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

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Abstract

SARS-CoV-2 501Y.V2, a novel lineage of the coronavirus causing COVID-19, contains multiple mutations within two immunodominant domains of the spike protein. Here we show that this lineage exhibits complete escape from three classes of therapeutically relevant monoclonal antibodies. Furthermore 501Y.V2 shows substantial or complete escape from neutralizing antibodies in COVID-19 convalescent plasma. These data highlight the prospect of reinfection with antigenically distinct variants and may foreshadow reduced efficacy of current spike-based vaccines.

Keywords: SARS-CoV-2 501Y.V2, COVID-19, Neutralization Escape, Monoclonal Antibodies, Reinfection, Vaccine Efficacy

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19), has resulted in substantial morbidity and mortality worldwide[1,2]. Infected individuals develop neutralizing antibodies that can persist for months[3-5]. Neutralizing antibodies are considered the primary correlate of protection against reinfection for most vaccines, including SARS-CoV-2[6,7], and are being actively pursued as therapeutics[8,9].

The SARS-CoV-2 receptor binding domain (RBD) is the dominant target of the neutralizing response during infection[7,10-13]. Consequently, the overwhelming majority of monoclonal neutralizing antibodies isolated thus far from infected individuals, immunoglobulin libraries, or immunized animal models, target this region[10,11,13,14]. These antibodies can be broadly divided into four main classes[15], of which class 1 and class 2 antibodies target site I[10] epitopes that overlap with the angiotensin converting enzyme 2 (ACE2) receptor binding site. Class 1 antibodies are most frequently elicited by SARS-CoV-2 infection and are comprised almost entirely of immunoglobulin V-gene (VH3-53/66) germline restricted antibodies, forming a multi-donor class with highly similar epitopes only accessible in the RBD 'up' conformation[11,15,16]. Class 2 antibodies also bind to site I[10,15], but can bind to both RBD 'up' and RBD 'down' conformations of spike. Although they can use a more diverse VH-gene pool, class 2 antibodies include VH1-2 germline restricted antibodies that form a second commonly elicited multi-donor class of public antibodies[17]. The RBD is also the target of several nanobodies/sybodies that are being considered for clinical development[18,19].

After the RBD, the N-terminal domain (NTD) of spike is the next most frequently targeted by neutralizing antibodies, and several potent monoclonal antibodies to this region also being considered for clinical development[20-22]. Remarkably, despite being derived from diverse VH-genes, almost all these antibodies target a single immunodominant site on NTD, involving the N1-loop (NTD N-terminus), N3-loop (supersite β-hairpin), and N5-loop (supersite loop). A subset of these antibodies also forms a third multi-donor class, with a common VH1-24 germline restricted mode of spike recognition.

We, and others, recently described the emergence of a new SARS-CoV-2 lineage in South Africa, 501Y.V2, that included nine changes in the spike protein. These can be divided into two subsets, one cluster in NTD that includes four substitutions and a deletion (L18F, D80A, D215G, Δ242-244, and R246I), and another cluster of three substitutions in RBD (K417N, E484K, and N501Y). Although the 501Y change has been associated with increased transmissibility, rather than immune pressure, the accumulation of mutations specifically within these two immunodominant regions of spike is highly suggestive of escape from neutralization. Indeed, mutations at 484 have been shown to reduce neutralization sensitivity[23,24]. Furthermore, mutations in these same regions have also been described in a new variant, which has recently emerged independently in Brazil[25]. Lastly, variants in the UK and USA, albeit with fewer potential antibody escape mutations have also been described, suggesting the global emergence of new SARS-CoV-2 variants[26].

Here we show that spike mutations in the 501Y.V2 lineage confer neutralization escape from multiple classes of SARS-CoV-2 directed monoclonal antibodies. Furthermore, we observe significantly increased neutralization resistance of 501Y.V2 to plasma from individuals previously infected with SARS-CoV-2, with implications for both rates of re-infection, and vaccine effectiveness.

