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Running title : Antibody therapies drive Spike evolution

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ABSTRACT

Background

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

Methods

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

Results

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

Conclusions

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

Key words

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

BACKGROUND

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

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

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

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

METHODS

Study design

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

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

SARS-CoV-2 viral RNA load

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

Whole genome sequencing

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

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

Statistical analysis

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

General population

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

RESULTS

Patients description

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

Emerging mutational profiles

Following the SARS-CoV-2 genome sequencing and SNVs calling, we have listed missense mutations who were absent at baseline but emerged in the S gene in patients' follow-up 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

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

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

Emerging mutations in the general population

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

AUTHOR CONTRIBUTIONS

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-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, F.C ; Technical experimentation : V.L, K.Z, E.G, S.S, C.S ; Statistical analysis : A.F, V.L ; Writing – original draft: V.L, K.Z, C.S, A.F, A-G.M, G.M-B ; Writing – review & editing: F.C, Y.Y.

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ETHICAL APPROVAL

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

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest.

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

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

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

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

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

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

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

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

<u>Supplementary figure 1</u>: **Results of selected logistic regression models.**

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

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