Transchromosomic bovine-derived human anti-SARS-CoV-2 polyclonal antibodies protect hACE2 transgenic Syrian hamsters against multiple SARS CoV-2 variants

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Abstract

Pandemic SARS CoV-2 has undergone rapid evolution resulting in the successive emergence of many variants with novel mutations in the Spike protein, some of which appear to evade antibody neutralization, transmit more efficiently and/or exhibit altered virulence. This raises significant concerns regarding the efficacy of protection elicited after primary infection or from vaccines derived from single virus Spike (S) genotypes, as well as the efficacy of anti-S monoclonal antibody based therapeutics. To address this concern, SAB-185, a human anti-SARS-CoV-2 polyclonal antibody (pAb) was generated in the DiversitAb^{TM} platform. This platform uses human artificial chromosome-transgenic bovines to produce human IgG preparations after hyper-immunization. The *in vitro* neutralizing capacity of SAB-185 was tested against ten variant SARS-CoV-2 strains including several Omicron variants. SAB-185 exhibited equivalent neutralization of the Munich, alpha, beta, gamma and D144-146 variants and retained neutralization of the delta variant AY.1 and omicron variants BA.1.1.529, BA.2.12.1, BA.4 and BA.5, with only modest losses of neutralization efficacy. For *in vivo* protection studies, we used a new human ACE2 (hACE2) transgenic Syrian hamster model that exhibits rapid lethality after intratracheal SARS-CoV-2 challenge with the Munich, Alpha, Beta, Delta, and D144-146 variants; the Omicron B.1.1529 variant resulted in a delayed, less severe and non-lethal disease. Prophylactic SAB-185 treatment protected the hamsters from death and minimized clinical signs of infection when challenged with the variant viruses tested. This suggests that SAB-185 may be an effective immunotherapy even in the presence of ongoing viral mutation.

Results

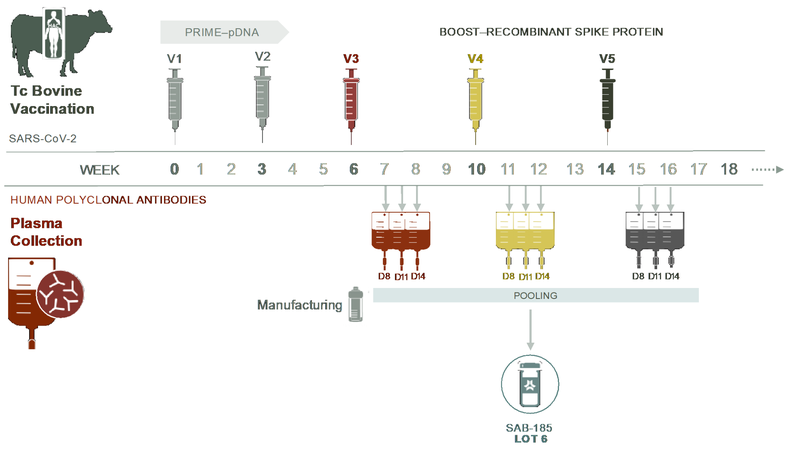

Figure 1. Tc bovine immunization schedule and SAB-185 production strategy using the DiversitAb^{TM} platform (as described in Liu et al., Hum. Vaccin. Immunother. 18:1940652, 2022 PMID34228597). Tc bovines were immunized twice with plasmid DNA expressing the Wuhan WA-1 strain spike protein at three-week intervals. Starting week six, animals were boosted three times with recombinant WA-1 spike ectodomain protein derived from insect cells. Plasma from weeks 7-8, 11-12 and 15-16 were pooled and human IgG was purified for the SAB-185 final preparation. Tc bovine plasma was thawed, pooled, fractionated by caprylic acid (CA), and clarified by depth filtration in the presence of Celpure P1000 filter aid. The clarified sample containing Tc bovine-derived human IgG is further purified by affinity chromatography, first using an anti-human IgG kappa light chain-specific column, KappaSelect (GE Healthcare Life Sciences) to capture hIgG followed by a low pH treatment, and second, by passing through an anti-bovine IgG heavy chain-specific affinity column, Capto HC15 (GE Healthcare Life Sciences). To further remove residual IgG that contains bovine heavy chain, the human IgG fraction was then subjected to a Q Sepharose chromatography polishing step to further reduce impurities, nanofiltration, final buffer exchange, concentration, and sterile filtration. Finally, the SAB-185 product was terminally filtered and filled into vials.