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Results

The SARS-CoV-2 501Y.V2 lineage escapes monoclonal antibodies to dominant RBD epitopes. To evaluate the impact of 501Y.V2 spike amino acid changes on commonly elicited neutralizing specificities, we first assessed neutralization sensitivity of the 501Y.V2 lineage to monoclonal antibodies. An analysis of twelve class I antibody structures revealed their epitopes to be centred on spike residue K417 (Fig.1a), which is one of three mutations in the RBD of the 501Y.V2 lineage. Class 1 antibodies buried between 60-100% of the K417 side chain accessible surface area in their paratopes and included key hydrogen bonds at this site (Fig.1b). Three representative class 1 antibodies, CA1, LyCoV016 (also known as CB6 or JS016), and CC12.1, were assessed by ELISA for binding to the RBD of both the original and 501Y.V2 lineages. All three antibodies achieved saturated binding to the original lineage but were unable to bind 501Y.V2 RBD at 10 µg/mL (Fig.1c). Similarly, when assessed in a lentivirus-pseudotyped SARS-CoV-2 neutralization assay, all three antibodies potently neutralized the original lineage, but were unable to neutralize the 501Y.V2 variant (at 25 µg/mL), confirming dependence on the K417 residue (Fig.1d).

An analysis of eight class 2 antibodies and five nanobodies/sybodies to this site, revealed key interactions with spike residue E484 which buried between 75%-100% of its side chain accessible surface area in their paratopes, and formed critical hydrogen bonds or charged interactions at this site (Fig.1e). As with the class 1 antibodies, three representative class 2 antibodies (BD23, C119, and P2B-2F6) failed to bind to 501Y.V2 RBD (Fig.1f) and were unable to neutralize the 501Y.V2 pseudovirus (Fig.1g). These data add to growing evidence implicating E484K (found in 501Y.V2) in escape from a broad array of class 2 antibodies. 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.

A three amino acid deletion in 501Y.V2 dramatically alters the antigenic landscape of a supersite in NTD. In addition to changes in RBD, 501Y.V2 is also defined by several changes in the NTD. These include a three amino acid deletion in the N-terminus of a relatively large, solvent exposed loop in NTD variably defined as the N5-loop (residues 246-260)[20,21], or the supersite loop (residues 245-264)[22]. An analysis of the monoclonal antibody 4A8-bound spike structure showed that roughly half (47%) of the neutralization interface with spike was buried in the N5-loop supersite (Fig.1h). 4A8 is part of the VH1-24 restricted class of NTD neutralizing antibodies, with a common mode of recognition involving key CDR-H1 mediated interactions with N5-loop residue R246[20]. Strikingly, most NTD-directed neutralizing antibodies to SARS-CoV-2 recognize epitopes that incorporate the N5-loop supersite and interact with key residues R246 or D253 (Fig.1i). Modelling the 501Y.V2 N5-loop onto the 4A8-bound spike structure revealed an apical loop displacement of at least 8Å away from the 4A8 paratope (Fig.1j). In addition, the deletion would shift position R246 three amino acids earlier in the registry, bringing it into proximity with R102 and creating a potential clash that would locally disrupt the N5-loop conformation and shift it even further from the 4A8 paratope. When assessed in ELISA, 4A8 was unable to bind to recombinant 501Y.V2 NTD at the highest concentration tested, suggesting that 501Y.V2 effectively escapes antibodies to the N5-loop supersite (Fig.1k).

