1 Replicative fitness SARS-CoV-2 20I/501Y.V1 variant in a human reconstituted 2 bronchial epithelium

3 Franck Touret^{1*}, Léa Luciani¹, Cécile Baronti¹, Maxime Cochin¹, Jean-Sélim Driouich¹,
4 Magali Gilles¹, Laurence Thirion¹, Antoine Nougairède^{1 #} and Xavier de Lamballerie^{1 #}

5 ¹ Unité des Virus Émergents (UVE: Aix-Marseille University -IRD 190-Inserm 1207),
6 Marseille, France.

7 # Co-last authors

8 *Correspondence: franck.touret@univ-amu.fr

9 Keywords: SARS-CoV-2; variant; replicative fitness; *ex vivo*

10 Abstract

11 Since its emergence in 2019, circulating populations of the new coronavirus continuously
12 acquired genetic diversity. At the end of 2020, a variant named 20I/501Y.V1 (lineage B.1.1.7)
13 emerged and replaced other circulating strains in several regions. This phenomenon has
14 been poorly associated to biological evidence that this variant and original strain exhibit
15 different phenotypic characteristics. Here, we analyse the replication ability of this new
16 variant in different cellular models using for comparison an ancestral D614G European strain
17 (lineage B1). Results from comparative replication kinetics experiments *in vitro* and in a
18 human reconstituted bronchial epithelium showed no difference. However, when both viruses
19 were put in competition in a human reconstituted bronchial epithelium, the 20I/501Y.V1
20 variant outcompeted the ancestral strain. Altogether, these findings demonstrate that this
21 new variant replicates more efficiently and could contribute to better understand the
22 progressive replacement of circulating strains by the SARS-CoV-2 20I/501Y.V1 variant.

23 Importance

24 The emergence of several SARS-CoV-2 variants raised numerous questions concerning the
25 future course of the pandemic. We are currently observing a replacement of the circulating
26 viruses by the variant from the United Kingdom known as 20I/501Y.V1 from B.1.1.7 lineage
27 but there is little biological evidence that this new variant exhibit a different phenotype. In the
28 present study, we used different cellular models to assess the replication ability of the
29 20I/501Y.V1 variant. Our results showed that this variant replicate more efficiently in a
30 human reconstituted bronchial epithelium, which may explain why it spreads so rapidly in
31 human populations.

32

33

34 Observation

35 The new coronavirus SARS-Cov-2 emerged in China by the end of 2019 and rapidly spread
36 worldwide. In a few months, D614G spike mutation was rapidly fixed in almost all SARS-
37 CoV-2 circulating populations without evidence for higher COVID-19 mortality or clinical
38 severity (1). It is still being debated whether it is due to a random founder effect (1) or, more
39 probably, if the mutation enhances viral loads in the upper respiratory tract increasing the
40 infectivity and stability of virions (2, 3).

41 In September 2020, emerged in the United Kingdom a variant named 20I/501Y.V1 from
42 lineage B.1.1.7 (initially named VOC 2 202012/01). It spread rapidly and is becoming
43 dominant in Western Europe (4) and the USA (5). There is consistent epidemiological
44 evidence that this so-called 'UK variant' is more efficiently transmitted than the pre-existing
45 European strains, in particular in young patients. However, this has been poorly associated
46 to biological evidence that the UK and original European variants exhibit different phenotypic
47 characteristics.

48 Here, we present a comprehensive analysis of the replication ability *in vitro* and *ex vivo* of the
49 20I/501Y.V1 variant (strain UVE/SARS-CoV-2/2021/FR/7b isolated in February 2021 in
50 Marseille, France; GISAID accession nb. EPI_ISL_918165), using for comparison the lineage
51 B.1 BavPat D614G strain that circulated in Europe in February/March 2020.

52 The first experiments, performed in two cell lines commonly used for SARS Cov-2 culture,
53 VeroE6/TMPRSS2 and Caco-2, revealed highly similar replication kinetics (Fig 1A-B). We
54 then assessed the replicative fitness of both strains using a previously described model of
55 reconstituted human airway epithelium (HAE) of bronchial origin (6). Following the
56 inoculation of the epithelia through their apical side at a multiplicity of infection (MOI) of 0.1 in
57 order to mimic the natural route of infection, we monitored the excretion of new virions at the
58 apical side between 2 and 4 days post-infection (dpi) and measured the intracellular viral
59 RNA yields at 4 dpi. Infectious titres (Fig.1D) and viral RNA yields (Fig.1E) at the apical side
60 at 3 and 4 dpi, as well as intracellular viral RNA yields at 4 dpi (Fig. 1G), were slightly higher
61 for the 20I/501Y.V1 variant. However, differences were not significant and estimated relative
62 virion infectivity (*i.e.*, the ratio of the number of infectious particles over the number of viral
63 RNA) were similar for both viruses at all sampling times (Fig1. F). Altogether, these results
64 are in line with our findings in common cell lines and with a recent report (7).

