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Supplementary appendix

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Supplementary Appendix

Safety and tolerability of a novel, polyclonal human anti-MERS coronavirus antibody produced from transchromosomic cattle: a phase 1 randomised, double-blind, single-dose-escalation study

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Full Inclusion / Exclusion Criteria

Inclusion Criteria

1. Age ≥18 years and ≤60 years
2. Body mass index (BMI) of 19-32 kg/m^2
3. Estimated glomerular filtration rate ≥70 mL/min at screening, calculated using the CKD-EPI formula
4. Subjects must agree to:
    - Not take any prescription or OTC medications with the exception of acetaminophen, ibuprofen, vitamins, seasonal allergy medications, and/or contraceptive medications for a period 7 days prior to study drug administration (i.e., Day 0)
5. One of the following in order to avoid pregnancy:
    - Females who are able to become pregnant (i.e., are not postmenopausal, have not undergone surgical sterilization, and are sexually active with men) must agree to use at least 2 effective forms of contraception from the date of the subject's signing of the informed consent form through 60 days after the last dose of study drug. At least one of the methods of contraception should be a barrier method.
    - Males who have not undergone surgical sterilization and are sexually active with women must agree to use condoms plus have a partner use at least one additional effective form of contraception from the date of the subject's signing of the informed consent form through 60 days after the last dose of study drug.

Exclusion Criteria

1. Any history of allergy, anaphylaxis, or severe reaction to beef products (including milk and gelatin)

2. Any history of allergy, anaphylaxis, or severe reaction to IGIV or human blood products
3. Any chronic medical problem that requires daily oral medications (except Tylenol, ibuprofen, oral contraceptives, vitamins, and seasonal allergy medications).
4. History of cardiovascular disease, cardiomyopathy, heart failure, or unexplained syncope
5. Subjects that have had confirmed MERS
6. Women who are breast-feeding
7. Positive urine or serum pregnancy test
8. Abnormal chemistry panel
    - defined as any clinically significant baseline Grade 1 or greater toxicity, or any Grade 3 or greater toxicity (regardless of clinical significance) by the toxicity table
        - evaluating only sodium (Na), potassium (K), serum bicarbonate (total CO2), blood urea nitrogen (BUN), creatinine, glucose, asp (ALT), aspartate aminotransferase (AST), total bilirubin, lactate dehydrogenase (LDH), and estimated glomerular filtration rate (GFR) by the CKD-EPI equation.
9. Abnormal complete blood count (CBC)
    - defined as any clinically significant baseline Grade 1 or greater toxicity, or any Grade 3 or greater toxicity (regardless of clinical significance) by the toxicity table
        - evaluating only the WBC (to include absolute neutrophil, lymphocyte, and eosinophil counts), hemoglobin, hematocrit, and platelets.
10. Abnormal urinalysis
    - defined as any clinically significant baseline Grade 1 or greater toxicity
        - evaluating only protein, and RBCs
11. Positive rheumatoid factor
12. IgA deficiency (defined as IgA < 7 mg/dL)
13. Participation in another research study with receipt of any investigational drug within 5 half-lives or 30 days, whichever is longer, prior to study drug administration (i.e., Day 0) and until completion of the study
14. Participation in any other research study for 30 days after study drug administration
15. Receipt of blood products within 2 months prior to study drug administration (i.e. Day 0)
16. Receipt of any vaccination within 30 days prior to study drug administration (i.e. Day 0)
17. Any acute or chronic condition that, in the opinion of the Investigator, would limit the subject's ability to complete and/or participate in this clinical study

Additional Methods

SAB-301 manufacturing

Plasma fractionation

The major contaminants in Tc bovine plasma are bovine serum albumin (BSA) and other bovine plasma proteins such as coagulation factors. Removal of these contaminating proteins to a clinically safe level is critical for SAB-301 manufacturing process. SAB has developed an effective plasma fractionation method using caprylic acid (CA) to retain a high yield recovery of IgG with contaminating protein levels being significantly reduced. Under conditions of controlled temperature (18-30°C) and pH (4.5-4.9), CA selectively precipitates BSA and other non-IgG bovine plasma proteins, and can substantially reduce concentrations of BSA and other bovine plasma proteins.

Chromatography steps for SAB-301 purification

There is about 80% of IgG produced in Tc bovine plasma that is fully human IgG and about 20% of IgG is chimeric IgG (cIgG) that contains human heavy chain and bovine light chain due to the fact that bovine light kappa has not been knocked out. Additionally, there are low levels of so-called trans-class switched bovine IgG (containing bovine constant domain and human variable domain) in Tc bovine plasma. To effectively separate fully human IgG from cIgG, a human IgG kappa chain specific affinity column, KappaSelect (from GE Healthcare), is used as a capture step. KappaSelect specifically binds fully human IgG with a minimum cross reactivity to bovine IgG Fc and cIgG. To further remove residual bovine IgG, a bovine IgG Fc specific affinity column, Capto HC15 (from GE Healthcare), is used as a negative affinity step to specifically clear bovine IgG. An anion exchange chromatography (Q Sepharose) step is incorporated as a polishing step to further reduce contaminants such as host DNA, endotoxin, IgG aggregates and leached affinity ligands. SAB-301 is formulated in 10mM L-glutamic acid monosodium salt, 262mM D-sorbitol, and 0·05mg/mL Tween 80, pH 5·5.