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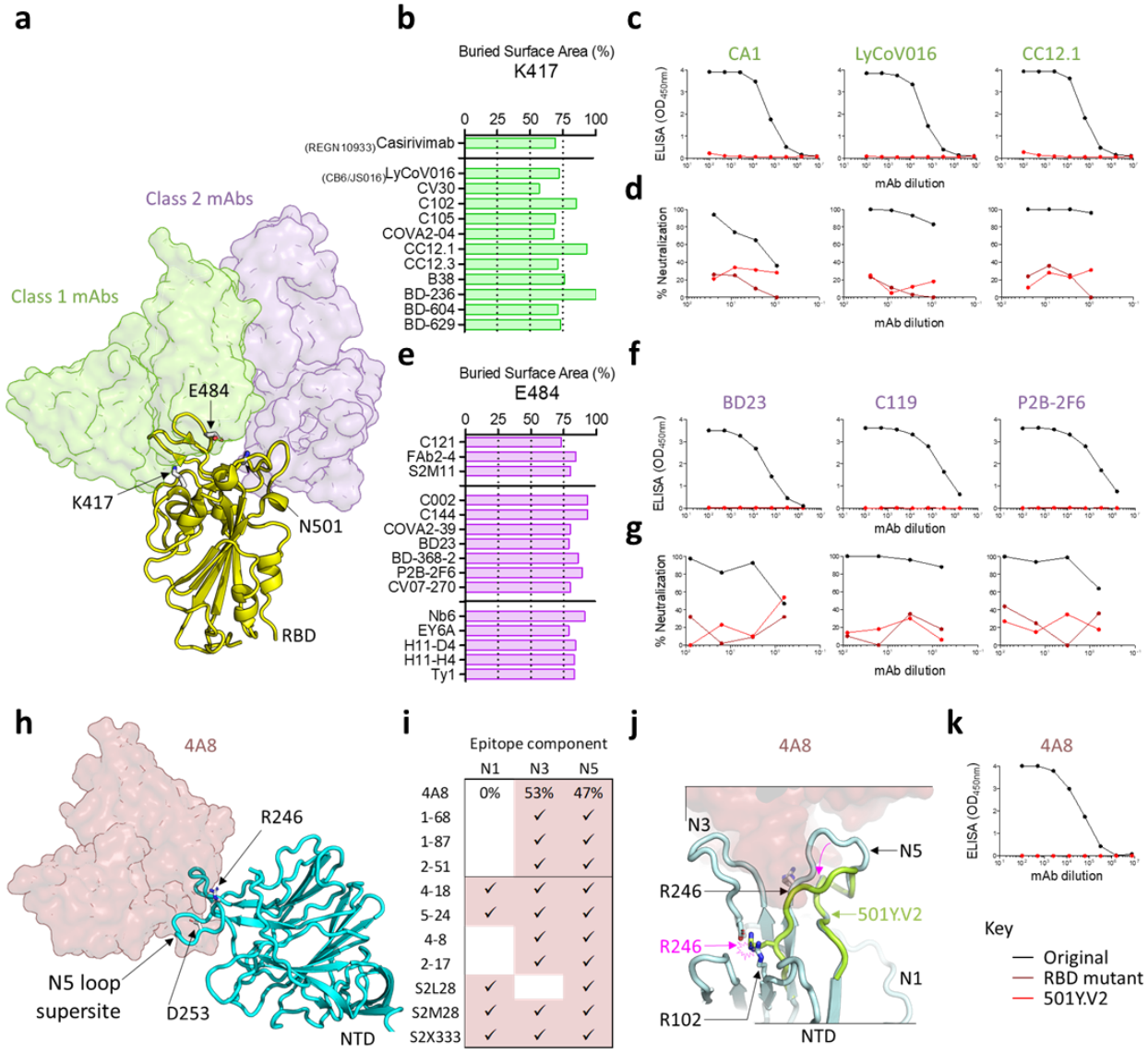

