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XAV-19, a swine glyco-humanized polyclonal antibody against SARS-CoV-2 Spike receptor-binding domain, targets multiple epitopes and broadly neutralizes variants

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XAV-19, a novel swine glyco-humanized polyclonal antibody against SARS-CoV-2 spike, efficiently neutralizes B.1.1.7 British and B.1.351 South-African variants.

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Abstract

Amino acid substitutions and deletions in spike (S) protein of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants can reduce the effectiveness of monoclonal antibodies (mAbs). In contrast, heterologous polyclonal antibodies raised against S protein, through the recognition of multiple target epitopes, have the potential to maintain neutralization capacities. We report on XAV-19, a swine "glyco-humanized" polyclonal antibody (GH-pAb) raised against the receptor binding domain (RBD) of the Wuhan-Hu-1 spike protein of SARS-CoV-2. XAV-19 target epitopes are distributed all over the RBD and particularly cover the receptor binding motives (RBM), on direct contact sites with the Angiotensin Converting Enzyme-2 (ACE-2). Using spike/ACE2 interaction assays, we analyzed in vitro the impact of punctual and grouped mutations in the S protein corresponding to the B.1.1.7 (British form; UK) and B.1.351 (South-African form, SA) variants and recorded that neutralization by XAV-19 exhibited little if any sensitivity to these mutations. These results were confirmed by two independent tissue culture infective doses assays (TCID) showing 100% neutralization of the variants at close concentrations. XAV-19, which is currently evaluated in patients hospitalized for coronavirus disease 2019 (Covid-19) in the phase 2a-2b of the POLYCOR study (ClinicalTrial.gov, NCT04453384), may provide a novel effective therapeutic tool to combat coronavirus disease 2019 (Covid-19), caused by the original Wuhan form and by the UK or SA variants of concern.

Introduction

Passive antibody therapies have demonstrated efficacy to reduce progression of mild COVID-19 patients to severe disease if administered early enough in the course of illness [1-3]. Three sources of antibodies have so far been assessed. Passive antibody therapy using the infusion of convalescent plasma (CP) with high SARS-CoV-2 antibody titers in hospitalized patients, administered within 72 hours after the onset of mild symptoms, reduced the relative risk of progression to severe disease by 73% if CP presented a titer of >1:3200 and by 31.4% with lower titer CP [1]. This was true with CP drawn between June and October 2020. However, CP raised against the original SARS-CoV-2 lineage had poor activity against the 501Y.V2 virus (South-African variant; SA). Indeed, nearly half of recipients had no detectable neutralization activity and this loss was attributed to a lack of recognition of only three 501Y.V2 mutations within the spike protein (K417N, E484K, N501Y) [4]. The site where mutations tend to have the largest effect on binding and neutralization is E484 [5]. Beside use of CP, more than 50 neutralizing monoclonal antibodies (mAbs) are in development against the spike and the N-terminal domain (NTD) domains of SARS-CoV-2 [6]. Those developed by Regeneron Pharmaceuticals (REGN-COV2 (casirivimab/imdevimab cocktail) and Eli Lilly (bamlanivimab/etesevimab) have proven to provide protection against the risk for severe Covid-19 when administered early in high-risk symptomatic patients with mild to moderate Covid-19 not requiring hospitalization, with 70% reduction in risk of hospitalization with bamlanivimab/etesevimab (Douglas 2021) and 71% reduction in related medically-attended visits with casirivimab/imdevimab cocktail (https://www.ema.europa.eu/en/documents/referral/regn-cov2-antibody-combination-casirivimab/imdevimab- covid19 - article - 53- procedure-assessment-report_en.pdf). Both combination of mAbs were recently authorized for emergency use by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA). However, viral mutations can escape mAbs used to treat Covid-19 [7]. B.1.1.7 is refractory to neutralization by most mAbs to the NTD of S protein and relatively resistant to several mAbs to the receptor-binding domain (RBD) [8]. Mutations in the 501Y.V2 lineage (K417N, E484K, and N501Y in RBD SA variant), especially mutations at spike E484 but also in the N-terminal Domain (NTD; L18F, D80A, D215G, Δ242-244, and R246I in SA variant [9,10]) have been shown to reduce neutralization sensitivity or confer neutralization escape from multiple mAbs [4,5,11-16]. There are also concerns about the selective pressure that could arise from the partial neutralizing activity of these mAbs against certain variants of concern. Polyclonal antibodies produced from humanized or glyco-humanized large animals have also proven efficacy in vitro in ELISA and in Plaque

