TITLE PAGE

Title:

A possible role of COVID-19 therapy in the selective sweep and emergence of new SARS-CoV-2 variants

Short title:

COVID-19 therapy and selection of SARS-CoV-2 variants

Authors: Philippe COLSON[1,2], Christian A DEVAUX[1,2,3], and Didier RAOULT[1,2]*

Affiliations: [1] IHU Méditerranée Infection, 19-21 boulevard Jean Moulin, 13005 Marseille, France; [2] Aix-Marseille Univ., Institut de Recherche pour le Développement (IRD), Assistance Publique-Hôpitaux de Marseille (AP-HM), Microbes Evolution Phylogeny and Infections (MEPHI), 27 boulevard Jean Moulin, 13005 Marseille, France; [3] CNRS, Marseille, France

*** Corresponding author:** Didier RAOULT, IHU - Méditerranée Infection, 19-21 boulevard Jean Moulin, 13005 Marseille, France. Tel.: +33 413 732 401, Fax: +33 413 732 402; email: didier.raoult@gmail.com

Keywords: SARS-CoV-2; COVID-19; remdesivir; Plasma therapy; anti-spike antibodies; variant; mutants

Word counts: summary: 195 words; text : 3,095 words

Figures: 2; **Table:** 0; **References:** 62

SUMMARY

Since summer 2020, SARS-CoV-2 strains at the origin of the COVID-19 pandemic have suddenly been replaced by new SARS-CoV-2 variants, and some of which are highly transmissible and spread at high rate. These variants include the Marseille-4 lineage (Nexstrain clade 20A.EU2) in Europe, the 20I/501Y.V1 variant first detected in the UK, the 20H/501Y.V2 variant first detected in South Africa, or the 20J/501Y.V3 variant first detected in Brazil. These variants are characterized by multiple mutations that particularly affect the viral spike domain involved in host cell surface receptor binding and targeted by neutralizing antibodies elicited in response to infection or vaccine immunization. The usual coronavirus mutation rate cannot account alone through genetic drift for such rapid changes. Recent reports of the occurrence of such mutations in immunocompromized patients who received remdesivir and convalescent plasma or monoclonal antibodies to treat prolonged SARS-CoV-2 infections led us to hypothesize that experimental therapies that fail to cure the patients from COVID-19 could favor the emergence of immune escape SARS-CoV-2 variants. We review the data that support this hypothesis and urge physicians and clinical trial promoters to systematically monitor viral mutations by whole-genome sequencing for patients who are administered these treatments.

TEXT

Since the start of the COVID-19 pandemic, whole-genome sequencing approaches have made it possible to demonstrate that SARS-CoV-2 variants have gradually replaced the original "Wuhan-Hu-1 strain". Since July 2020, these variants have caused several overlapping or successive epidemic waves, and some of them have become the majority strains in different geographical areas (**Figure 1**). This is the case of the Marseille-4 lineage (Nexstrain clade 20A.EU2) in Southern France and some parts of Europe[1], and of variants 20I/501Y.V1 (Pangolin lineage B.1.1.7) in the United Kingdom[2,3], 20H/501Y.V2 (lineage B.1.351) in South Africa[4], and 20J/501Y.V3 (lineage B.1.281.1) in Brazil[5]. Compared to the original Wuhan-Hu-1 strain, these variants harbor multiple non-synonymous mutations in their genomes, including some causing amino acid substitutions in the spike glycoprotein (S) that interacts with the ACE2 cellular receptor and is the target of neutralizing antibodies[6,7]. Until autumn 2020, SARS-CoV-2 genetic diversity was most often deemed to be low, as reported in the study of 27,977 SARS-CoV-2 genomes from 84 countries[8] or in analyzes suggesting that the diversity in the surface glycoproteins of influenza A viruses was 437-fold greater than that measured in the SARS-CoV-2 spike[9]. Thereby, the rapid emergence of several SARS-CoV-2 strains with increased number of mutations, particularly in the spike-encoding gene, that has been reported in some recent observations[10-12]. (**Table 1**) questions whether an event (e.g., genetic or ecologic) has occurred which may have modified the virus replication process or the host-dependent virus selection, to promote and spread variant viruses more transmissible than the previously-circulating strains and capable of escaping immune responses in some cases[2,4,13]. It raises fear of an over-amplification of the pandemic worldwide. Of particular concern are the mutations within the viral spike that attaches the virion to the ACE2 cell-surface receptor[14] and serves as major target for neutralizing antibodies[7, 15].

