SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma

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SARS-CoV-2 501Y.V2 (B.1.351), a novel lineage of coronavirus causing COVID-19, contains substitutions in two immunodominant domains of the spike protein. Here, we show that pseudovirus expressing 501Y.V2 spike protein completely escapes three classes of therapeutically relevant antibodies. This pseudovirus also exhibits substantial to complete escape from neutralization, but not binding, by convalescent plasma. These data highlight the prospect of reinfection with antigenically distinct variants and foreshadow reduced efficacy of spike-based vaccines.

Individuals infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19), develop neutralizing antibodies that can persist for months1–5. Neutralizing antibodies are considered the primary correlate of protection from infection and are being pursued as therapeutics6–8. Interim analyses with monoclonal neutralizing antibodies have shown success, facilitating their authorization for emergency use5,6.

The SARSCoV-2 receptor binding domain (RBD) exists in either an ‘up’ (receptor-accessible) or ‘down’ (receptor-shielded) conformation. RBD is the dominant neutralization target for this and other human coronaviruses4,9. These antibodies can be broadly divided into four main classes, of which two overlap with the angiotensin converting enzyme 2 (ACE2) receptor binding site (Fig. 1a and Supplementary Fig. 1a)4. Class 1 antibodies are most frequently elicited in SARS-CoV-2 infection and include a public antibody contact that spans 40–100% of the E484 side-chain-accessible surface area, including key hydrogen bonds at this site (Fig. 1b). Three representative antibodies were assessed by ELISA and achieved saturated binding and confirmed dependence on the K417 residue (Fig. 1d).

A structural analysis of 15 class 2 antibodies and 6 nanobodies revealed key interactions with spike residue E484 (Fig. 1e). Each contacted 40–100% of the E484 side-chain-accessible surface area and formed critical hydrogen bonds or charged interactions at this site. As with class 1 antibodies, three representative class 2 antibodies failed to bind 501Y.V2 RBD (Fig. 1f) and were unable to neutralize the 501Y.V2 pseudovirus (Fig. 1g). Thus, the SARS-CoV-2 501Y.V2 lineage has effectively escaped two major classes of neutralizing antibodies targeting an immunodominant, highly antigenic site in the RBD of the spike protein.

501Y.V2 is also defined by several changes in the NTD, including a three-amino-acid deletion preceding the N5-loop supersite (Supplementary Fig. 1b) relative to the Wuhan-1 D614G spike mutant that previously dominated in South Africa (here referred to as the original lineage)9. These changes include N501Y, which confers enhanced affinity for ACE2 (ref. 10) and clusters of substitutions in two immunodominant regions of spike, suggesting escape from neutralization. Indeed, substitutions at E484 reduce neutralization sensitivity to convalescent plasma11. We therefore compared neutralization by monoclonal antibodies and convalescent plasma of 501Y.V2 to Wuhan-1 D614G, using a spike-pseudotyped lentivirus neutralization assay.

An analysis of 17 class 1 antibody structures revealed their epitopes to be centered on spike residue K417, one of three substitutions in the RBD of the 501Y.V2 lineage. These antibodies contact 60–100% of residue K417 side-chain-accessible surface area, including key hydrogen bonds at this site (Fig. 1b). Three representative antibodies were assessed by ELISA and achieved saturated binding to recombinant RBD from the original lineage but not 501Y.V2 RBD (Fig. 1c). Similarly, all three antibodies potently neutralized the original lineage, but not the 501Y.V2 pseudovirus (at 25 μg ml−1), confirming dependence on the K417 residue (Fig. 1d).

A structural analysis of 15 class 2 antibodies and 6 nanobodies revealed key interactions with spike residue E484 (Fig. 1e). Each contacted 40–100% of the E484 side-chain-accessible surface area and formed critical hydrogen bonds or charged interactions at this site. As with class 1 antibodies, three representative class 2 antibodies failed to bind 501Y.V2 RBD (Fig. 1f) and were unable to neutralize the 501Y.V2 pseudovirus (Fig. 1g). Thus, the SARS-CoV-2 501Y.V2 lineage has effectively escaped two major classes of neutralizing antibodies targeting an immunodominant, highly antigenic site in the RBD of the spike protein.