Fig.1|SARS-CoV-2 501Y.V2 is resistant to monoclonal antibodies to both RBD and NTD a. A structure of the SARS-CoV-2 RBD is shown in yellow cartoon view, modelled in complex with a representative neutralizing antibody from class 1 (CV30) and class 2 (C104). The VH domains of the representative class 1 antibody are shown in translucent green surface view, while those of the class 2 antibody are shown in translucent purple. The side chains of residues K417, E484, and N501 are indicated with arrows. **b.** A plot showing the percentage of accessible surface area for the RBD K417 side chain (x-axis) that is buried in the paratopes of several class 1 neutralizing antibodies (listed on the y-axis). VH3-53/66 antibodies are separated below the horizontal line. **c.** ELISA binding curves for antibodies CA1, LyCoV016, and CC12.1 to the original RBD (black) or the 501Y.V2 RBD (red). **d.** Neutralization curves for the same antibodies shown in c, against the original virus (black), 501Y.V2 (red), or a chimeric construct that includes only the RBD mutations K417N, E484K, and N501Y (maroon). **e.** Percentage of accessible surface area for the RBD E484 side chain (x-axis) that is buried within the paratopes of several class 2 neutralizing antibodies (listed on the y-axis). VH1-2 (top) or VH-diverse (middle) antibodies, and nanobodies (bottom) are separated with the horizontal lines. **f.** ELISA binding curves for antibodies BD23, C119, and P2B-2F6 to

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the original RBD (black) or the 501Y.V2 RBD (red). **g.** Neutralization curves for the same antibodies shown in (f), against the original virus (black), 501Y.V2 (red), or the chimeric RBD construct (maroon). **h.** A structure of the SARS-CoV-2 NTD is shown in cyan cartoon view, modelled in complex with a VH1-24 neutralizing antibody 4A8 (shown in translucent maroon). The N5-loop supersite, and side chains of residues R246 and D253 are indicated with arrows. **i.** The relative percent contribution of NTD loops N1, N3, and N5 to the 4A8 epitope were calculated, and contributions for additional NTD-directed neutralizing antibodies are shown. **j.** Modelling of the 501Y.V2 Δ242-244 deletion (lime green cartoon view), in the context of the 4A8 epitope. NTD loops N1, N3, and N5 are shown and the position of R246 in the original NTD or 501Y.V2 NTD is labelled with black or pink, respectively. The minimum displacement for 501Y.V2 loop N5, and the accompanying clash with R102 are indicated with the pink arrows. **k.** ELISA binding curves for 4A8 to the original NTD (black) or the 501Y.V2 NTD (red).

The SARS-CoV-2 501Y.V2 lineage shows high levels of resistance to convalescent plasma/sera. We next sought to evaluate the effect of 501Y.V2 spike mutations on polyclonal plasma/sera derived from individuals infected with SARS-CoV-2, including individuals who were hospitalised for severe COVID-19. Samples were divided into two groups, half with higher titre neutralizing antibodies (22 of 44, ID_{50} >400) and half with lower titres (22 of 44, 400 >ID_{50} >25) to the original SARS-CoV-2 D614G lineage (Fig.2a - left). Consistent with previous studies[27], when stratified by disease severity, convalescent individuals who reported mild to moderate disease developed substantially lower neutralizing antibody titres (average ID_{50} titre 488, n=30) than severely ill individuals from the hospitalized cohorts (average ID_{50} titre 4212, n=14).

When these same samples were assessed against the 501Y.V2 virus, nearly half (21 of 44, 48%) had no detectable neutralization activity, with only three samples (7%) retaining titres of ID_{50} >400 (Fig.2a - right). Notably, all three of these samples were obtained from individuals reporting severe disease who had some of the highest neutralization titres against the original virus. To define the location of dominant escape mutations, neutralization was also assessed against the RBD chimeric viral construct containing only three 501Y.V2 mutations (K417N, E484K, N501Y) (Fig.2a - middle). A 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, while only 23% retained higher titres of ID_{50} >400. These data provide more evidence for the dominance of Class 1 and Class 2 neutralizing antibodies in polyclonal sera. However, the differences in neutralization between the RBD-only chimera and 501Y.V2 also highlight the contribution of 501Y.V2 NTD mutations (L18F, D80A, D215G, and Δ242-244) to neutralization escape. This was particularly evident in the higher titre samples, which retained an average titre of ID_{50} 680 against the RBD-only mutant.