Mutations in the spike associated with a risk of neutralizing immune response escape

Several mutations in the spike protein, including those in its receptor binding domain (RBD; amino acids 333 to 527 of the spike) that binds to ACE2, increase the affinity of the trimeric form of this spike to ACE2 and thus increase viral replication or worsen viral cytopathic effects[16]. In addition, some were reported to reduce the sensitivity to anti-SARS-CoV-2 antibodies[17,18]. The variants that we detected in July 2020 and named Marseille-4 (later classified as Nextstrain clade 20A.EU2)[19], harbor amino acid substitution S477N located in the spike RBD[1, 19-20]. This substitution was reported to confer resistance to neutralization to multiple monoclonal antibodies[13]. Regarding the rapidly-spreading SARS-CoV-2 variants from the United Kingdom (20I/501Y.V1) and South Africa (20H/501Y.V2), both harbor a substitution N501Y in the RBD that increases affinity to ACE2[3-6], plus six additional substitutions including P681H for the 20I/501Y.V1 variant[3], or K417N and E484K for the 20H/501Y.V2 variant[4], which reduce the sensitivity to anti-SARS-CoV-2 antibodies [17,18]. Beyond, these mutations can lead to immune escape, as shown in several *in vitro* studies [6,12-13,17-18]. Accordingly, beyond the uncertainty that remains about the duration of protective immune response against SARS-CoV-2 after a first episode of infection, a major concern comes from the appearance of amino acid changes in the spike of new variants. Genomic evidences of SARS-CoV-2 reinfections with 20I/501Y.V1, 20H/501Y.V2, and 20J/501Y.V3 variant were documented[21-23]. Regarding for instance the first of these reports, it involved a 78-year-old man with type-2 diabetes mellitus, diabetic nephropathy on haemodialysis and chronic obstructive pulmonary disease in the UK[21]. This patient was diagnosed with SARS-CoV-2 during the first episode of the pandemic and exhibited mild illness. Then, 8 months later, he was reinfected with the 20I/501Y.V1 variant as documented by whole-genome sequencing, which caused a critical illness. In addition, we observed in our institute the case

of several patients who had been infected in March-April 2020, then experienced clinical recovery and viral clearance as documented by qPCR negativity, but were infected again during summer 2020 or later with a Marseille-4 variant that carries the S477N substitution in the spike[24]. Such clinical observation of reinfection with a Brazilian variant is corroborated by the observation that culturing *in vitro*, in presence of neutralizing plasma, a SARS-CoV-2 isolate sensitive to highly-neutralizing plasma from a COVID-19 convalescent patient, was associated with the occurrence of a deletion of amino acid F140 in the N-terminal domain (NTD, loop N3) of the spike after 45 days, and of the E484K substitution after 73 days, followed by an insertion in the NTD, loop N5[25]. Computational modeling was also reported to predict that such variant should escape neutralization antibodies[25]. Furthermore, such mutations could make the vaccine approach less effective or even harmful through the generation of antibody-dependent enhancement of viral uptake through binding to Fc or complement receptor-bearing cells[26].

Intriguing high error rate in the new SARS-CoV-2 variants

We can wonder about the mechanisms of emergence and selection of these new highly transmissible SARS-CoV-2 variants. SARS-CoV-2, like other coronaviruses and RNA viruses, is evolving according to the quasi-species (mixtures of different viral populations) model characterized by continuous genetic variation as a result of a high error rate of RNA-dependent RNA polymerase (RdRp)[27,28]. Under positive selective pressure from the host, spontaneously-generated mutations can be selected, leading to the emergence of variant viruses able to escape the host's defense mechanisms[29]. These variants are strains that differ from all others by a set of several mutations, and have reached a detectable population size. Among RNA viruses, coronaviruses appear to be quite complex since they have the largest genome (almost 30,000 bases). They harbor two characteristics that allow them to maintain

such large genome[30,31]: a RdRP with a proofreading activity (conferred by the Nsp14 exoribonuclease) and a homologous recombination mechanism associated with replication. This should prevent high mutation rates and deleterious mutations through the corrective action of Nsp14 that is able to remove nucleoside analogues after their incorrect insertion into the nascent RNA[30]. Although the capacity of SARS-CoV-2 to evolve under the host's immune pressure remains to be further characterized, this virus is likely to have a reduced tolerance to genetic drift in order to maintain the integrity of its large RNA genome, under the action of its Nsp14 exonuclease that counteracts the low RdRP fidelity[31]. As a result, the recent emergence of some variants with large sets of mutations is surprising and difficult to explain without evoking a particular induction and selective pressure. It can either result from a natural origin (such as an intra-host species evolution under the immune response pressure or a change in species before reintroduction into humans) or be induced by a therapy (such as an antiviral therapy inducing escape variants, as previously reported decades ago for AIDS monotherapy[32]).