Neutralization Reduction Tests to neutralize SARS-CoV-2 (Vanhove et al [17] and https://www.news-medical.net/news/20210210/Polyclonal-SARS-CoV-2-antibody-shows-potent-neutralizing-activity-in-vitro.aspx). Their tolerability in humans has been confirmed recently in two clinical trials (NCT04453384, NCT04469179), contrasting with wild-type polyclonal antibodies that induce serum sickness and allergic reactions (including fever and skin rashes) in 20 to 30% of the patients, excepting for patients who concomitantly receive immunosuppression [18,19]. Polyclonal antibodies recognize an array of epitopes on the target antigen and their global binding should theoretically be less affected by antigen variations.

Here we investigated whether XAV-19, a glyco-humanized polyclonal antibody previously shown to present neutralizing activity against SARS-CoV-2 Wuhan-D614G [17] also maintains activity against the UK (B.1.1.7) and SA (B.1.351) variants of concern.

Methods

Reagents

XAV-19 is a swine glyco-humanized polyclonal antibody against SARS-CoV-2 obtained by immunization of pigs double knocked out for GGTA1 and CMAH genes, as previously described [17]. Intermediate R&D preparations of swine glyco-humanized polyclonal antibody against SARS-CoV-2 have been prepared, presenting variable anti-SARS-CoV-2 binding activities [17]. Comparator bamlanivimab is from Lilly (Indianapolis, In, USA). Recombinant Spike molecules of the Wuhan type (Sino Biologicals ref 40591-V08H), mutation-containing RBD (Y453F ref 40592-V08H80; N501Y, ref 40592-V08H82; N439K, ref 40592-V08H14; E484K, ref 40592-V08H84), UK (ref 40591-V08H12; containing mutations HV69-70 deletion, Y144 deletion, N501Y, A570D, D614G, P681H) and SA (ref 40591-V08H10; containing mutations K417N, E484K, N501Y, D614G) forms and recombinant human Fc-tagged ACE-2 were purchased by Sino Biological Europe, Eschborn, Germany.

SARS-CoV-2 Wuhan (D614G variant), UK and SA strains were isolated from SARS-CoV-2 infected patients in the Pitié-Salpêtrière Hospital and Toulouse University hospital.

Spike/ACE-2 neutralization assay

An assay was developed to assess the properties of anti-SARS-CoV-2 spike antibodies to inhibit binding of ACE-2 to immobilized spike. SARS-CoV-2 spike S1 (either Wuhan, UK or SA) was immobilized on Maxisorp plates at 1 µg/mL in carbonate/bicarbonate buffer pH 9.0 at 4°C

overnight. The plates were washed in PBS-Tween-0.05% and saturated with PBS-Tween-0.05%-2% skimmed milk for 2h at room temperature (RT). Anti-Spike RBD antibodies diluted in PBS-Tween-0.05%-1% skimmed milk (dilution range between 50 and 0.39 µg/mL) were then added and incubated for 30 min. Then, ligand human ACE-2-mFc tag (Sino Biological; 125 ng/mL final concentration) was added in the same dilution buffer. After 1h incubation at room temperature and 3 washes, the mouse Fc tag was revealed with a specific HRP-conjugated anti-mouse IgG secondary antibody (diluted in in PBS-Tween-0.05%-1% skimmed milk powder at 1:1000, incubated 1h at RT and washed 3 times). TMB reagent was added into the plate, incubated 6 minutes in the dark and stopped with 50 µl 1M H_2SO_4. The plate was read at 450 nm.