501Y.V2 is also defined by several changes in the NTD, including a three-amino-acid deletion preceding the N5-loop supersite (Fig. 1h and Supplementary Fig. 1b). An analysis of NTD-bound antibody structures showed that roughly half their neutralization interfaces with spike comprised the N5-loop supersite, often involving...
key residue R246 (Fig. 1i). Modeling the 501Y.V2 N-loop deletion onto the antibody 4A8-bound spike structure revealed an apical loop displacement of at least 8 Å away from the 4A8 paratope (Fig. 1j). In addition, the deletion would shift position R246 three amino acids earlier, bringing it into proximity with R102 and creating a potential clash that could locally disrupt the N5-loop conformation and shift it even further from the 4A8 paratope. Consequently, when assessed by ELISA, 4A8 was unable to bind to recombinant 501Y.V2 NTD (Fig. 1k) and failed to neutralize the 501Y.V2 pseudovirus (Fig. 1l), showing escape from N5-loop targeted neutralizing antibodies.

We next sought to evaluate the effect of 501Y.V2 spike substitutions on polyclonal plasma/sera derived from individuals with
Fig. 2 | SARS-CoV-2 501Y.V2 increased resistance to neutralization by convalescent plasma/serum. Plasma/serum collected from individuals infected with SARS-CoV-2 was assessed for neutralization to the original lineage ( Wuhan-1 D614G, left), an RBD chimeric mutant containing K417N, E484K and N501Y substitutions only (middle) or the 501Y.V2 lineage pseudovirus. Twelve of the samples were collected from donors hospitalized for >10d with COVID-19 (black). The graph is colored according to the magnitude of neutralization titer, with ID₅₀ greater or lesser than 1:400 colored dark or light blue, respectively and titer <100 colored orange. The limit of detection (knockout) was an ID₅₀ <20 (red).

PCR-confirmed SARS-CoV-2 infection, including individuals who were hospitalized with severe COVID-19. Samples were divided into two groups, half with higher titer neutralizing antibodies (22 of 44, 50% inhibitory dilution (ID₅₀) >400) and half with lower titers (22 of 44, 400 ≥ ID₅₀ > 25) to the original SARS-CoV-2 D614G lineage (Fig. 2 and Supplementary Fig. 2a). Consistent with previous studies, when stratified by disease severity, convalescent individuals who reported mild-to-moderate disease developed substantially lower neutralizing antibody titers (average ID₅₀ titer 488, n = 30) than severely ill individuals from the hospitalized cohorts (average ID₅₀ titer 4,212, n = 14).

When these same samples were assessed against the 501Y.V2 pseudovirus, nearly half (21 of 44, 48%) had no detectable neutralization activity (and 71% had ID₅₀ <100). Only three samples (7%) retained titers of ID₅₀ >400 (Fig. 2 and Supplementary Fig. 2a). Notably, these three samples were obtained from individuals reporting severe disease and had among the highest neutralization titers against the original virus. Conversely, four samples with border-line neutralization of the original virus were unaffected by 501Y.V2 substitutions, perhaps representing additional, less-potent specificities. To define the location of dominant escape substitutions, neutralization was also assessed against the RBD chimeric pseudovirus containing only three 501Y.V2 substitutions (K417N, E484K and N501Y) (Fig. 2 and Supplementary Fig. 2a). Substantial loss of neutralization was also observed against the RBD-only mutant, with 27% of the samples losing all activity against the RBD triple mutants (63% had ID₅₀ <100) and only 23% retaining higher titers of ID₅₀ >400. These data provide more evidence for the dominance of class 1 and class 2 neutralizing antibodies in polyclonal sera; however, differences in neutralization between RBD-only chimera and 501Y.V2 also highlight the contribution of 501Y.V2 NTD substitutions (L18F, D80A, D215G and Δ242-244) to neutralization escape. This was particularly evident in higher titer samples, which retained an average ID₅₀ titer of 680 against the RBD-only mutant.