Selection of highly transmissible variants: 'natural hypothesis'

A first hypothesis for the emergence and selection of new SARS-CoV-2 variants, the 'natural hypothesis', is linked to the selective pressure of the host's immune system. In this model, if the virus is exposed to the host's immune response including neutralizing and non-neutralizing antibodies, this can select for spike variants among the viral quasi-species[27]. One SARS-CoV-2 variant with substitution D614G in the spike, which increases the stability of S trimer complex and renders the virus more infectious, progressively increased in prevalence worldwide and was almost the only variant since the epidemic onset in Europe[33]. It corresponds to the selection of a new beneficial mutation that increases its frequency and fixes it in the population[34]. It has been for instance reported that functional SARS-CoV-2

spike variants with mutations in the RBD and N-terminal domain that confer resistance to monoclonal antibodies or convalescent plasma can be selected *in vitro*[35]. In another work, Li et al. studied the infectivity and reactivity to neutralizing antibodies and convalescent patients' sera of 106 SARS-CoV-2 spike mutants including 26 with deletions at putative N-linked glycosylation sites, and they identified changes in infectiousness and sensitivity to neutralizing antibodies, including resistance in some cases[36]. In addition, Thomson et al. reported that the natural spike variant N439K can escape antibody-mediated immunity while maintaining fitness[37].

Selection of highly transmissible variants: 'interventionist therapy hypothesis'

A second hypothesis to explain the emergence and selection of new SARS-CoV-2 variants, the 'interventionist therapy hypothesis', is linked to the selective pressure of treatments. In order to treat severe COVID-19, some patients received immunoglobulin-based immunotherapy with hyper-immune sera from convalescent COVID-19 patients[10-11, 38] (**Table 1**). However, a study reported that of the sera from 26 patients who recovered from COVID-19 and exhibited high titers of anti-SARS-CoV-2 immunoglobulins, only 3 effectively blocked the binding of the SARS-CoV-2 spike protein to ACE2[39]. Studies by Greaney et al. and Liu et al. also reported that neutralization of spike harboring changes at some amino acid positions, including positions 484, 456 and 477, had decreased sensitivity to convalescent serum antibodies[6,13]. Recent *in vitro* results from Andreano et al.[25] also suggested that SARS-CoV-2 can evade suboptimal concentrations of neutralizing antibodies. These authors reported that after 45 days of culture (6 subcultures) of wild-type SARS-CoV-2 with serial two-fold dilutions of neutralizing plasma, a variant with several spike mutations among which the E484K substitution emerged, and resisted high levels of neutralizing antibodies. Weisblum et al. also reported the selection of SARS-CoV-2 with mutations in the spike RBD

by culturing in presence of convalescent plasma[35]. In addition, it was reported that 15 patients with hematological malignancies, one patient with multiple sclerosis and one patient with common variable immune deficiency, who had prolonged COVID-19 symptoms, improved their clinical symptoms and showed decreased SARS-CoV-2 RNA load between 7 and 14 days after receiving 4 units of COVID-19 convalescent plasma therapy (some of them having also received remdesivir)[40]. However, 5 of these patients remained positive for SARS-CoV-2 on nasopharyngeal swab, and unfortunately their viruses were not monitored by whole-genome sequencing and information regarding the possible selection of variants is therefore missing. Besides, the 20H/501Y.V2 and 20J/501Y.V3 variants were reported to escape from therapeutic antibodies and antibodies elicited by infection and vaccine immunization[41]. These data indicate that administration of sera from convalescent patients may favor the emergence of SARS-CoV-2 variants evading the immune response.