Convalescent plasma/serum retains high levels of binding antibodies to 501Y.V2 RBD. 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[10,12]. To determine whether 501Y.V2 is still recognized by non-neutralizing antibodies, the binding of polyclonal sera (from Fig.2a) to a recombinant 501Y.V2 RBD+SBD1 protein and an RBD+SBD1 from the original lineage was assessed by ELISA (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 ≥five-fold reduction, 32%). Most of the convalescent plasma/serum suffered less than four-fold reductions in total binding activity (as measured by area under the curve), suggesting a considerable non-neutralizing antibody component are still able to bind the 501Y.V2 spike antigen.

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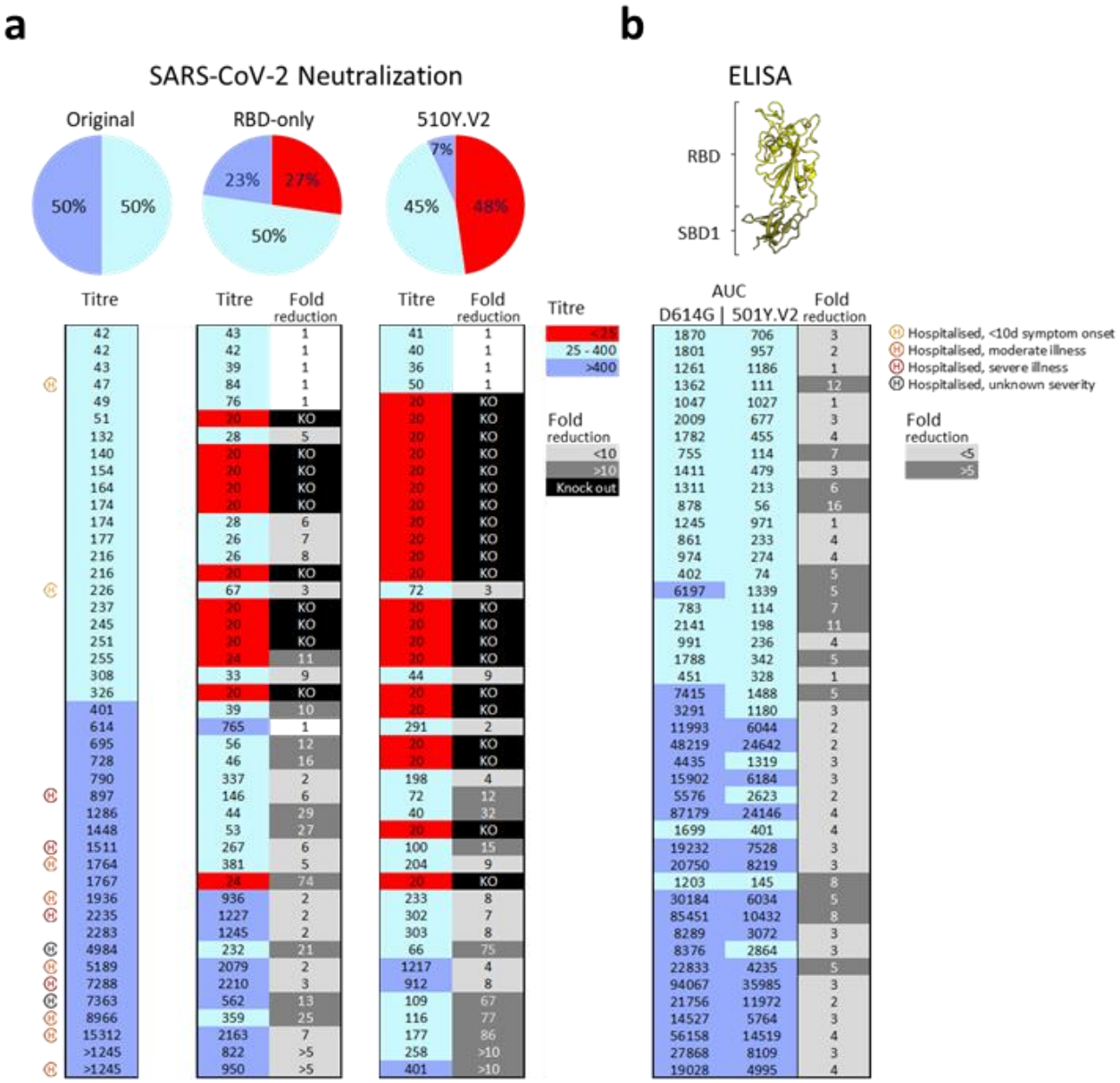