While neutralizing antibodies to SARS-CoV-2 are dominated by the specificities defined above, non-neutralizing antibodies are also elicited during SARS-CoV-2 infection. To determine whether 501Y.V2 is still recognized by non-neutralizing antibodies, the binding of polyclonal sera (from Fig. 2) to a recombinant protein that includes the RBD and subdomain 1 (RBD + SBD1) of 501Y.V2 or the original lineage was assessed by ELISA (Supplementary Fig. 2b). These data revealed that binding of polyclonal plasma to 501Y.V2 RBD + SBD1 was only substantially affected in a minority of cases (14 of 44 with more than a fivefold reduction, 32%). Most of the convalescent plasma/serum suffered less than a four-fold reduction in total binding activity (as calculated by area under the curve), suggesting a considerable non-neutralizing antibody component is still able to bind the 501Y.V2 spike.

Among previous emerging lineages, only D614G has subsequently become globally dominant. The repeated, independent evolution of spike position 501 in 501Y.V1 (https://virological.org/t/576), 501Y.V2 (ref. 17) and 501Y.V3 (https://virological.org/t/586), strongly argues for a selective advantage, likely enhanced transmissibility, of these new variants. Here we have shown that the 501Y.V2 lineage, containing nine spike substitutions, and rapidly emerging in South Africa during the second half of 2020, is resistant to neutralizing antibodies found in 48% of individuals infected with previously circulating lineages. These data, showing a 13-fold reduction in mean titer, are corroborated by vesicular stomatitis virus-pseudotyped and live virus assays showing an 11- to 33-fold and 6- to 204-fold reduction in mean titer (including complete knock out) relative to the original lineage, respectively. The 501Y.V3 lineage has similar changes including 417T and 484K (in RBD) as well as 18F and 20N (in NTD), thus also having strong potential for high levels of neutralization resistance. The independent emergence and subsequent selection of 501Y lineages with key substitutions conferring neutralization resistance strongly argues for selection by neutralizing antibodies as the dominant driver for SARS-CoV-2 spike diversification and makes these lineages of considerable public health concern. This suggests that, despite the many people who have already been infected with SARS-CoV-2 globally and are presumed to have accumulated some level of immunity, new variants such as 501Y.V2 may pose a substantial re-infection risk.

While higher titers of neutralizing antibodies are common in hospitalized individuals, most people infected with SARS-CoV-2 develop low-to-moderate neutralization titers. Therefore, the data herein suggest that most individuals infected with previous SARS-CoV-2 lineages will have greatly reduced neutralization activity against 501Y.V2. This dramatic effect on plasma neutralization can be explained by the dominance of RBD-directed neutralizing antibodies, supported by studies showing reduced plasma neutralization titers mediated by the E484K change alone. Notably, here we show that the K417N change also has a crucial role in viral escape, effectively abrogating neutralization by a well-defined, multidonor class of VH3-53/66 germline-restricted public antibodies that comprise some of the most common and potent neutralizing antibodies to SARS-CoV-2 (ref. 19).

The marked loss of neutralization against 501Y.V2 pseudovirus compared to the RBD-only chimeric pseudovirus demonstrates the important role that substitutions in the NTD play in mediating immune escape. For 501Y.V2 this resistance to neutralization is likely mediated by a three-amino-acid deletion that completely disrupts a dominant public antibody response to the N5-loop
This deletion predominates among 501Y.V2 variants and occurs either alone or with an R246I substitution that is also important for neutralization by several NTD-directed neutralizing antibodies.