Cytopathogenic Effect (CPE) assay

Vero cells (CCL-81) and Vero E6 cells (CRL-1586) were obtained from the American Type Culture Collection (ATCC CCL-81) and maintained at 37°C with 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 5% heat-inactivated fetal bovine serum (FBS) and 1X Penicillin-Streptomycin solution (Thermo Fisher Scientific, USA). SARS-CoV-2 clinical isolates Wuhan (D614G variant; GenBank accession number MW322968), UK (GenBank accession number MW633280) and SA (GenBank accession number MW580244) were isolated from SARS-CoV-2 RT-PCR confirmed patients by inoculating Vero cells with sputum sample or nasopharyngeal swabs in the biosafety level-3 (BSL-3) facility of the Pitié Salpêtrière University Hospital. Viral stocks were generated using one passage of isolates on Vero cells. Titration of viral stock was performed on Vero E6 by the limiting dilution assay allowing calculation of tissue culture infective dose 50% (TCID50). The neutralizing activity of the polyclonal antibody batches was assessed with a whole virus replication assay using the three SARS-CoV-2 isolates. Each batch of polyclonal antibodies were subjected to serial two-fold dilution ranging from 50 µg/ml to 0.05 µg/ml in fresh medium. 50 µl of these dilutions were incubated with 50 µl of diluted virus (2 x 10^3 TCID50/ml) per well in a 96-well plate at 37°C for 60 min in 8 replicates. We then added 100 µl of a Vero E6 cell suspension (3 x 10^5 cells/ml) to the mixture and incubated at 37°C under an atmosphere containing 5% CO_2 until the microscopy examination on day 4 to assess the CPE. IC50 were analyzed by nonlinear regression using a four-parameter dosage-response variable slope model with the GraphPad Prism 8.0.2 software (GraphPad Software, USA).

To further analyze the neutralization potency and to confirm data with another independent virus strain, a CPE assay was carried out independently on Vero E6 cells at the BSL-3 facility of VibioSphen, University Paul Sabatier, Toulouse, France. SARS-CoV-2 Wuhan and UK strains were isolated from patients with laboratory-confirmed COVID-19 from the Toulouse University hospital. The viral isolates were amplified by one additional passage in VeroE6 cells to make working stocks of the virus. Vero E6 Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10%v/v fetal bovine serum, 1% v/v penicillin-streptomycin supplemented with 1% v/v sodium pyruvate at 1x 10^5 cells per well in 12-well tissue culture plates. At 100% confluence (2 days post-seeding), the cells were washed twice with PBS and six serial dilutions of the virus (1/10 each time) were added to the cells. Following infection with 0.3 ml per well of each dilution, plates were incubated at 37°C for 1 h, and the cells were washed with PBS before the addition of 2% w/v agar containing 1 µg/mL-5 tosyl phenylalanyl chloromethyl ketone-trypsin (Sigma-Aldrich,) to the cell surface. Plates were left at room temperature for 20–30 min to allow for the overlay to set and were then incubated at 37°C for 72 h. Cells were fixed with 4% v/v paraformaldehyde before both the fixative and agar will be removed and the cells stained with 0.1%w/v Crystal Violet (Fisher) in 20% v/v ethanol. Plaque titers were determined as plaque forming units per mL. CPE reduction assay was performed as follows: Vero E6 cells were seeded in 96-well clusters at a density of 5,000 cells/well 2 days before infection. Two-fold serial dilutions, starting from 100 µg/mL of XAV-19 were mixed with an equal volume of viral solution containing 300 pfu of SARS-CoV-2 (final volume 200 µL). The serum-virus mixture was incubated for 1 hour at 37°C in a humidified atmosphere with 5% CO_2. After incubation, 100 µL of each dilution were added in 8 wells of a cell plate containing a semi-confluent Vero E6 cells monolayer. Control cells were infected with Covid-19 at MOI 0.01. Remdesivir (25 µM) was used as a positive control. After 3 days of incubation, the plates were inspected by an inverted optical microscope. Viable cells were quantified with CellTiter-Glo 2.0 luminescent cell viability assay. A Tissue Culture Infectivity Index has been calculated on the following basis: for each of the 8 replicate wells tested for one condition, an infectivity score has been assigned: 0, no cytopathic effect; 1, a fraction of cells was affected; 2, 100% cells affected. The addition of the scores in the 8 replicates was then transformed in % of the maximal scoring (ex. Score of 16 = 100%) and reported on a graph.
The highest product dilution that protected 100% of cells (Tissue Culture Infectivity Index of 0%) was considered the neutralization titer.