Fig.2|SARS-CoV-2 501Y.V2 increased resistance to neutralization by convalescent plasma/serum. a. Plasma/serum samples collected from SARS-CoV-2 infected individuals who were (n=14) or were not (n=30) hospitalized with COVID-19, ranked by titre against the original SARS-CoV-2 D614G lineage (left pie chart / column 1). Neutralization titre is coloured according to magnitude, where titres greater or lesser than 1:400 are coloured dark or light blue, respectively. Neutralization titre and fold decrease relative to the original lineage are shown for an RBD-only chimeric virus containing the K417N, E484K, and N501Y substitutions (middle pie chart / columns 2 and 3), and the 501Y.V2 lineage virus (right pie chart / column 4 and 5). Neutralization titres <1:25 are coloured red, while a complete knock out of neutralization activity is highlighted in black. **b.** Binding of plasma samples (from Fig.2a) against RBD+SBD1 (shown in yellow and olive cartoon view) from the original virus (column 1) or the 501Y.V2 lineage (column 2) and plotted as area under the curve. The fold reduction in AUC is shown in column 3.

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Discussion

SARS-CoV-2, the virus responsible for the COVID-19 pandemic is evolving, with new lineages being reported all over the world. Amongst previous lineages, D614G was shown to have faster growth *in vitro*, enhanced transmission in small animals, and has subsequently become globally dominant[28-30]. N501Y has been shown to increase affinity for the human ACE2 receptor, which together with the repeated and independent evolution of 501Y containing lineages[25,26,31], strongly argues for enhanced transmissibility of these new variants. Here we show that the 501Y.V2 lineage, that contains nine spike mutations, and rapidly emerged in South Africa during the second half of 2020, is largely resistant to neutralizing antibodies elicited by infection with previously circulating lineages. 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 pose a significant re-infection risk.

While higher titres of neutralizing antibodies are common in hospitalized individuals, however most SARS-CoV-2 infected people develop moderate neutralization titres[3-5]. Therefore, the data herein suggest that most individuals infected with previous SARS-CoV-2 lineages will have minimal or no detectable neutralization activity against 501Y.V2. This dramatic effect on plasma neutralization can be explained by the dominance of RBD-directed neutralizing antibodies[10,11,13]. These data are supported by studies showing reduced plasma neutralization titres mediated by the E484K change alone[23,24]. Importantly, here we show that the K417N change also has a crucial role in viral escape, effectively abrogating neutralization by a well-defined, multi-donor class of VH3-53/66 germline restricted public antibodies that comprise some of the most common and potent neutralizing antibodies to SARS-CoV-2[11,17].

The marked loss of neutralization against 501Y.V2 compared to the RBD-only chimeric virus demonstrates the important role that mutations in the NTD play in mediating immune escape. While the L18F change (found in 501Y.V2) has previously been linked to neutralization resistance[22], we also define an important role for a relatively small, three amino acid deletion in the 501Y.V2 NTD domain that completely disrupts a dominant public antibody response to the N5-loop supersite[20-22]. This deletion predominates among 501Y.V2 variants, and occurs either alone or with an R246I substitution that has also been shown to abrogate neutralization by several NTD-directed neutralizing antibodies[22].