The relatively rapid acquisition of a comprehensive suite of neutralization escape substitutions likely occurred because of the large number of commonly shared public antibodies (such as VH3-53/66, VH1-2 and VH1-24) to both the RBD and NTD of spike, together with high levels of SARS-CoV-2 transmission. The sporadic emergence of escape substitutions in long-term viral shudders, including immunocompromised individuals, may also contribute to the emergence of neutralization-resistant viruses\(^3\). Altogether, these data highlight the need for increased, ongoing genomic surveillance during the SARS-CoV-2 pandemic. 

Crucially, it is from these same public antibody responses that many therapeutic strategies currently under development have been derived\(^1\). The overwhelming majority of monoclonal antibodies already on the path to licensure target residues K417 or E484 and are therefore likely to be futile against 501Y.V2. In addition, emerging variants may limit the use of recently identified neutralizing antibodies that target the NTD N5-loop supersite. Some of these monoclonal antibodies have already been granted emergency use authorization in the United States (Regeneron Pharmaceuticals and Eli Lilly and Company), including antibodies ineffective against 501Y.V2 (such as REGN10933 and LY-CoV555) as well as antibodies likely to retain neutralization of this variant (REGN10987 and VIR-7831), some of which are being engineered to potentially enhance virus-specific T cell function (VIR-7832).

These data also have implications for the effectiveness of SARS-CoV-2 vaccines, largely based on immune responses to the original spike protein. Indeed, sera from the Moderna and Pfizer-BioNTech vaccinees show significantly reduced neutralization of 501Y.V2 (ref. 14). Furthermore, compared to global efficacy estimates, vaccine efficacy in South Africa (overlapping with the emergence of 501Y.V2) has been significantly reduced for several vaccines. Conversely, these same trials seem to retain efficacy against severe COVID-19. While antibody effector functions elicited by infection and vaccination have been implicated in protecting from reinfection and disease\(^6\), the role of non-neutralizing antibodies and the efficacy of T cell responses to 501Y.V2 remain to be elucidated. We did not measure the extent of these responses to 501Y.V2 but did show that a substantial proportion of non-neutralizing antibodies remain active against 501Y.V2 recombinant RBD protein. Ultimately, the correlates of protection against SARS-CoV-2 infection and severe COVID-19 disease remain undetermined and rely upon ongoing large-scale clinical trials. Nevertheless, these data highlight the urgent requirement for rapidly adaptable vaccine design platforms and the need to identify less-mutable viral targets for incorporation into future immunogens.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-021-01285-x.

Received: 21 January 2021; Accepted: 10 February 2021; Published online: 02 March 2021

**References**

Brief Communication

Methods

Samples and ethics approvals. Plasma/serum samples were obtained from individuals without human immunodeficiency virus (HIV), enrolled into one of three studies described below. All participants provided informed consent.

Hospitalized Steve Biko cohort. This study has been given ethics approval by the University of Pretoria, Human Research Ethics Committee (Mediical) (247/2020). Serum samples were obtained (longitudinally) from hospitalized patients with PCR-confirmed SARS-CoV-2 infection, known HIV status and aged ≥18 years. Samples from six participants with symptom onset between May and August 2020 were used.

Novel coronavirus (COVID-19) viral shedding and clinical characterization study. This study has been given ethics approval by the University of the Witwatersrand Human Research Ethics Committee (Mediical) M160667. Serum samples were obtained (longitudinally) from hospitalized patients with PCR-confirmed SARS-CoV-2 infection, known HIV status and aged ≥18 years. Samples from six participants with symptom onset between May and September 2020 were used.

South African National Blood Service. Plasma was obtained from blood donors of the South African National Blood Service (ethics clearance from South African National Blood Service Human Research Ethics Committee 2019/0519), who had PCR-confirmed SARS-CoV-2 infection, had recovered and were at least 28 d post-symptom onset. All donors met the standard eligibility criteria for donors who donate source plasma, which includes being generally healthy, being older than 18 years of age, weighing more than 55 kg and leading a lifestyle that reduces risk of acquiring transfusion transmissible infections. Only male and nulliparous females were accepted as COVID-19 convalescent plasma donors.