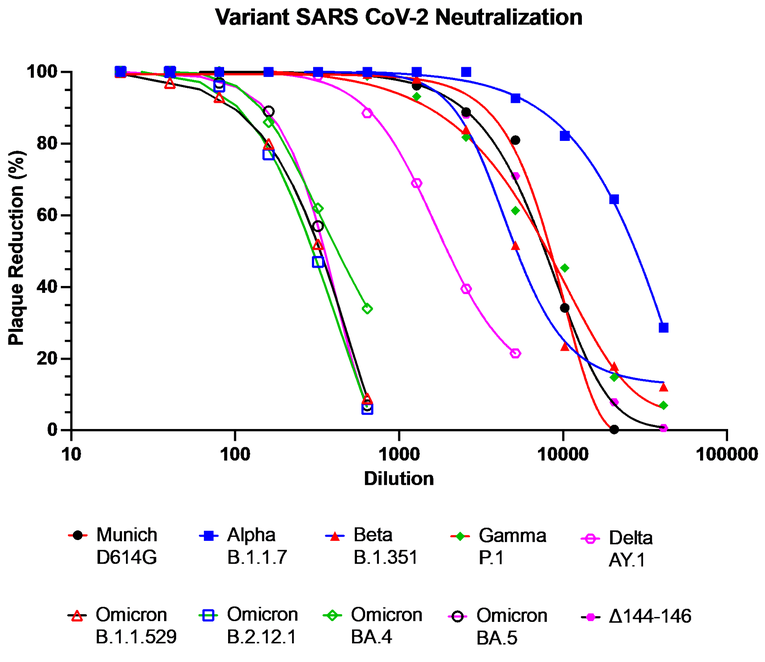

Figure 2. SAB-185 neutralization potential *versus* the Munich variant (Spike D614G) and other variants. Neutralization capacity of SAB-185 was assayed by Vero E6 or Vero hAce2/TMPRSS2 cell plaque neutralization assay. SAB-185 was diluted to 1mg/ml in PBS and then diluted serially two-fold before reaction with viruses. All Ab samples were heat inactivated by incubation at 56°C for 30 minutes. Viruses were diluted in OPTI-MEM (Gibco) with 2% FBS to approximately 200 PFU in 250 ml and reacted with an equal volume of serial two-fold dilutions of each antibody (in PBS) for 1 hour at 37°C followed by infection of Vero E6 monolayers for 1 hour at 37°C. A solution of 0.1% immunodiffusion agarose (MP Bio) in 2X Vero E6 growth medium was then added and plaques were developed at 37°C for 72-96 hours followed by removal of agarose, staining of cells with crystal violet (Fisher Scientific) and counting of plaques. Data points are averages of results from 3 replicates with 2 duplicate wells at each dilution. Error bars are omitted for clarity.