Also worth noting are the cases of patients who received the antiviral nucleoside (adenosine) analogue prodrug GS-5734, remdesivir, which sterically interacts with the viral Nsp12/RdRp to induce delayed chain termination[42]. The structure of the SARS-CoV-2 Nsp12/RdRp in complex form with Nsp7 and Nsp8, the template primer RNA, remdesivir, and Mg^{2+} ions was determined recently[43]. Only low-level resistance to remdesivir has been observed *in vitro*, in association with 2 amino acid substitutions (F480L and V557L) in the Nsp12/RdRp[44], while RdRp substitution D484Y has been observed *in vivo* in association with treatment failure[45]. This corroborates the observation that under subclinical concentrations of remdesivir, a variant of Ebola virus emerged with a single F548S substitution that confers fourfold to fivefold reduced susceptibility to remdesivir[46]. This mutation lies in the F-motif of the RdRp active site where mutations that confer remdesivir resistance occur in coronaviruses. A mechanism of action similar to that of remdesivir has been reported for the prodrug T-705 (favipiravir), a drug that is orally administered and reported to inhibit SARS-CoV-2 by lethal

mutagenesis escaping the coronavirus repair machinery[47]. Similarly, the ribonucleoside analog NHC/EIDD-2801 antiviral activity is associated with increased viral mutation rates[48]. Altogether, these results suggest that nucleoside analog prodrugs may increase the mutation frequency of SARS-CoV-2, allowing the emergence of fast-spreading SARS-CoV-2 variants. Interestingly, remdesivir may also interact with and hinder the action of the Nsp14 exonuclease that has proofreading activity and excises mis-incorporated nucleotides[49]. Therefore, remdesivir could shut down the correcting activity and increase the mutation rate. A mutagenic effect was also predicted for remdesivir[50]. Remdesivir is an analog of adenine and is believed to compete with adenine triphosphate (ATP) during the viral replication. The mechanism of action of this molecule could lead to (i) RNA chain termination; (ii) non-obligate chain termination with modification of neighboring side chain; or (iii) delayed chain termination. Both non-obligate chain termination and delayed chain termination have been proposed for remdesivir[51-53]. In addition, it was reported that tautomers (structural isomers that differ from one another regarding the position of protons and double bonds) of RNA bases could play a crucial role in mutagenesis[54]. Adenine has the ability to adopt amino and imino tautomeric forms involving the exocyclic group at the 6-position. In Jena's article[50], the role of different tautomers in their base-pairing abilities was studied to further understand the role of remdesivir in the generation of mutations. It was found that remdesivir can adopt both amino and imino tautomeric conformations to base-pair with RNA bases. While the insertions of G and U appeared as preferred pairs against the amino tautomers of this drug, the insertion of C is mainly possible against the imino tautomers. The author concluded that both amino-remdesivir: G and imino-remdesivir: C pairs could be quite mutagenic. An experimental work by Szemiel and colleagues[55] recently demonstrated how serial *in vitro* passages of SARS-CoV-2_{Eng12} in cell culture media supplemented with remdesivir selected for drug-resistant viral populations. They found that remdesivir triggers the selection of SARS-CoV-2 variant

with a E802D mutation in the RNA-dependent RNA polymerase (Nsp12) sufficient to confer decreased sensitivity to remdesivir without affecting viral fitness. Another mutation I168T was observed in the Nsp6. The analysis of more than 200,000 sequences also revealed the occurrence of 22 mutations in the spike including changes at amino acids E484 and N501 corresponding to mutations identified in emerging SARS-CoV-2 variants in the UK (20I/501Y.V1) and in South Africa (20H/501Y.V2). These results clearly indicate that E484 and N501 mutations can arise *in vitro* in the absence of immune selection, under the sole selection pressure of remdesivir.

Could remdesivir and/or convalescent plasma experimental therapy promote the emergence of highly transmissible SARS-CoV-2 variants?