Results

Assessment of Spike/ACE-2 interaction by ELISA is predictive of the neutralization assessed using whole viruses

In order to assess whether testing spike/ACE-2 interaction in ELISA is predictive of live virus neutralization, we used 4 R&D batches of swine glyco-humanized IgG antibodies presenting variable anti-RBD binding activity levels and assessed their neutralizing potential in parallel, using both methods. The batches presented IC50 values by ELISA of 1.3, 1.34, 2.2 and 12 µg/ml and the corresponding values in TICD assays were of 3, 2, 12.5 and 25 µg/ml. These values presented a correlation coefficient of 0.97 (Figure 1).

ELISA to assess inhibition of Spike from Wuhan, UK and SA variants interaction with ACE-2

XAV-19 was tested in a spike/ACE-2 binding competition assay, where the spike protein was of the original Wuhan type or contained the RBD mutations N501Y, N439K, Y453F described in the UK and SA variants, or the mutation E484K show to induce resistance to mAbs. Combined mutations gathering the variations present in the Spike UK (HV69-70 deletion, Y144 deletion, N501Y, A570D, D614G, P681H) or SA (K417N, E484K, N501Y, D614G) variants were also tested.

All single mutation forms of the spike could be fully neutralized at concentrations not significantly different (slightly lower for the E484K mutation) from the Wuhan type (Figure 2A). XAV-19 also demonstrated 100% inhibitory capacity on the 2 spike proteins fully representative of the UK and SA variants, similar to the Wuhan spike, with IC50 values of 6.4, 4.0 and 4.5 µg/ml, respectively (Figure 2B). Bamlanivimab, tested in parallel, demonstrated a potent inhibitory capacity against the Wuhan and the UK variants, with a IC50 value of 0.01µg/ml but, as described [20], failed to inhibit binding of SA spike to ACE-2, even at high concentration (Figure 2C).

Neutralization of live SARS-CoV-2 Wuhan, UK and SA variants in cytopathic assays (CPE)

The neutralizing effect of XAV-19 was determined by a CPE assays on the La Pitié-Salétrière platform using Wuhan, UK and SA SARS-CoV-2 clinical isolates, as previously described [21]. The test assesses inhibition of live viruses with sensitive human Vero E6 cells and records infection after 4 days by CPE. Data (Figure 3A) showed global similar behavior of XAV-19 on the 3 strains with absence of neutralizing activity below 1.5 µg/ml and 100% neutralizing

activity above 5 µg/ml. IC50 against Wuhan, UK and SA strains were respectively of 2.2, 2.2, and 3.2 µg/ml, respectively. Interestingly, the comparator mAb bamlanivimab presented IC50 values against Wuhan and UK strains of 0.1 µg/ml but failed to inhibit the SA variant at any concentration (Figure 3B).

In order to confirm these data, the assay has been repeated independently on another platform (Vibiosphen, Toulouse University), using other SARS-CoV-2 clinical isolates. XAV-19 presented a $TCID_{100}$ (tissue culture infective dose 100; represents the concentration required to fully inhibit the cytopathogenic effect) of 1.56 µg/ml against the Wuhan strain and of 0.78 against the UK strain. At the concentration below 0.78 and down to 0.1 µg/ml, a partial neutralization of the cytopathogenic effect caused by the UK variant was still visible whereas the neutralization of the cytopathogenic effect caused by the Wuhan variant was mostly absent (Figure 4).

Discussion

In this paper, we report that XAV-19, a swine glyco-humanized polyclonal antibody raised against the spike RBD protein of the SARS-CoV-2 original Wuhan strain [17], also fully neutralizes UK and SA variants.

The two independent cytopathic assays with live viruses were run in parallel in different locations by different teams. They showed similar findings, that a concentration of XAV-19 of approximatively 1 to 5 µg/ml is required to fully neutralize all the variants. There was a clear tendency of a lower capacity at low concentration to neutralize the SA variant, as compared with the Wuhan and UK forms in the neutralization assay. However, in the cytopathic effect assay, we found that the UK strain was more susceptible to neutralization by XAV-19. Whether this difference is real or reflects method variability has not been determined and will require further measures. In any case, it was clear that SARS-CoV-2 variants could be fully neutralized by XAV-19, and a clear difference was evident when comparing with bamlanivimab, which has no neutralizing effect on the SA variant.