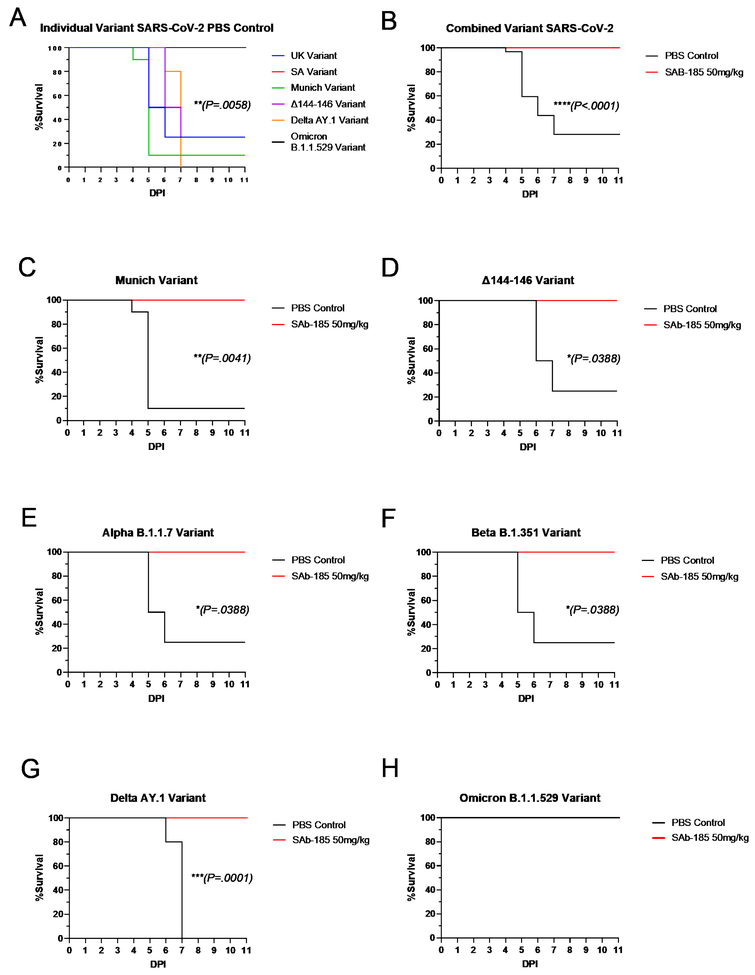

Figure 3. SAB-185 protection from mortality in hamsters challenged with six variant SARS CoV-2 isolates. Hamsters were administered SAB-185 or PBS intramuscularly and then challenged intratracheally 24 hours later with 1000 plaque forming units of variant viruses. Mortality for individual variant PBS controls (A) and for combined (all SARS-CoV-2 variants tested) PBS control *versus* SAB-185 treated groups (B). Individual mortality data for Munich (C), D144-146 (D) Alpha (E), Beta (F), Delta (G), and Omicron (H) viruses. Mantel-Cox log-rank significance is indicated within each panel. * p<0.05, **p<0.01, ***p<0.005. .

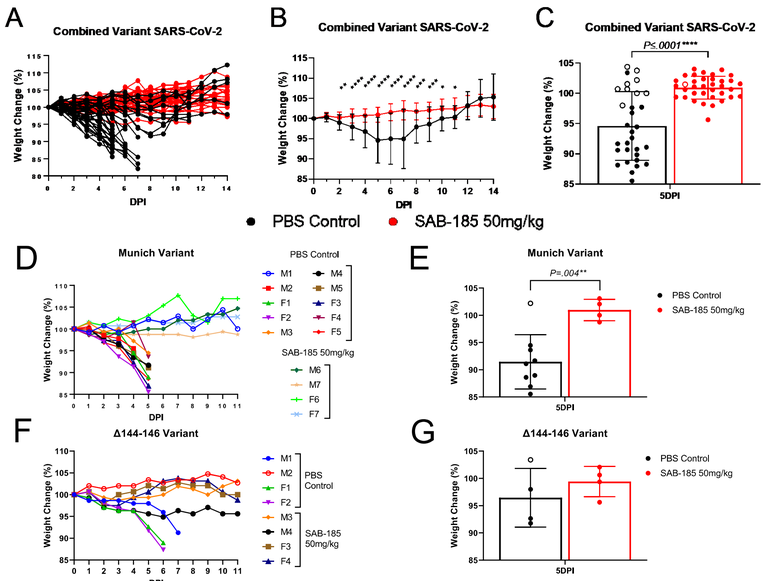

Figure 4. SAB-185 protection from weight loss in hamsters challenged with six variant SARS CoV-2 isolates. (A) Weight loss for individual hamsters in all groups. (B) Combined (all SARS-CoV-2 variants tested) average weight loss data for SAB-185-treated and PBS control hamsters. (C) Combined average weight loss data for SAB-185-treated and PBS control hamsters on D5 (last day all animals were alive). Omicron infected animals are omitted due to delayed disease development. Individual weight loss data for Munich (D), D144-146 (F), Alpha (H), Beta (J), Delta (L) and Omicron (N) viruses. Combined average weight loss data for Munich (E), D144-146 (G), Alpha (I), Beta (K) and Delta (M) variants on D5 (last day all animals were alive) post challenge or D8 post challenge for Omicron-infected animals (O) (peak weight loss). * p<0.05, **p<0.01, ***p<0.005. Open circles are surviving animals (controls and Omicron) and the SAB-185 treated animal that exhibited delayed replication (data not shown).