A recently identified lineage in Brazil also has changes at key positions shown here to affect neutralizing antibodies (417T, 484K in RBD and 18F, 20N in NTD - the last mutation adding a glycosylation sequon)[25]. Our data suggest that this lineage is also likely to exhibit significant levels of neutralization resistance, making both lineages of considerable public health concern. The independent emergence and subsequent selection for two distinct 501Y lineages with key mutations conferring neutralization resistance strongly argues for selection by neutralizing antibodies as the dominant driver for SARS-CoV-2 spike diversification. The relatively rapid acquisition of a comprehensive suite of neutralization escape mutations likely occurred because of the large number of commonly shared public antibodies (eg: VH3-53/66, VH1-2, VH1-24) to both the RBD and NTD of spike, together with high levels of SARS-CoV-2 transmission around the world. The sporadic emergence of escape mutations in long-term viral shedders, including immunocompromised individuals, may also contribute to the emergence of neutralization resistant viruses[32]. Altogether, these data highlight the need for increased, ongoing surveillance and sequencing 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[8,9,33]. The overwhelming majority of monoclonal antibodies already on

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the path to licensure target residues K417 or E484 and will therefore be ineffective against 501Y.V2. Amongst these antibodies, some have already been granted emergency use authorization in the USA (Regeneron Pharmaceuticals and Eli Lilly and Company). In addition, a next generation of potent neutralizing antibodies that target the NTD N5-loop supersite have been suggested for clinical development but are also likely to be futile against 501Y.V2.

These data also have implications for the effectiveness of SARS-CoV-2 vaccines, which are principally based on immune responses to the spike protein. Neutralizing antibodies have repeatedly been demonstrated as the primary correlate of protection for most vaccines, including those designed to prevent infection with respiratory pathogens[34]. Despite neutralization escape, we show here that a significant proportion of non-neutralizing, RBD binding antibodies remain active against 501Y.V2. While antibody effector functions elicited by infection and vaccination have been implicated in protecting from reinfection and disease[35,36], the role of non-neutralizing antibodies and the efficacy of T cell responses to 501Y.V2 remain to be elucidated. 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, the speed and scope of 501Y.V2 mediated immune escape from pre-existing neutralizing antibodies highlight the urgent requirement for rapidly adaptable vaccine design platforms, and the need to identify less mutable viral targets for incorporation into future immunogens.

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Materials and Methods

Samples and ethics approvals

Plasma/serum samples were obtained from HIV negative individuals enrolled into one of three studies described below.

Hospitalised Steve Biko Cohort. This study has been given ethics approval by the University of Pretoria, Human Research Ethics Committee (Medical) (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-August 2020 and 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 (Medical) 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-September 2020 and were used.

SANBS. Plasma were obtained from blood donors of the South African National Blood Service (ethics clearance from SANBS Human Research Ethics Committee 2019/0519), who had PCR confirmed SARS-CoV-2 infection, had recovered and were at least 28 days 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 55kg and leading a lifestyle that reduces risk of acquiring transfusion transmissible infections. Only male and nulliparous females were accepted as CCP donors.

Structural modelling

The closed prefusion SARS-CoV-2 Spike protein was modelled on PDBID 6VXX[37], while a model of the RBD was averaged from Nab bound structures from PDBID 6LZG, 6WPS, 6XE1, 7BZ5, 7C01, 7JMP, 7K8M, 7K8S, 7K8U, 7K8V, 7K8W, 7JMO, 7K8X, 7K8Z, 7K90, 7BYR, 6WPS, and 6XDG. The 501Y.V2 NTD domain was modelled using both antibody constrained (PDBID 7C2L[21]) and unconstrained structures of Spike deposited in the protein databank[38]. Buried interfaces were calculated using PDBePISA (https://www.ebi.ac.uk/pdbe/pisa/pistart.html) and structures were visualized in PyMol[39].