Our data also indicated that for a given variant, neutralization assessment of antibodies in the ELISA format, using recombinant spike RBD protein interaction with human ACE-2, was predictive of the neutralizing activity assessed with living viruses and to some extent could predict preservation or loss of activity against SARS-CoV-2 variants. This observation

confirmed data showing that a neutralizing ELISA is predictive of SARS-Cov-2 neutralization assessed in a lentivirus-pseudotyped SARS-CoV-2 neutralization assay [4].

Early after Covid-19 outbreak onset, many labs have been able to rapidly develop neutralizing antibodies. One year later, as of March 2021, more than 93 clinical trials assessing the clinical safety and benefit of mAbs and more than 7 of polyclonal antibodies can be found in the Clinicaltrials.gov repository. In mid-2020, variants of concern started to spread in the population and now represent most of the infections. However, antibodies that are now assessed clinically have been mostly raised against the initial, Wuhan strain. It has therefore become essential to revisit their potential to also neutralize variants. XAV-19 is currently being tested in the POLYCOR (ClinicalTrial.gov, NCT04453384) and the EUROXAV (Eudract Number: 2020-005979-12) studies. The Phase 2a of the POLYCOR study [22] demonstrated that a single intravenous perfusion of XAV-19 at 2 mg/kg was safe, achieving a median serum Cmax of 50.4 µg/ml and D8 concentration of 20.3 µg/ml with a elimination half-life (T1/2) estimated at 11.4 days (Gaborit B. et al, MedRxiv, In Press). The data presented here, that XAV-19 expected neutralizing level against Wuhan, UK and SA variants does not exceed 5 µg/ml, together with these pharmacokinetic data, indicate that XAV-19 can provide high and sustained therapeutic activity with an inhibitory quotient well above 10. These data warrant continuation of clinical studies with XAV-19, especially in a context where the UK variant becomes dominant and other variant of concerns emerge.

Key words

polyclonal antibodies, pig, Covid-19, SARS-CoV-2, Spike, neutralization, variant of concern

Authorship

Conceived the study: OD, BV, FR

Designed and supervised some experiments: OD, BV, BG, SM, AGM, VC

Performed the experiments: GE, AGM, SM, SD

Analyzed data: BV, OD, AGM, VP, BG, FR

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Competing Interests

The authors of this manuscript have conflicts of interest to disclose: OD, PJR, CC, GE, EL, BV are employees of Xenothera, a company developing glycol-humanized polyclonal antibodies as those described in this manuscript.

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Figure legends

Figure 1

Correlation between neutralizing ELISA and PRNT assay. Four R&D batches of swine anti-SARS-CoV-2 Spike RBD have been produced, that presented different binding activities against SARS-CoV-2 spike (described in Vanhove et al, EJI 2021). These samples were evaluated in parallel in neutralizing ELISA, as described in Figure 1, and by PRNT. IC50 in ELISA and $PRNT_{100}$ have been plotted to assess for correlation. R^2 after linear extrapolation was 0.9392.

Figure 2

Neutralization assay in the ELISA format: assessment of SARS-CoV-2 spike/ACE-2 interaction and its anti-Spike antibody-mediated inhibition. Spike-HIS containing the indicated mutations (A) or corresponding to the UK and SA variants (B, C) was immobilized on plastic and binding to ACE2-Fc was revealed with a secondary antibody against Fc. 100% inhibition represents absence of spike/ACE-2 interaction. (A, B) Means of triplicate analyses run in a single experiment assessing XAV-19 at the indicated concentration. (C) Means of triplicate analyses run in a single experiment assessing bamlanivimab at the indicated concentration.

Figure 3

Neutralization activity against three SARS-CoV-2 isolates assessed by inhibition of cytopathogenic effect (CPE). . XAV-19 (A) and bamlanivimab (B) have been tested in a whole virus replication assay against the indicated SARS-CoV-2 variant as described in the Material and Methods section. CPE percentage was assessed by microscopy examination and calculated on 8 replicates. 100% represent absence of CPE inhibition at the studied concentration, as found in the control (no inhibitor) situation.