65 Based on these results, we performed competition experiments, which have been previously
66 demonstrated to be effective to detect moderate replicative fitness differences(8).
67 Accordingly, we inoculated epithelia with both viruses simultaneously as described above,
68 sampled the apical side between 2 and 4 dpi and extracted intracellular viral RNA yields at 4
69 dpi. Three infection inoculum ratios (20I/501Y.V1/BavPat D614G: 70/30, 50/50 and 30/70)
70 were used. Using two specific RT-qPCR assays, we estimated the proportion of each viral
71 genome in the viral population (expressed as 20I/501Y.V1/BavPat D614G ratio in Figure 1H-
72 I). Results revealed that regardless the initial ratio, a similar pattern was observed in which
73 Bavpat D614G was outcompeted by the 20I/501Y.V1 variant: all 20I/501Y.V1/BavPat D614G
74 ratio values estimated from apical side washes were above 1 and over 57, 22 and 8 at 4 dpi
75 for epithelia inoculated for initial ratios 70/30, 50/50 and 30/70 respectively (Fig1. H). Notably,
76 20I/501Y.V1/BavPat D614G ratios measured at 4 dpi were significantly higher than initial
77 50/50 and 30/70 inoculum ratios ($p= 0.0475$ and $p=0.0082$ respectively; Kruskal-Wallis test
78 with uncorrected Dunn's post-hoc analysis). Similar result were observed when estimating
79 the 20I/501Y.V1/BavPat D614G ratios from intracellular viral RNAs (Fig1. I):
80 20I/501Y.V1/BavPat D614G ratios measured at 4 dpi were significantly higher than initial
81 50/50 and 30/70 inoculum ratios ($p= 0.0208$, $p=0.0082$ and $p=0.0475$ with 70/30, 50/50 and

30/70 inoculum ratios respectively; Kruskal-Wallis test with uncorrected Dunn's post-hoc analysis).

Our results demonstrated that the 20I/501Y.V1 is more fit than the BavPat D614G in a reconstituted bronchial human epithelium. This may be explained by the presence of the N501Y mutation in the receptor binding domain (RBD) of the spike protein which enhances viral particle binding to the ACE2 receptor (9). This may translate into a fitness advantage as demonstrated in a recent study with engineered viral strains (10). Similar observations have been made with the D614G mutation, where the new G614 strains overcame the D614 original strains when put in competition (2). Altogether, these findings could contribute to better understand the progressive replacement of circulating strains by the SARS-CoV-2 20I/501Y.V1 variant (11).

Acknowledgments

We thank Pr C Drosten for providing the SARS-CoV-2 BavPat strain through EVA GLOBAL. This work was supported by Inserm through the REACTing (REsearch and ACTION targeting emerging infectious diseases) initiative. This work was supported by the European Virus Archive Global (EVA GLOBAL) funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 871029. This work was supported by the Fondation de France "call FLASH COVID-19", project TAMAC.

