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1 **Influence of treatment with neutralizing monoclonal antibodies on the**
2 **SARS-CoV-2 nasopharyngeal load and quasispecies**

3 Camille VELLAS ^{1 2 3*}, Arnaud DEL BELLO ^{2 3 4}, Alexa DEBARD⁵, Zara STEINMEYER ⁶,
4 Laure TRIBAUDEAU ^{3 7}, Noémie RANGER ¹, Nicolas JEANNE ¹, Guillaume MARTIN-
5 BLONDEL ^{2 3 5}, Pierre DELOBEL ^{2 3 5}, Nassim KAMAR ^{2 3 4} and Jacques IZOPET ^{1 2 3}

6

7 ¹ CHU de Toulouse, Virology Laboratory, Toulouse, F-31300 France

8 ² INSERM UMR1291 - CNRS UMR5051 - Université Toulouse III, Toulouse Institute for
9 Infectious and Inflammatory Diseases (Infinity), Toulouse, F-31300 France

10 ³ Université Toulouse III Paul Sabatier, Toulouse, F-31300 France

11 ⁴ CHU de Toulouse, Département de Néphrologie, Dialyse et Transplantation d'Organes,
12 Toulouse, F-31300 France

13 ⁵ CHU de Toulouse, Service des Maladies Infectieuses et Tropicales, Toulouse, F-31300
14 France

15 ⁶ CHU de Toulouse, Geriatrics Department, Toulouse, F-31300 France

16 ⁷ CHU de Toulouse, C.O.M.E.D.I.M.S, Toulouse, F-31300 France

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22 * **Corresponding author:**

23 Camille Vellas, PharmD

24 IFB, Laboratoire de Virologie,

25 330 av de Grande Bretagne, 31052 Toulouse, France

26 Tel 0567690457 Email: vellas.c@chu-toulouse.fr

27 ORCID: 0000-0002-8637-6695

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29 **Short running title:** SARS-CoV-2 evolution after mAbs therapy

30 **ABSTRACT (219 words)**

31 **TEXT (1198 words)**

32 **ABSTRACT (219 words)**

33 **Objectives:** We aimed to evaluate the impact of neutralizing monoclonal antibodies (mAbs)
34 treatment and to determine whether the mAbs selective pressure could facilitate the
35 proliferation of virus variants with spike protein mutations that might attenuate mAb
36 effectiveness.

37 **Patients and methods:** We therefore evaluated the impact of mAbs on the nasopharyngeal
38 (NP) viral load and virus quasispecies of mAb-treated patients using single molecule real time
39 sequencing (Pacific Biosciences). The mAbs used were: Bamlanivimab alone (4 patients),
40 Bamlanivimab/Etesevimab (23 patients), and Casirivimab/Imdevimab (5 patients).

41 **Results:** The NP SARS-CoV-2 viral load of mAb-treated patients decreased from 8.2 log₁₀
42 copies/ml before administration to 4.3 log₁₀ copies/ml 7 days after administration. Five
43 immunocompromised patients given Bamlanivimab/Etesevimab were found to have mAbs
44 activity-reducing spike mutations. Two patients harbored SARS-CoV-2 variants with a
45 Q493R spike mutation 7 days after administration, as did a third patient 14 days after
46 administration. The fourth patient harbored a variant with a Q493K spike mutation 7 days
47 post-treatment, and the fifth patient had a variant with a E484K spike mutation on day 21.
48 The emergence of the spike mutation was accompanied by stabilization or rebound of the NP
49 viral load in 3/5 patients.

50 **Conclusion:** Two-mAb therapy can drive the selection of resistant SARS-CoV-2 variants in
51 immunocompromised patients. Patients given mAbs should be closely monitored and
52 measures to limit virus spread reinforced.

53

54 **Keywords:** SARS-CoV-2, neutralizing monoclonal antibodies, quasispecies, COVID-19,
55 RBD, mutations, spike protein, SMRT sequencing

56 **TEXT (1198 words)**

57 **INTRODUCTION**

58

59 Neutralizing monoclonal antibodies (mAbs) are promising tools for protecting at-risk
60 patients from a severe COVID-19 infection. They prevent the virus attaching to human ACE2
61 receptors and entering cells by targeting the receptor-binding domain (RBD) of the virus
62 spike-protein [1]. The mAbs available in France were Bamlanivimab (LY-CoV555) until
63 March 2021, then combinations of Bamlanivimab/Etesevimab (LYCoV016), and
64 Casirivimab/Imdevimab (REGEN-COV) [2,3]. New variants containing key changes in the
65 RBD have recently appeared that can be more transmissible, more pathogenic, and resistant to
66 endogenous or exogenous antibodies, than the original virus [4,5]. The full spectrum of key
67 spike mutations associated with resistance to mAbs is not yet established, but mutations
68 K417N, E484D/K/Q, Q493R/K and S494P seem to be involved in virus escape and resistance
69 to mAbs [6–8]. It is not clear how frequently these mutations occur in mAbs-treated-patients
70 or how they influence virus clearance.

71 We evaluated the impact of neutralizing mAb therapy on the nasopharyngeal viral
72 load and the diversity of the virus genome encoding the spike protein by single-molecule real-
73 time (SMRT) sequencing.

