5.63 - 7.31]	5.58 [4.96 - 6.41]	6.81 [6.07 - 7.64]	6.52 [5.81 - 7.66]	< 0.001
Viral RNA load [log ₁₀ cp/ml] at day 14 – median [Q1-Q3]	4.72 [3.50 - 6.53]	3.56 [2.96 - 4.11]	5.50 [3.88 - 6.85]	5.53 [4.45 - 6.13]	< 0.001
Covid-19-related hospitalisation at day 28 (%)	11 (4)	7 (9)	4 (2)	0 (0)	0.040