Declaration of interest statement

The authors declare that there is no conflict of interest

References

1. Volz E, Hill V, McCrone JT, Price A, Jorgensen D, O'Toole Á, Southgate J, Johnson R, Jackson B, Nascimento FF, Rey SM, Nicholls SM, Colquhoun RM, da Silva Filipe A, Shepherd J, Pascall DJ, Shah R, Jesudason N, Li K, Jarrett R, Pacchiarini N, Bull M, Geidelberg L, Siveroni I, COG-UK Consortium, Goodfellow I, Loman NJ, Pybus OG, Robertson DL, Thomson EC, Rambaut A, Connor TR. 2021. Evaluating the Effects of SARS-CoV-2 Spike Mutation D614G on Transmissibility and Pathogenicity. *Cell* 184:64-75.e11.
2. Plante JA, Liu Y, Liu J, Xia H, Johnson BA, Lokugamage KG, Zhang X, Muruato AE, Zou J, Fontes-Garfias CR, Mirchandani D, Scharton D, Bilello JP, Ku Z, An Z, Kalveram B, Freiberg AN, Menachery VD, Xie X, Plante KS, Weaver SC, Shi P-Y. 2020. Spike mutation D614G alters SARS-CoV-2 fitness. *Nature* 1–6.
3. Zhang L, Jackson CB, Mou H, Ojha A, Peng H, Quinlan BD, Rangarajan ES, Pan A, Vanderheiden A, Suthar MS, Li W, Izard T, Rader C, Farzan M, Choe H. 2020. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. 1. *Nat Commun* 11:6013.
4. 2020. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations - SARS-CoV-2 coronavirus / nCoV-2019 Genomic Epidemiology. *Virological*.
5. Galloway SE. 2021. Emergence of SARS-CoV-2 B.1.1.7 Lineage — United States, December 29, 2020–January 12, 2021. *MMWR Morb Mortal Wkly Rep* 70.
6. Pizzorno A, Padey B, Julien T, Trouillet-Assant S, Traversier A, Errazuriz-Cerda E, Fouret J, Dubois J, Gaymard A, Lescure F-X, Dulière V, Brun P, Constant S, Poissy J, Lina B, Yazdanpanah Y, Terrier O, Rosa-Calatrava M. 2020. Characterization and Treatment of SARS-CoV-2 in Nasal and Bronchial Human Airway Epithelia. *Cell Rep Med* 1:100059.

7. Brown JC, Goldhill DH, Zhou J, Peacock TP, Frise R, Goonawardane N, Baillon L, Kugathasan R, Pinto AL, McKay PF, Hassard J, Moshe M, Singanayagam A, Burgoyne T, the ATACCC Investigators, PHE Virology Consortium, Barclay WS. 2021. Increased transmission of SARS-CoV-2 lineage B.1.1.7 (VOC 202012/01) is not accounted for by a replicative advantage in primary airway cells or antibody escape. preprint, Microbiology.
8. de Fabritus L, Nougairède A, Aubry F, Gould EA, de Lamballerie X. 2015. Attenuation of tick-borne encephalitis virus using large-scale random codon re-encoding. PLoS Pathog 11:e1004738.
9. Zahradník J, Marciano S, Shemesh M, Zoler E, Chiaravalli J, Meyer B, Rudich Y, Dym O, Elad N, Schreiber G. 2021. SARS-CoV-2 RBD *in vitro* evolution follows contagious mutation spread, yet generates an able infection inhibitor. preprint, Biochemistry.
10. Weaver S, Liu Y, Liu J, Plante K, Plante J, Xie X, Zhang X, Ku Z, An Z, Scharton D, Schindewolf C, Menachery V, Shi P-Y. 2021. The N501Y spike substitution enhances SARS-CoV-2 transmission. preprint, In Review.
11. World Health Organization. Weekly epidemiological update - 23 February 2021. Emergency Situational Updates; <https://www.who.int/publications/m/item/weekly-epidemiological-update---23-february-2021>.

Figure 1: *In vitro* and *ex vivo* replication ability of a 20I/501Y.V1 variant in comparison with a lineage B.1 D614G strain.

(A-B) Replication kinetics in VeroE6 TMPRSS2 (A) and Caco-2 (B) cells. Viral replication was assessed using a RT-qPCR assay. (C) Graphical representation of experiments with reconstituted human airway epithelium (HAE) of bronchial origin. (D-E) Kinetics of virus excretion

149 at the apical side of the epithelium measured using a TCID₅₀ assay (D) and a RT-qPCR assay
150 (E). (F) Estimation of virion infectivities (*i.e.*, the ratio of the number of infectious particles over the
151 number of viral RNA). (G) Intracellular viral RNA yields measured at 4 dpi using a RT-qPCR
152 assay. (A-G) Data represent mean±SD of a triplicate. No statistical difference was observed
153 between both viral strains ($p>0.05$; unpaired Mann-Whitney test). (H) Follow-up of the
154 20I/501Y.V1/BavPat D614G ratios at the apical side. Each line represents results from an HAE
155 insert. (I) Individual 20I/501Y.V1/BavPat D614G ratios estimated from intracellular viral RNAs at
156 4dpi (I). (H-I) p-values were determined against the initial ratios using Kruskal-Wallis test followed
157 by uncorrected Dunn's post-hoc analysis. The graphical representation was created with
158 BioRENDER.