74

75 **METHODS**

76

77 Nasopharyngeal samples (NPs) were taken from infected SARS-CoV-2 patients not requiring
78 oxygen who were treated with neutralizing mAbs at the Toulouse-University-Hospital. These
79 patients were immunosuppressed, aged >80 years, or had a combination of risk-factors for
80 severe COVID-19. Bamlanivimab (700mg) was used from 27 February to 15 March, 2021,
81 and Bamlanivimab/Etesevimab (700mg/1400mg) or Casirivimab/Imdevimab
82 (1200mg/1200mg) from 16 March to 20 May, 2021. NPs were collected before treatment
83 (Day 0), 7 days after infusion (Day 7), and weekly until the viral load reached the 31 cycle-
84 threshold (Ct). The controls for solid-organ-transplant (SOT) patients were an historical
85 control group of untreated SARS-CoV-2-infected SOT patients.

86 NP SARS-CoV-2 RNA was extracted with MGI Easy Nucleic Acid Extraction kits (MGI,
87 Shenzhen, China) and quantified using the Ct values obtained with the TaqPath COVID-19
88 RT-PCR assay (Thermo Fisher Scientific, Waltham, MA) and digital-droplet-RT-PCR
89 (BioRad, Hercules, CA). SARS-CoV-2 RNA from NPs with N-gene values of <25Ct was
90 amplified and sequenced (PacBio SMRT; Pacific BioSciences, Menlo Park, CA)
91 (Supplementary material). Haplotypes were aligned on the reference genome (NC_045512.2)
92 to detect mutations associated with reduced mAb activity [6,7].

93 Changes in the NP viral load of treated patients were compared using the Wilcoxon matched-
94 pairs signed rank test. Differences between the viral loads of patients and controls were
95 compared using the Mann-Whitney U-test (GraphPad Prism 8.0; GraphPad Software, San
96 Diego, CA). These analyses were part of the national SARS-CoV-2 surveillance. French law
97 (CSP Art.L1121-1.1) does not require institutional review board approval for anonymous
98 retrospective studies.

99 **RESULTS**

100 **Patient characteristics**

101 32 SARS-CoV-2-infected patients were treated with neutralizing mAbs at the
102 Toulouse-University-Hospital (average age: 69 years; range: 23-95; 56% men). The 17/32
103 who were immunocompromised including 11/17 SOT patients (Table S1). Most patients (31;
104 97%) harbored the B.1.1.7 variant; one had the B.1, clade 20A. Four patients were given
105 Bamlanivimab, 23 were given Bamlanivimab/Etesevimab, and 5 were given
106 Casirivimab/Imdevimab.

107

108 **Changes in NP viral load after mAb administration**

109 The RT-PCR N-gene values became greater than 31Ct a median of 21 days after
110 treatment (range: 7-28 days). The median SARS-CoV-2 viral load of mAb-treated patients
111 decreased from 8.2 log₁₀copies/ml [IQR: 7.1-9.0] on day 0 to 4.3 log₁₀copies/ml [IQR: 3.4-
112 5.2] on day 7 ($p<0.001$) (Figure 1A). The viral loads of all three mAb groups decreased
113 similarly: by 3.65 log₁₀copies/ml [IQR: 1.53-6.30] for Bamlanivimab patients, 3.67
114 log₁₀copies/ml [IQR: 2.40-4.90] for Bamlanivimab/Etesevimab patients, and 3.60
115 log₁₀copies/ml [IQR: 2.95-5.15] for Casirivimab/Imdevimab patients (Figure 1B).
116 We compared the decreases in viral load of the 11 treated SOT-patients and 10 untreated
117 SOT-patients to determine the antiviral effect of mAb therapy in a homogeneous group. The
118 median decreases in the NP viral load were: 3.63 log₁₀copies/ml [IQR: 2.85-4.91] for treated
119 patients and 2.47 log₁₀copies/ml [IQR: 2.14-3.31] for controls ($p=0.03$) (Figure S1).

120

121 **SARS-CoV-2 quasispecies evolution after mAbs administration**

122 We followed-up with spike-sequencing 23 (78%) mAb-treated patients. We detected
123 no key mutation associated with reduced mAb activity in treated patients on day 0 (Table S2).

124 However, several spike-haplotypes appeared over time in 2 (50%) Bamlanivimab-treated
125 patients, 5 (31%) Bamlanivimab/Etesevimab-treated patients and one (33%)
126 Casirivimab/Imdevimab-treated patient (Table S2). SARS-CoV-2 variants with mAb activity-
127 reducing spike protein mutation appeared in five (31%) Bamlanivimab/Etesevimab-treated
128 patients, all of whom were SOT-patients. A variant with the Q493R spike mutation was first
129 detected in Patient#1 and Patient#2 on day 7 and in Patient#3 on day 14. The Q493K spike
130 mutation was detected in Patient#4 on day 7 and a variant with the E484K spike mutation was
131 detected in Patient#5 on day 21. The SARS-CoV-2 viral loads of these patients decreased
132 over time, but the viral loads of Patient#3 and Patient#5 rebounded when the mutation was
133 first detected; the load of Patient#1 first stabilized and then decreased (Figure 2).