Figure 4

Cytopathogenic Effect (CPE) assay. XAV-19 has been evaluated in a CPE assay where tissue culture infectious dose (TCID) was recorded after infection with SARS-CoV-2 Wuhan or UK variants. A Tissue Culture Infectivity Index has been calculated on the following basis: for each of the 8 replicate wells tested for one condition, an infectivity score has been assigned: 0, no cytopathic effect; 1, a fraction of cells was affected; 2, 100% cells affected. The addition of the scores in the 8 replicates was then transformed in % of the maximal scoring (ex. Score of 16 = 100%) and reported on the graph.

Figure 1

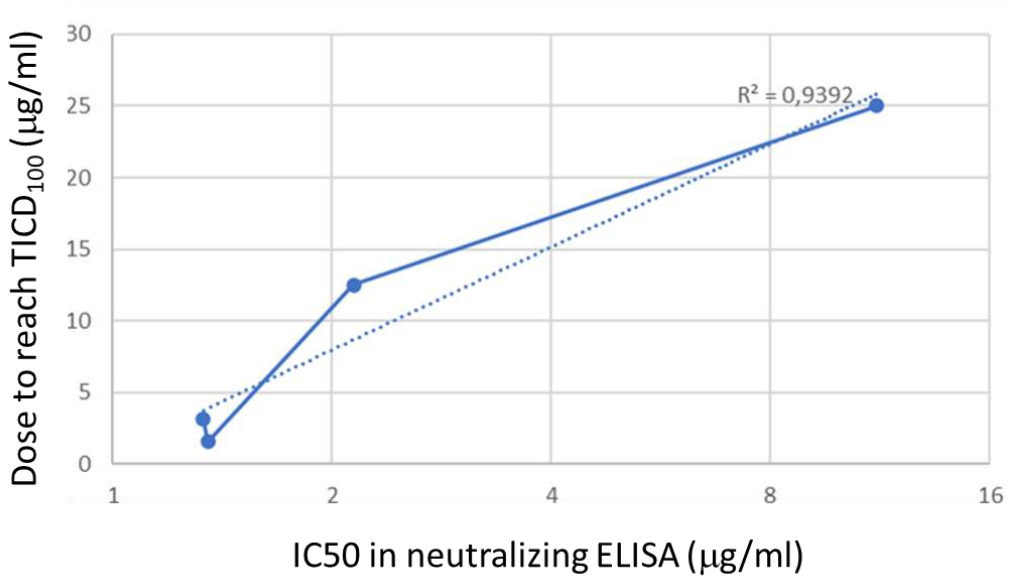

Figure 2

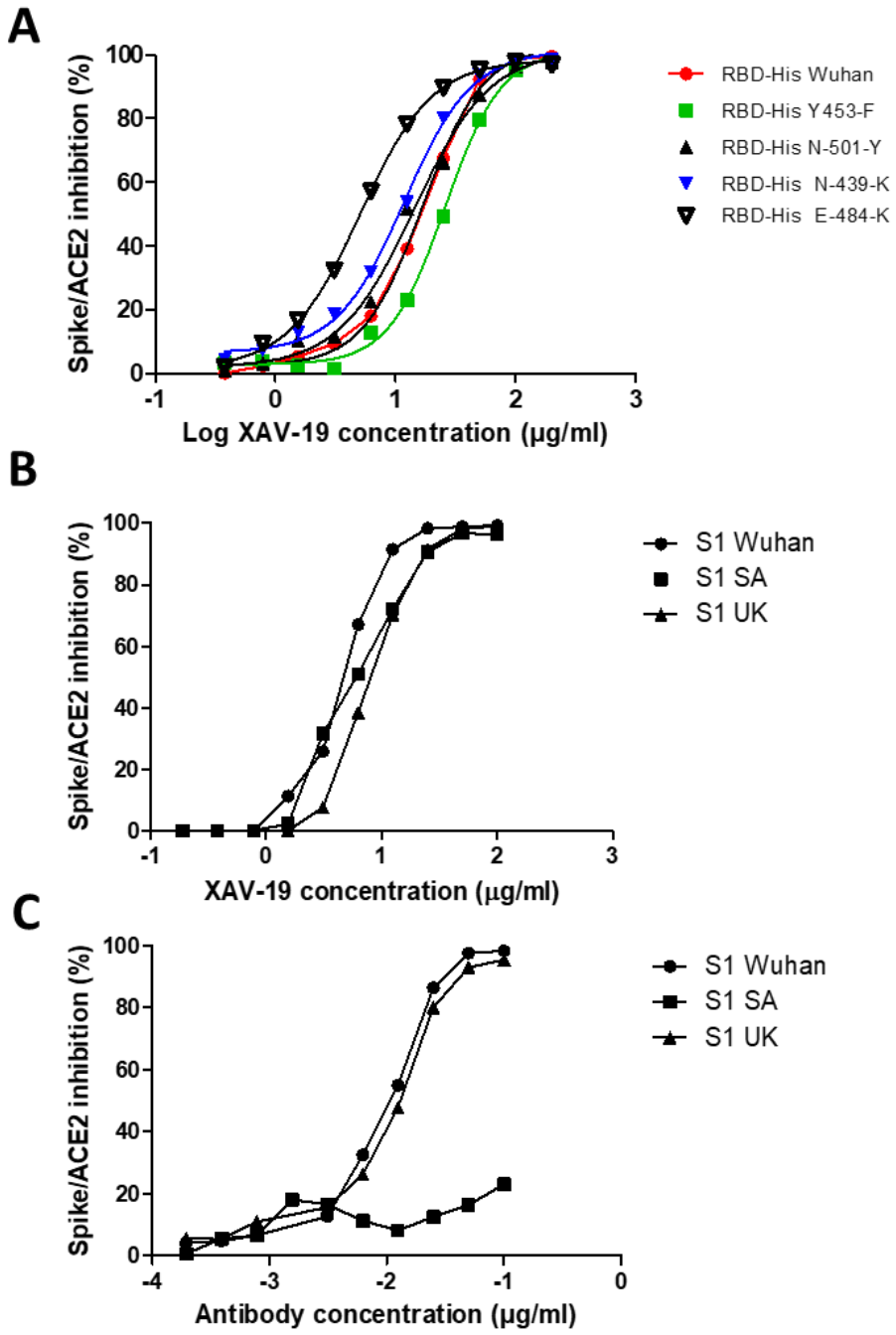

Figure 3

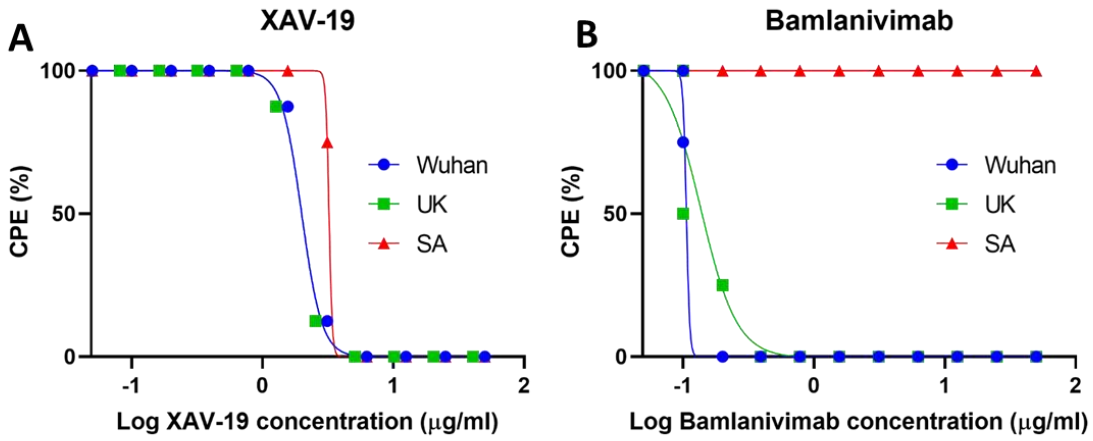

Figure 4