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Fully Human Antibody Immunoglobulin from Transchromosomic Bovines is Potent Against SARS-CoV-2 Variant Pseudoviruses

Abstract

SAB-185 is a fully human polyclonal anti-SARS-CoV-2 immunoglobulin produced from the plasma of transchromosomic bovines that are hyperimmunized with recombinant SARS-CoV-2 Wuhan-Hu-1 Spike protein. SAB-185 is being evaluated for efficacy in an adaptive phase 2/3 clinical trial. The World Health Organization (WHO) has identified multiple Variants-of-Concern and Variants-of-Interest (VOC/VOI) that have mutations in their Spike protein that appear to increase transmissibility and/or reduce the effectiveness of therapeutics and vaccines, among other parameters of concern. SAB-185 was evaluated using a lentiviral-based pseudovirus assay performed in a BSL2 environment that incorporates a stable 293T cell line expressing human angiotensin converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2). The results indicate that SAB-185 retained neutralization potency against multiple SARS-CoV-2 pseudovirus variants, including the Delta, Kappa and Lambda variants, that are supplanting other VOC/VOI in many countries and regions around the world.

Introduction

Multiple variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have rapidly arisen since the progenitor strain was first identified in Wuhan, China in late 2019 (1). The World Health Organization (WHO) has categorized some of these variants as being Variants of Concern (VOC) or Variants of Interest (VOI) due to their transmissibility, virulence, or their ability to reduce the effectiveness of vaccines, therapeutics, and diagnostics (2). WHO currently identifies four SARS-CoV-2 variants as VOCs which include the Alpha, Beta, Gamma and Delta variants (formerly known as the United Kingdom, South African, Brazilian, and Indian' variants respectively) and four VOIs which include the Eta, Iota, Kappa and Lambda' variants.

Currently, the Delta variant is or has rapidly supplanted the Alpha variant that previously supplanted other variants in some countries and regions (3). Other VOC/VOIs, such as the Gamma and Lambda variants, continue to cause a large proportion of infections and/or are currently predominate in a particular country or region. Consequently, therapeutic countermeasures must be potent against existing dominate SARS-CoV-2 variants but also be continuously and rapidly assessed for potency against emerging variants. Lastly, the therapeutic production platform must be able to rapidly respond to emerging variants while covering previous dominant strain(s).

Multiple passive immunotherapeutic products have been developed and are being used to treat SARS-CoV-2 infections in clinical trials or in a clinical setting under Emergency Use Authorization (EUA). The primary target for these therapeutic products is the Spike

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protein (S). In the case of monoclonal antibodies (mAbs), their corresponding epitope targets are typically found in the receptor binding domain (RBD) and/or the N-terminal domain (NTD) which are highly immunogenic regions but also susceptible to rapid mutational drift. Convalescent plasma (CP) obtained from SARS-CoV-2 survivors is a polyclonal antibody (pAb) product that contains antibodies to multiple epitopes of the infecting variant's S protein and other viral proteins. Mutations in the S protein of Delta and other VOC/VOIs has been shown to severely reduce or eliminate the ability of many mAbs therapeutics and CP to neutralize these variants in vitro (4-6).

A fully human polyclonal antibody immunoglobulin (SAB-185) produced from the plasma of Transchromosomic bovines (Tc bovines) hyperimmunized with recombinant Wuhan-Hu-1 SARS-CoV-2 S protein is currently being evaluated in an adaptive phase 2/3 clinical trial to treat patients with COVID-19 infections (ClinicalTrials.gov Identifier: NCT04518410). The in vitro potency of SAB-185 in comparison to a mAb and CP against emerging SARS-CoV-2 variants was previously reported for a vesicular stomatitis virus (VSV) pseudovirus assay with strains incorporating single and double-point mutations in the S protein (7). The VSV pseudovirus platform avoided the difficulties of rapid acquisition, transnational transport, and testing of multiple "wild-type" VOC/VOI under challenging BSL-3 environments. In that study, SAB-185 retained potency and did not result in the development of escape mutants as was observed with the mAb and CP.