Expression and purification of SARS-CoV-2 antigens

Monoclonal antibodies or recombinant SARS-CoV-2 RBD+SBD1 or NTD proteins were transfected in FreeStyle 293F suspension cells (Life Technologies) using PEIMAX transfection reagent (Polysciences). Transfections were incubated at 220 rpm, 37 °C, 9% CO_2 for 6-7 days, and clarified supernatants were purified using nickel affinity or protein A and size-exclusion chromatography.

ELISA

Recombinant RBD+SBD1 proteins were coated at 2 µg/ml onto 96-well, high-binding plates, and incubated overnight at 4 °C. The plates were then washed and incubated at 37 °C for 1-2 hours in blocking buffer (5% skimmed milk powder, 0.05% Tween 20, 1x PBS). Plasma samples were added at 1:100 and serially diluted 5-fold in blocking buffer. Following a 1-hour incubation at 37 °C, an anti-human horseradish peroxidase-conjugated antibody was added for 1-hour at 37 °C. The signal was developed with TMB substrate (Thermofisher Scientific) for 5 minutes at room temperature, followed by the addition of 1M

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H_2SO_4 stop solution. Absorbance at 450nm was measured and used to calculate area under the curve (AUC) using Graphpad Prism v8.4.2.

Lentiviral pseudovirus production and neutralization assay

293T/ACE2.MF cells modified to overexpress human ACE2 were kindly provided by Dr Mike Farzan, Scripps Research. Cells were cultured in DMEM (Gibco BRL Life Technologies) containing 10% heat-inactivated FBS and 3 µg/mL puromycin at 37°C, 5% CO_2. Cell monolayers were disrupted at confluency by treatment with 0.25% trypsin in 1 mM EDTA (Gibco BRL Life Technologies).

The SARS-CoV-2, Wuhan-1 spike, cloned into pCDNA3.1 was mutated using the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies) to include D614G (original) or K417N, E484K, N501Y, D614G (RBD only) or L18F, D80A, D215G, Δ242-244, K417N, E484K, N501Y, D614G, A701V (501Y.V2). Pseudoviruses were produced by co-transfection with a lentiviral backbone (HIV-1 pNL4.luc encoding the firefly luciferase gene) and either of the SARS-CoV-2 spike plasmids with PEIMAX (Polysicences). Culture supernatants were clarified of cells by 0.45µM filter and sored at -70°C.

Plasma/serum samples were heat-inactivated and clarified by centrifugation. Pseudovirus and serially diluted plasma/sera were incubated for 1 hour at 37°C, 5% CO_2. Cells were added at $1x10^4$ cells per well after 72 hours incubation at 37°C, 5% CO_2, luminescence was measured using PerlinElmer Life Sciences Model Victor X luminometer. Neutralization was measured as described by a reduction in luciferase gene expression after single-round infection of 293T/ACE2.MF cells with spike-pseudotyped viruses. Titers were calculated as the reciprocal plasma dilution (ID50) causing 50% reduction of relative light units (RLU). Equivalency was established through participation in the SARS-CoV-2 Neutralizing Assay Concordance Survey (SNACS) Concordance Survey 1 (CS1) run by EQAPOL and VQU, Duke Human Vaccine Institute.

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the SARS-CoV-2 pseudotyped neutralization assay, and enabling equivalency and proficiency testing. We thank Drs Devin Sok, Elise Landais, Dennis Burton, Nicole Doria-Rose and Peter Kwong for SARS-Co-V-2 directed monoclonal antibodies. We are grateful to Thandeka Moyo-Gwete and Zanele Molaudzi for expresssing monoclonal antibodes. We thank the informal 501Y.V2 consortium of South African scientists, chaired by Drs Willem Hanekom and Tulio D'Oliveira for suggestions and discussion of data.