Structural modeling. The closed prefusion SARS-CoV-2 spike protein from Nextstrain clade 501Y.V2 (PANGOLin lineage B.1.351, https://cov-lineage.org/). The 501Y.V2 NTD domain was modeled using both antibody-constrained with Fab domains from CV30 (6XE1), C104 (7k8u), VIR-7831 (6WPS) and EY6A or recombinant SARS-CoV-2 RBD
Monoclonal antibodies
FreeStyle 293F suspension cells (Life Technologies) using PEIMAX transfection 0.05% Tween 20, 1 PBS). Plasma samples were added at 1:100 dilution and × and incubated at 37 °C for 1–2 h in blocking buffer (5% skimmed milk powder, [43x276]CO2. Cells were added at 1 [43x50]Victor X luminometer. Neutralization was measured as described by a reduction in
Pseudovirus and serially diluted plasma/sera were incubated for 1 h at 37 °C, 5% M filter and stored at [43x93]×0.45-
Membrane-1 (M160667) who contributed samples and the GERMS-SA clinical staff for their contributions to sample and data collection. We acknowledge funding from the South African Medical Research Council (ref. nos. 96825, SHIIPNCD 7675 and DST/CON 0250/2012), the Wellcome Trust (grant no. 221033/Z/20/Z), the United States for Disease Control and Prevention (grant no. 5 U01IP001048-05-00) and the ELMA Foundation (grant no. 20-ESA011). P.L.M. is supported by the South African Research Chairs Initiative of the Department of Science and Innovation and the National Research Foundation of South Africa (grant no. 98341). C.K.W. is supported by Fogarty International Center of the National Institutes of Health under Award Number R21TW011454 (this work is solely the responsibility of the authors and does not
Brief Communication

necessarily represent the official views of the National Institutes of Health) as well as the FLAIR Fellowship Program under award number FLR/R1/201782 (the FLAIR Fellowship Program is a partnership between the African Academy of Sciences and the Royal Society funded by the UK Government’s Global Challenges Research Fund). K.v.d.B., is supported in part by the Fogarty International Centre or the National Institutes of Health under award no. 1D43TW010345. We thank N. Doria-Rose, D. Montefiori, E. Landais and M. Farzan for reagents and assistance in establishing the SARS-CoV-2 pseudotyped neutralization assay and enabling equivalency and proficiency testing. We thank D. Sok, E. Landais, D. Burton, N. Doria-Rose and P. Kwong for SARS-CoV-2-directed monoclonal antibodies. We are grateful to T. Moyo-Gwete and Z. Molaudzi for expressing monoclonal antibodies. We thank the informal 501Y.V2 consortium of South African scientists, chaired by W. Hanekom and T. D’Oliveira for suggestions and discussion of data. We also thank all NGS-SA laboratories in South Africa that were responsible for producing the SARS-CoV-2 genomes that enabled the rapid dissemination of SARS-CoV-2 sequences and the identification of 501Y.V2.

Author contributions

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-021-01285-x.

Correspondence and requests for materials should be addressed to P.L.M.

Peer review information Nature Medicine thanks Rogier Sanders and the other, anonymous, reviewers for their contribution to the peer review of this work. Alison Farrell is the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

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Policy information about availability of computer code

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Life sciences study design

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- Sample size: 44 Plasma donors
- Data exclusions: n/a
- Replication: Samples were tested in duplicate in neutralization assays and ELISAs. Each assay was repeated twice.
- Randomization: n/a
- Blinding: n/a

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### Methods

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### Antibodies

- Antibodies used: CA1, Ly-CoV016, CC12.1, BD23, C119, P2B-2F6, 4A8, CC12.23, Palivizumab
- Validation: Antibodies were cloned, expressed and quality controlled according to published IC50 data.

### Eukaryotic cell lines

- Policy information about cell lines: Mike Farzan, The Scripps Research Institute
- Cell line source(s): Flow cytometry was used to confirm increased ACE-2 expression as reported
- Authentication: Cell line tested negative for mycoplasma
- Commonly misidentified lines (See CRCLAC register): None
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