The above results lead to question whether the N501Y-harboring SARS-CoV-2 variants may have arisen due to remdesivir administration and concurrent or subsequent selection by the host's immune response and/or the administration of antibodies from convalescent patients' sera (**Figure 2)**. As a matter of fact, several cases have been reported for which emergence of SARS-CoV-2 variants with mutations within the viral spike was evidenced in immunocompromized patients with prolonged SARS-CoV-2 infection who had received remdesivir and/or anti-spike antibodies or convalescent plasma (**Table 1**). The first case occurred in a 45-year-old man with a severe antiphospholipid syndrome who was recurrently diagnosed with SARS-CoV-2 during approximately 5 months in Boston, USA[10]. He was treated with glucocorticoids, cyclophosphamide and eculizimab. This patient received four 5-day or 10-day courses of remdesivir around days 0, 72, 105 and 151 after first viral detection. He also received an antibody cocktail targeting the SARS-CoV-2 spike. Sequencing of sequential samples showed that amino acid changes had occurred within the spike and its RBD in 57% and 38% of cases, these regions being over-mutated since they represent only

13% and 2% of the viral genome, respectively. These changes included substitutions P9L and Q183H and deletion delta142/144 in the N-terminal domain; substitutions I870V and A1020S in the C-terminal domain; as well as substitutions N440D, T478K, E484K/A, F486I, Y489H, Q493K, S494P, and N501Y in the RBD. Strikingly, this latter change that was found from day 128 until day 152 is the one harbored by SARS-CoV-2 variants that have expanded since October mostly in the United Kingdom[3]. One non-synonymous mutation occurred in the envelope and three in the nucleocapsid. A single synonymous mutation was observed in the RdRp. SARS-CoV-2 in respiratory samples was found to be infectious when tested at days 75 and 143. A second case was in a hypogammaglobulinemia patient who was treated with B cell depletion and who was followed over 101 days with viral genome sequencing from 23 sequential respiratory samples[11]. This patient received two courses of remdesivir at days 41 and 54 and SARS-CoV-2 convalescent patients' plasma at days 63, 65, and 93. Low-frequency variant analysis showed the occurrence of spike variant as soon as on day 45, then of N501Y variant at 33% frequency on day 55, that was no more detected on day 66 when variants with changes in Nsp2 and RdRp (V157L) occurred. This latter variant was later replaced after convalescent plasma administration by variants that differed according to the time point and harbored spike substitutions Y200H, T240I, P330S, D796H, and the Delta69/70 double deletion found in the 20I/501Y.V1 UK variant. The Delta69/70 single mutant had two-fold higher infectivity compared to wild type. These findings could possibly explain why such variants harboring Delta69/70 deletion and N501Y substitution emerged in countries such as the UK where remdesivir is routinely used for the treatment of hospitalized SARS-CoV-2 patients with chronic infection. Four other studies reported cases of 31-75-year-old immunocompromized males with hematological malignancies or agammaglobulinaemia who experienced viral shedding during between 62 and 268 days and received two to four cures of remdesivir, as well as one or two administrations of convalescent plasma[56-59]. Three

patients also received steroids. SARS-CoV-2 qPCR positivity persisted between 29 and 227 days after first remdesivir administration. Two of these four patients eventually died, at days 74 and 271 after SARS-CoV-2 diagnosis. Viral genome sequencing showed in three of the patients, who were infected for 62, 71 and 268 days, the occurrence of 1, 7 and 9 amino acid changes in the spike, respectively. These included a deletion of amino acid Y144 and a substitution N501T in one patient, and substitutions H69Y/P and V70G in another patient, all these changes having occurred at spike positions that also harbor mutations in variants 20I/501Y.V1.

Conclusion

It is urgent to explore why new SARS-CoV-2 variants with spike mutations suddenly arose in France, the UK, South Africa, or Brazil. One hypothesis is a genetic change due to an ecological accident such as transmission from humans to another species and then reinfection of humans, as it is suspected with minks[60-61]. Another hypothesis that could shed light on these aberrant events involves chronically ill patients treated with experimental therapies at concentrations that did not allow to eliminate the virus and favored emergence of variants from the residual viral load. Some immunocompromized patients with persistent SARS-CoV-2 infection[10,11] were administered remdesivir that possibly increases the frequency of mutations and have received convalescent plasma or anti-spike antibodies that were reported to drive the occurrence of spike escape mutations. While waiting to learn more, it is essential to use experimental therapies with extreme caution and to monitor the possible emergence of SARS-CoV-2 variants in these patients. Characterization of variants relies on whole-genome sequencing that has been performed until now only in a minority of SARS-CoV-2 infections[62] and may be therefore drastically under-reported. An evolution of clinical practices is required and the promoters of immunotherapy and antiviral therapy trials in COVID-19 patients should

imperatively organize the monitoring of SARS-CoV-2 variants by whole-genome sequencing, in particular for patients who are immunosuppressed.