Following that SAB-185 specific study, the US Food and Drug Administration (FDA) reported on the ability of antibody-based therapeutics provided by many manufacturers to retain neutralization potency in recombinant HIV-based lentivirus pseudoviruses that express the full-length S protein of multiple VOC/VOIs. This BSL-2 assay utilizes a stably transduced 293T-ACE2 cell line expressing both ACE2 and TMPRSS2 (293T-ACE2.TMPRSS2$_S$) and determines the IC50 ratio of the antibody products to neutralize SARS-CoV-2 variants compared to an early pandemic wild-type progenitor strain (D614G) (8-9). These studies were performed as part of a package of standardized assays established by the U.S. government's COVID-19 Therapeutics response efforts. The assays utilized product samples provided by the manufacturers, and the results were blinded to the products tested and published (8-9). Multiple therapeutic products did not retain the ability to neutralize multiple variants including the Alpha, Beta and Gamma variants that were previously supplanting other earlier SARS-2 variants. That study did not report on recent VOC/VOI such as Delta, Kappa and Lambda among others because of their recently rapid ascent to be regionally and/or globally predominate variants.

Here, we report unblinded data showing that SAB-185 retained potency to neutralize recombinant S protein lentiviral pseudoviruses including the Delta, Kappa and Lambda variants in the pseudovirus assay.

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Discussion

Multiple SARS-CoV-2 VOC/VOIs have sequentially arisen with numerous mutations in the S protein that confer enhanced transmission and the ability to supplant previous variants to become the dominant global, regional, or national circulating variant. Producers of therapeutic and vaccine countermeasures that target the S protein have the challenge to quickly understand the impact of S protein mutations on their potency. This is complicated by the fact that wild-type SARS-CoV-2 variants are BSL-3 agents that result in significant expense, safety protocols, and a host of regulatory hurdles to safely study. Furthermore, the acquisition, identification, storage, transport, and testing of globally sourced wild-type BSL-3 or 4 agents is difficult even when the world is not experiencing a pandemic event that is causing mass infections and disrupting supply-lines (10).

A potential alternative to the use of wild-type SARS-CoV-2 variants (under BSL-3 conditions) is to assess/screen potency using a pseudovirus system (under BSL-2 conditions) that rapidly and accurately screens evolving variants. To explore the utility of this approach for SAB-185, a previous study investigated recombinant VSV pseudoviruses that expressed single D614G, N501Y, E484K, S477N, or double E484K-N501Y S protein mutations in comparison to mAbs and CP (7). In this current study, an alternative pseudovirus platform using recombinant lentivirus pseudoviruses expressing the multiple mutations in VOC/VOI S proteins (among others) was conducted using a stably transduced 293T-ACE2 cell line expressing both ACE2 and TMPRSS2 (293T-ACE2.TMPRSS2$_S$) (8). This pseudovirus system may have the attributes of safety, genetic stability, and scalability for screening assays. As shown in the results, SAB-185 retained in vitro potency against these variants including the Delta and Lambda variants that appear to be supplanting other variants in multiple countries.

Pseudovirus potency screening assays could be particularly relevant to the Tc bovine therapeutic production platform. As was done in this study, VOC/VOIs could be early and rapidly assessed using recombinant S protein pseudoviruses as they are identified. As previously reported, Tc bovines are continuously hyperimmunized with the target antigen(s) every 21-28 days and large volumes of hyperimmune plasma are obtained for rapid production of drug product (7). This means that the Tc bovines could be vaccinated with one or more variant S proteins to optimize its potency against emerging current or future SARS-CoV-2 variants should potency be compromised. This same framework could be used in future pandemics of coronavirus, influenza or other pathogens that are highly mutable.

In conclusion, SAB-185 maintained neutralization activity in a pseudovirus assay against multiple SARS-CoV-2 VOC/VOIs including the Delta, Kappa and Lambda variants that appear to be supplanting other variants.