134 **DISCUSSION**

135

136 The impact of neutralizing anti-SARS-CoV-2 mAbs on the development of resistant
137 variants is still unclear. Our analysis of the SARS-CoV-2 spike protein evolution in infected
138 patients treated with mAbs indicated that key mAb activity-reducing mutations (Q493R/K,
139 E484K) appeared in 5 SOT-patients treated with Bamlanivimab/Etesevimab.

140 Previous in vitro studies showed that mAbs can induce the production of SARS-CoV-
141 2 variants with mutation E484K and/or Q493R/K [9–11]. We have demonstrated that
142 exposure to mAbs in vivo induces the emergence of variants harboring these mutations in the
143 spike-RBD of immunocompromised-patients 7 to 21 days after Bamlanivimab/Etesevimab
144 treatment. The Q493R mutation has also been reported in one patient with
145 cholangiocarcinoma [12].

146 A clinical study of Bamlanivimab and Bamlanivimab/Etesevimab (2800mg/2800mg)
147 treatment found that new spike variants emerged in 11% of Bamlanivimab/Etesevimab-
148 treated patients [13]. However, this study only looked for mutations E484K/Q, F490S and
149 S494P. We found that a spike mutation in position 493 was selected in four mAbs-treated
150 patients. The high proportion of patients who developed key mutations could be due to the
151 lower dose of Bamlanivimab/Etesevimab (700mg/1400mg) used or to patient characteristics.
152 Key mutations emerged in five of our mAb-treated SOT patients, suggesting that
153 immunosuppression could restrict virus elimination, enhancing the risk of virus mutation
154 under mAbs. Published clinical studies of Casirivimab/Imdevimab treatment have not looked
155 for new treatment-induced variants [14] and we found no new key mutations in our
156 Casirivimab/Imdevimab patients.

157

158 The PacBio SMRT system has been invaluable for analyzing virus diversity, as it
159 provides all the haplotypes, enabling us to detect all variants, even minor ones. This is the
160 first time this approach has been used to evaluate the impact of neutralizing anti-SARS-CoV-
161 2 mAbs on a patient's virus population. We tracked the changes in viral load in addition to
162 monitoring the emergence of new variants. Although we found that mAbs reduced the viral
163 load of treated patients, this change did not occur in all the patients who harbored key
164 mutations.

165

166 The limitation of this study is the small number of patients in each group, all of whom
167 were treated at a single center. However, we have shown that key mutations emerged in a
168 substantial number of Bamlanivimab/Etesevimab-treated patients. Only five patients were
169 treated with Casirivimab/Imdevimab and we detected no key mutation, in agreement with a
170 recent study [15]. While we had no control for all the patient categories, we could compare
171 the decreases in the viral loads of mAb-treated and untreated SOT-patients.

172 Our findings highlight the need for close virological monitoring of mAbs-treated
173 patients to limit the spread of SARS-CoV-2 variants.

174 **Footnotes:**

175

176 **Contributions**

177 CV and JI designed the study. GMB, PD, NK; ADB, AD, ZS and LT provided medical care
178 to the participants and collected NPs; CV and NR collected biological data; NJ processed the
179 data, CV analyzed the results, prepared the figures and performed statistical analyses; CV and
180 JI drafted the initial version of the paper. All the authors revised the manuscript and approved
181 the final version.

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184

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189

190 **Conflict of interest**

191 The authors declare no conflict of interest.

192

193 **Corresponding author:**

194 Camille Vellas, PharmD

195 IFB, Laboratoire de Virologie,

196 330 av de Grande Bretagne, 31052 Toulouse, France

197 Tel 0567690457. Email: vellas.c@chu-toulouse.fr

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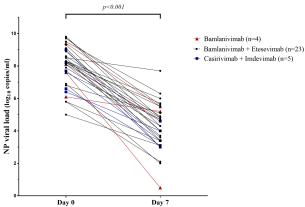
Figure legends

Figure 1:

1.A Evolution of the viral load in NPs from mAb-treated patients between days 0 and 7. The horizontal black line of the histogram indicates the median of difference (day 7 – day 0). Red: Bamlanivimab alone (n=4), dark grey: Bamlanivimab/Imdevimab (n=23) and blue: Casirivimab/Imdevimab (n=5).

1.B Differences in the viral load in NPs from mAb-treated patients between days 0 and 7. The horizontal black line of the histogram indicates the median of difference (day 7 – day 0). Red: Bamlanivimab alone (n=4), dark grey: Bamlanivimab/Etesevimab (n=23) and blue: Casirivimab/Imdevimab (n=5).

Figure 2: Changes in NP viral load, detection of spike protein mutations and SARS-CoV-2 genetic diversity in 5 Bamlanivimab/Etesevimab-treated patients. NP samples were analyzed for: clade, spike-protein mutations in the RBM, and percentage of haplotypes detected. Red stars indicate the appearance of key mAb activity-reducing mutations. Red: key mutation, blue: minor mutations.



Delta NP viral load
(log₁₀ copies/ml)