Acknowledgments:

This manuscript has been edited by a native English speaker.

Author contributions

PC, CD and DR wrote the first draft of the manuscript. DR supervised the work. All co-authors revised the manuscript and added key content. All co-authors read and approved the final manuscript.

Funding

This work was supported by the French Government under the "Investments for the Future" program managed by the National Agency for Research (ANR), Méditerranée-Infection 10-IAHU-03.

Competing interests

CD declares a link of interest with Sanofi and Merck pharmaceutical companies. PC and DR declare that they have no competing interests.

REFERENCES

1. Fournier, P. E. et al. Emergence and outcome of the SARS-CoV-2 "Marseille-4" variant. *IHU pre-prints* doi: https://doi.org/10.35081/xcrm-6t77 (2021).
2. Davies, N. G. et al. Estimated transmissibility and severity of novel SARS-CoV-2 Variant of Concern 202012/01 in England. *MedRxiv* doi: https://doi.org/10.1101/2020.12.24.20248822 (2020).
3 Leung, K. et al. Early transmissibility assessment of the N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020. *Euro Surveill* **26**, 2002106. (2021).
4 Tegally, H. et al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. *MedRxiv* 2020 Preprint not peer reviewed. doi: https://doi.org/10.1101/2020.12.21.20248640. (2020).
5. Faria, N. R. et al. Genomic characterisation of an emergent SARS-CoV-2 lineage in Manaus: preliminary findings. *Virological org* https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-manaus-preliminary-findings/586 (2021).
6. Greaney, A. J. et al. Comprehensive mapping of mutations to the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human serum antibodies. *Cell Host & Microbe* Feb 8;S1931-3128(21)00082-2. doi: 10.1016/j.chom.2021.02.003. Online ahead of print (2021).
7. Piccoli, L. et al. Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**, 1024-1042 (2020).

8 Dearlove, B. et al. A SARS-CoV-2 vaccine candidate would likely match all currently circulating variants. *Proc Natl Acad Sci USA* **117**, 23652–23662 (2020).

9 Rausch, J. W. et al. Low genetic diversity may be an Achilles heel of SARS-CoV-2. *Proc Natl Acad Sci USA* **117**, 24614-24616 (2020).

10 Choi, B. et al.Persistence and Evolution of SARS-CoV-2 in an Immunocompromised. *N Engl J Med* **383**, 2291-2293 (2020).

11 Kemp, S. A. et al. SARS-CoV-2 evolution during treatment of chronic infection. *Nature* Feb 5. doi: 10.1038/s41586-021-03291-y. Online ahead of print 2021.

12. Khatamzas, E. et al. Emergence of multiple SARS-CoV-2 mutations in an immunocompromised host. *MedRxiv* doi: https://doi.org/10.1101/2021.01.10.20248871 (2021).

13. Liu, Z. et al. Landscape analysis of escape variants identifies SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization. *BioRxiv* doi: https://doi.org/10.1101/2020.11.06.372037 (2020).

14 Qiu, Y. et al.Predicting the angiotensin converting enzyme 2 (ACE2) utilizing capability as the receptor of SARS-CoV-2. *Microbes Infect* **22**, 221-225 (2020).

15 Barnes, C. O. et al.SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. *Nature* **588**, 682-687 (2020).

16 Nguyen, H. T. et al.Spike glycoprotein and host cell determinants of SARS-CoV-2 entry and cytopathic effects. *J Virol* 2020. JVI.02304-20. doi: 10.1128/JVI.02304-20. Online ahead of print. (2020).

17. Wibmer, C. K. et al. SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. *bioRxiv* doi**:** https://doi.org/10.1101/2021.01.18.427166 (2021).

18. Collier, D. A. et al. SARS-CoV-2 B.1.1.7 sensitivity to mRNA vaccine-elicited, convalescent 2 and monoclonal antibodies . *MedRxiv* doi: https://doi.org/10.1101/2021.01.19.21249840; (2021).

19. Hodcroft, E. B. et al. Emergence in late 2020 of multiple lineages of SARS-CoV-2 Spike protein variants affecting amino acid position 677. *MedRxiv* 2021.02.12.21251658; doi: https://doi.org/10.1101/2021.02.12.21251658 (2021).