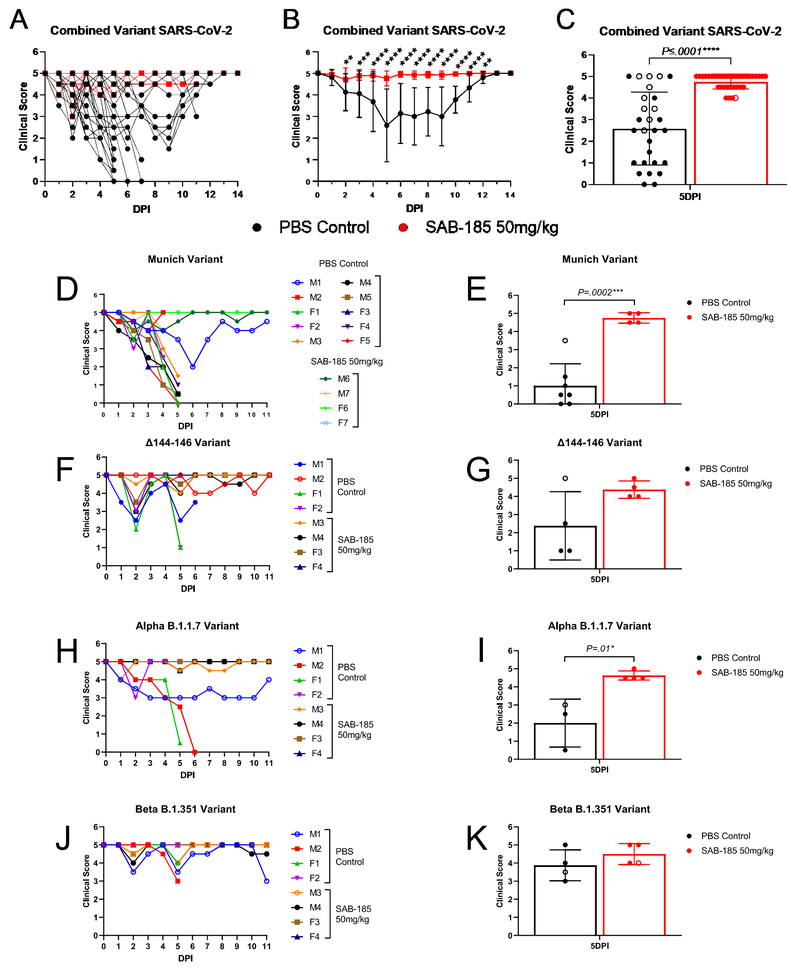

Figure 5. SAB-185 protection from clinical signs in hamsters challenged with six variant SARS CoV-2 isolates. Data is presented as the inverse of the clinical score sum values, as described in Materials and Methods, to be comparable to weight loss data. Each datum point represents an average of morning and afternoon observations. A) Clinical sign scoring for individual hamsters in all groups. B) Combined clinical sign scoring data for SAB-185-treated and control hamsters. (C) Combined clinical sign scoring data for SAB-185-treated and control hamsters on D5 (last day all animals were alive) post challenge or D8 post challenge for Omicron-infected animals (peak clinical signs). Individual clinical sign scoring data for Munich (D), D144-146 (F) Alpha (H), Beta (J), Delta (L) and Omicron (N) viruses. Individual clinical sign scoring data for Munich (E), D144-146 (G) UK (I), SA (K), Delta (M) and Omicron (O) variants on D5 (last day all animals were alive) post challenge or D8 post challenge for Omicron (peak clinical signs). * p<0.05, **p<0.01, ***p<0.005. Open circles are surviving (controls and Omicron) and the SAB-185 treated animal that exhibited delayed replication (data not shown).

Conclusions

The occurrence of successive waves of SARS CoV-2 variants with novel spike protein mutations throughout the COVID-19 pandemic has reduced the prophylactic efficacy of vaccines and abrogated the efficacy of many antibody-based therapeutics. SAB-185 is a human polyclonal IgG generated from the DiversitAb^{TM} platform produced using spike protein from the WA-1 SARS CoV-2. The current studies demonstrated that a single IM injection of SAB-185 protected recombinant hACE2 hamsters from mortality and/or severe morbidity when intratracheally infected with successive SARS CoV-2 variants including omicron. Although reduced *in vitro* SAb-185 $PRNT_{50}$ and $PRNT_{80}$ neutralization titers were observed with delta and omicron variants, SAB-185 was still highly protective at human-relevant doses *in vivo*. Therefore, reduced in *vitro* neutralization titers of SAB-185 against SARS CoV-2 variants were not associated with any reduction of in *vivo* efficacy.

Reasons underlying the protective efficacy of SAB-185 *versus* multiple strains, may include the hyper-immunization of Tc bovines with full length spike pDNA for priming and recombinant spike ectodomain protein for boosting, which may increase stimulation of polyclonal antibodies reactive with subdominant epitopes that are less likely/able to mutate during widespread human infection. Loss of reactivity of monoclonal antibodies that bind various epitopes in the S protein has been demonstrated clearly and the broad reactivity provided by polyclonal Ab products may have an advantage in neutralization and protection against variants. In addition, other factors such as non-neutralizing anti-spike antibodies and/or innate immune mechanisms such as effector cell function(s) could be important in *vivo*.

In summary, the DiversitAb^{TM} platform represents a highly scalable system that produces high neutralizing titer, fully human polyclonal antibodies. The data in this study suggest that human anti-SARS-CoV-2 polyclonal antibody, SAB-185, may have broad efficacy in preventing or treating SARS CoV-2 variant infections in humans.

Acknowledgments

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