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Results

Table 1: Potency determination of SAB-185 against SARS-CoV-2 variants and variant lineages

Variants	WT IC50 (ng/ml)	Mutation IC50 (ng/ml)	IC50 ratio (Mu:WT)
B.1.617.1 (Kappa)	48.09	120.9	2.6
B.1.617.1 (-T95I) +V382L+D1153Y) (Kappa lineage)	48.09	120.9	2.6
B.1.617.2 (Delta)	49.68	138.9	2.8
B.1.617.2 + K417N (Delta lineage)	77.20	272.8	3.6
C.37 (Lambda)	78.22	74.4	1.0
B	80.94	279.0	3.4
B.1.523	80.10	229.3	3.0
B.1.525 (Eta)	80.94	279.4	3.5

Note: Variant lineage strains with additional mutations from the WHO variant-defining sequences shown in Table 2 are designated by -/+.

All variants and variant lineages of SARS-CoV-2 tested against SAB-185 demonstrated retention of potency as defined as being less than an IC50 ratio (Mu:WT) of 5. Moderate or complete loss of potency is defined as being 5-50 or greater than 50 (Mu:WT), respectively, in the FDA developed pseudovirus assay.

Methods and Materials

Tc Bovines and SAB-185

The production of Tc-bovines and SAB-185 was previously described (7).

Plasmids and cell lines

Codon optimized full-length open reading frames of the S genes of SARS-COV2 variants were cloned into pCDNA3.1(+) by GenScript (Piscataway, NJ). 293T-ACE2.TMPRSS2s cells stably expressing human angiotensin converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) were established as previously described (8). The 293T and 293T-ACE2.TMPRSS2s cells were maintained at 37°C in Dulbecco's modified eagle medium (DMEM) supplemented with high glucose, L-Glutamine, minimal essential media (MEM) non-essential amino acids, penicillin/streptomycin and 10% fetal bovine serum (FBS).

SARS-CoV-2 pseudovirus production and neutralization assay

Pseudovirus production and the neutralization assay were previously described (8-9). The inverse of the dilutions or mAb concentrations causing a 50% and 80% reduction of

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RLU compared to control (ID50 and ID80) were reported as the neutralizing antibody titer. Titers were calculated using a nonlinear regression curve fit (GraphPad Prism software Inc., La Jolla, CA). The mean titer from at least two independent experiments each with intra-assay duplicates was reported as the final titer. See Table 2 below for variants evaluated.

Table 2: WHO SARS-CoV-2 Variants

VARIANT NAME PANGOLIN [WHO LABEL] (NEXTSTRAIN)	LOCATION OF EARLIEST DOCUMENTED SAMPLES	SPIKE SUBSTITUTIONS
B.1.617.1 [Kappa] (20A*/S:154K)	India	T95I, G142D, E154K, **L452R, E484Q**, D614G, P681R, Q1071H
B.1.617.2 [Delta] (21A/S:478K)	India	T19R, G142D, E156deletion, F15deletion, R158G, **L452R, T478K**, D614G, P681R, D950N
C.37 [Lambda] (21G)	Peru	G75V, T76I, R246deletion, S247deletion, Y248deletion, L249deletion, T250deletion, P251deletion, G252deletion, D253N, **L452Q, F490S**, D614G,T859N
B**	Italy	D80Y, Y144deletion, I210deletion, D215G, H245deletion, R246deletion, S247deletion, Y248H, L249M, W258L, R346K, **T478R, E484K**, H655Y, P681H, Q957H
B.1.523**	United Kingdom	H69deletion, V70deletion, T95I, Y144deletion, F157L, **N440K, E484K**, D614G, D950N, V1228L
B.1.525 [Eta] (20A***/S:484K)	Nigeria	Q52R, A67V, H69deletion, V70deletion, Y145deletion, **E484K**, D614G, Q677H, F888L

Note: Bold letters indicate substitutions in the receptor binding domain.

*CDC :20A; WHO: 21B.
**B and B.1.523 are not VOC/VOI.
*** CDC:20A; WHO:21D

Acknowledgments and Disclosure of Potential Conflicts of Interest

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necessarily representing the official policies or endorsements, either expressed or implied, of the US. Government. E.J.S, C.L.B, H.W, K.A.E, and T.C.L are employees of SAB Biotherapeutics, Inc.

Limitations

SAB-185 is in human clinical trials but efficacy against SARS-CoV-2 infections in humans has not been established.

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