20 Lan, J. et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* **581**, 215-220 (2020).

21 Harrington, D. et al. Confirmed reinfection with SARS-CoV-2 variant VOC-202012/01. *Clin Infect Dis* 2021 Jan 9;ciab014. doi: 10.1093/cid/ciab014. Online ahead of print (2021).

22. Zucman, N., Uhel, F., Descamps, D., Roux, D., Ricard, J. D. (2021). Severe reinfection with South African SARS-CoV-2 variant 501Y.V2: A case report. *Clin Infect Dis* Feb 10;ciab129. doi: 10.1093/cid/ciab129. Online ahead of print. (2021).

23 Vasques Nonaka, C. K. et al. Genomic evidence of a SARS-CoV-2 reinfection case with E484K spike mutation in Brazil. *Preprints* 2021. doi: 10.20944/preprints202101.0132.v1 (2021).

24. Brouqui, P. et al. COVID-19 re-infection. *Eur J Clin Invest* Mar 6;e13537. doi: 10.1111/eci.13537. Online ahead of print. (2021).

25 Andreano, E. et al.SARS-CoV-2 escape in vitro from a highly neutralizing COVID-19 convalescent plasma. *bioRxiv* preprint (2020). Preprint not peer reviewed. doi: 10.1101/2020.12.28.424451 (2020).

26 Wen, J. et al. Antibody-dependent enhancement of coronavirus. *Int J Infect Dis* **100**, 483-489 (2020).

27 Karamitros . et al. SARS-CoV-2 exhibits intra-host genomic plasticity and low-

frequency polymorphic quasispecies. *J Clin Virol* **131**, 104585 (2020).

28 Jary, A. et al.Evolution of viral quasispecies during SARS-CoV-2 infection. *Clin Microbiol Infect* **26**, 1560E1-1560E4 (2020).

29 Woo, H. J., Reifman, J. A quantitative quasispecies theory-based model of virus escape mutation under immune selection. *Proc Natl Acad Sci USA* **109**, 12980-12985 (2012).

30 Ferron, F. et al. Structural and molecular basis of mismatch correction and ribavirin excision from coronavirus RNA. *Proc Natl Acad Sci USA* **115**, E162–E171 (2018).

31 Snijder, E. J., Decroly, E., Ziebuhr, J. The nonstructural proteins directing coronavirus RNA synthesis and processing. *Adv Virus Res* **96**, 59-126 (2016).

32 Larder, B. A., Kemp, S. D. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). *Science* **246**, 1155-1158 (1989).

33 Korber, B. et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. *Cell* **182**, 812-827 (2020).

34 Biswas, N. K. et al. Analysis of RNA sequences of 3636 SARS-CoV-2 collected from 55 countries reveals selective sweep of one virus type. *Indian J Med Res* **151**, 450-458 (2020).

35 Weisblum, Y. et al. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. *eLife* **9**, e61312 (2020).

36 Li, Q. et al. The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity. *Cell* **182**, 1284-1294 (2020).

37 Thomson, E. C. et al. The circulating SARS-CoV-2 spike variant N439K maintains fitness while evading antibody-mediated immunity *BioRxiv* https://www.biorxiv.org/content/10.1101/2020.11.04.355842v1 (2020).

38 Nguyen, A. A. et al. Immunoglobulins in the treatment of COVID-19 infection: Proceed with caution! *Clin Immunol* **216**, 108459 (2020).

411 39 Chen, X. et al. Human monoclonal antibodies block the binding of SARS-CoV-2 spike
412 protein to angiotensin converting enzyme 2 receptor. *Cell Mol Immunol* **17**, 647-649
413 (2020).
414 40 Huesco, T. et al. Convalescent plasma therapy for B-cell-depleted patients with
415 protracted COVID-19. *Blood* **136**, 2290-2295 (2020).
416 41. Hoffmann, M. et al. SARS-CoV-2 variants B.1.351 and B.1.1.248: Escape from
417 therapeutic antibodies and antibodies induced by infection and vaccination. *BioRxiv* doi:
418 https://doi.org/10.1101/2021.02.11.430787 (2021).
419 42 Gordon, C. J. et al. Remdesivir is a direct-acting antiviral that inhibits RNA-dependent
420 RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high
421 potency. *J Biol Chem* **295**, 6785-6797 (2020).
422 43 Yin, W. et al. Structural basis for inhibition of the RNA-dependent RNA polymerase
423 from SARS-CoV-2 by remdesivir. *Science* **368**, 1499-1504 (2020).
424 44 Agostini, M. L. et al. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is
425 mediated by the viral Polymerase and the proofreading exoribonuclease. *mBio* **9**,
426 e00221-18 (2018).
427 45 Martinot, M. et al. Remdesivir failure with SARS-CoV-2 RNA-dependent RNA-
428 polymerase mutation in a B-cell immunodeficient patient with protracted Covid-19.
429 *Clin Infect Dis* 2020. ciaa1474. doi: 10.1093/cid/ciaa1474. Online ahead of print (2020).
430 46 Lo, M. K. et al. Remdesivir targets a structurally analogous region of the Ebola virus
431 and SARS-CoV-2 polymerases. *Proc Natl Acad Sci USA* **117**, 26946-26954 (2020).
432 47 Shannon, A. et al. Rapid incorporation of Favipiravir by the fast and permissive viral
433 RNA polymerase complex results in SARS-CoV-2 lethal mutagenesis. *Nat Comm* **11**,
434 4682 (2020).
435 48 Sheahan, T. et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2

in human airway epithelial cell cultures and multiple coronaviruses in mice. *Science Transl Med* **12**, eabb5883 (2020).

49 Shannon, A. et al. Remdesivir and SARS-CoV-2: structural requirements at both nsp12 RdRp and nsp14 exonuclease active-sites. *Antiviral Res* **178**, 104793 (2020).

50 Jena, N. R. Role of different tautomers in the base-pairing abilities of some of the vital antiviral drugs used against COVID-19. *Phys Chem Chem Phys* **22**: 28115-28122 (2020).

51. Tchesnokov, E. P., Feng, J. Y., Porter, D. P., Gotte, M. Mechanism of inhibition of Ebola virus RNA-dependent RNA polymerase by Remdesivir. *Viruses* **11**, 326 (2019).

52. Win, W. et al.Structural basis for inhibition of the RNAdependent RNA polymerase from SARS-CoV-2 by remdesivir. *Science* **368**, 1499–1504 (2020).

53. Gordon, C. J. et al. Remdesivir is a directacting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *J Biol Chem* **295**, 6785–6797 (2020).

54. Singh, V., Fedeles, B. I., Essigmann, J. M. Role of tautomerism in RNA biochemistry. *RNA* **21**, 1–13 (2015).

55. Szemiel, A. M. et al. In vitro evolution of Remdesivir resistance reveals genome plasticity of SARS-CoV-2. *BioRxiv* doi: https://doi.org/10.1101/2021.02.01.429199 (2021).

56. Bang, J. H. et al. Prolonged Severe Acute Respiratory Syndrome Coronavirus 2 Replication in an Immunocompromised Patient. *J Infect Dis* **223,** 23-7 (2021).

57. . Hensley, M. K. et al. Intractable COVID-19 and Prolonged SARS-CoV-2 Replication in a CAR-T-cell Therapy Recipient: A Case Study. *Clin Infect Dis* Jan 28;ciab072. doi: 10.1093/cid/ciab072. Online ahead of print. (2021).

58. . Sepulcri, C. et al. The longest persistence of viable SARS-CoV-2 with recurrence of viremia and relapsing symptomatic COVID-19 in an immunocompromised patient – a case study. *MedRxiv* doi: https://doi.org/10.1101/2021.01.23.21249554 (2021).

59. Buckland, M. S. et al. Treatment of COVID-19 with remdesivir in the absence of humoral immunity: a case report. *Nat Commun* **11,** 6385 (2020).

60. Oude Munnink, B. B. et al. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science* **371**, 172-177. doi: 10.1126/science.abe5901 (2021).

61. Fenollar, F. et al. Mink, SARS-CoV-2, and the human-animal interface. *IHU Pre-prints* doi : https://doi.org/10.35088/gwvd-p422 (2021).

62 Colson, P., Raoult D. Global discrepancies between numbers of available SARS-CoV-2 genomes and human 6 development indexes at country scales. *IHU Pre-prints* doi: https://doi.org/10.35088/tny6-v454 (2021).

FIGURE LEGENDS

Figure 1: Examples of the emergence of three SARS-CoV-2 spike variants in 2020

Number of sequences available from the GISAID database (https://www.gisaid.org/).

Figure 2: Possible mechanism of occurrence and selection of SARS-CoV-2 spike variants