HPLC Size Exclusion Chromatography (SEC): The integrity and purity of SAB-301 were determined by HPLC SEC using a Dionex UltiMate 3000 Series HPLC system with Chromeleon software installed and a Thermo Scientific BioBasic SEC-300 column (7·8 mm x 300 mm, 5µm). The column was equilibrated with PBS at a flow rate of 1·0 mL/minute, loaded with 50 µg of SAB-301 and the effluent is monitored for absorbance at 280nm and 214nm using a UV detector. Peak areas (integrated using Chromeleon analytical software) were used to determine the percentage of IgG aggregates, dimers, and monomers.

Residual Bovine Serum Albumin (BSA): BSA concentration in SAB-301 was measured by using a commercial BSA Immunoenzymetric Assay Kit purchased from Cygnus Technologies (Catalog number F030) per manufacturer's instruction. The assay has a sensitivity of measuring 0·5ng/mL of BSA.

Residual Bovine Plasma Proteins (BPP): BPP concentration in SAB-301 was measured by using a commercial BPP Immunoenzymetric Assay Kit purchased from Cygnus Technologies (Catalog number F290) per manufacturer's instruction. The assay has a sensitivity of measuring 2·0ng/mL of BPP. Chimeric IgG: A specific ELISA was used to detect the level of residual chimeric IgG containing human IgG heavy chain and bovine IgG light chain. In the chimeric IgG ELISA, sheep anti-bovine IgG (H+L) antibody (catalog number A10-115A, from Bethyl Laboratories) is used as a capture antibody and donkey anti-human IgG (H+L) antibody conjugated HRP (catalog number 709-035-149, from Jackson Laboratory) was used a detection antibody. The assay has a sensitivity of measuring 7·81ng/mL of chimeric IgG.

Residual Affinity Ligand: KappaSelect ligand, which was used to capture human IgG, was measured by a commercial Capture Select Kappa Select Leakage ELISA Kit purchased from Life Technologies (catalog number 810083301) per manufacture's instruction. The assay has a sensitivity of measuring 1·56ng/mL of KappaSelect ligand.

Thrombin Generation Activity (TGA): TGA is an assay to assess thromboembolic risks associated with residual blood coagulation factor XI in human IGIV products. TGA assay kit manufactured by Technoclone (Vienna, Austria) and Factor XI Deficient Plasma (Catalog number FIX-ID, Haematologic Technologies) were used to measure thrombin generation in SAB-301 per manufacture's instruction. The Technoclone TGA assay kit contains Technothrombin TGA SUB (Catalog number 5006235), Technothrombin TGA CAL Set (Catalog number 5006345), Technothrombin TGA CONT HIGH (Catalog number 5006320) and Technothrombin TGA RC HIGH (Catalog number 5006214). The formulation buffer of SAB-301 and human IGIV product were used as a negative control and reference, respectively, in the TGA assay.

Anti-Complementary Activity (ACA): Complement activation is typically triggered by specific antibody-antigen complex. However, IgG aggregates or distorted IgG can also bind to complement and thus can induce non-specific complementary activity. The activation of complement by non-specific IgGs is called anti-complementary activity (ACA). An ELISA method was used to measure ACA activity in SAB-301. In this assay, complement component C1q (Catalog number C1740, from Sigma) is used as a capture reagent to bind IgG aggregates or distorted IgG, and Protein A peroxidase (Catalog# P8651, from Sigma) was used as a detection reagent. BSA and human IGIV product were used as a negative control and reference, respectively, in the ACA assay.

Hemagglutination Assay

Hemmaglutination assay is a qualitative method to measure the agglutination properties in SAB-301. Specifically, this assay detects the presence of anti-A, anti-B, or anti-RhD antibodies in SAB-301. Hemmaglutination assay was performed by using human red blood cells and the Ortho

Confidence System purchased from Ortho Clinical Diagnostics (Catalog number 6902096) per manufacture's instruction.

Human IgG subclass proportion by ELISA

For detection of human IgG1, IgG2, IgG3, or IgG4 in clinical lot SAB-301, human IgG subclass ELISAs were performed in Maxisorp Immuno 96-well ELISA plates in accordance with the anti-human IgG subclass antibody manufacturer's instructions. Briefly, mouse anti-human IgG1 Fc, mouse anti-human IgG2 Fc, mouse anti-human IgG3-hinge, or mouse anti-human IgG4 Fc (Hybridoma Reagent Laboratory) was used as a capture antibody, respectively. Mouse anti human IgG HRP (Southern Biotech) for IgG1 and IgG2 detection, mouse anti-human IgG HRP (Invitrogen) for IgG3 detection, and mouse anti-human IgG HRP (Jackson ImmunoResearch) for IgG4 detection were used. Human IgG subclass standard serum (Bethyl) was used as the reference. Human IGIV (Grifols) was used as a positive control.

Additional methods for pharmacokinetics, pharmacodynamics, and immunogenicity

Serum SAB-301 levels

Serum SAB-301 levels were measured using qualified enzyme-linked immunosorbent assays (ELISA) with a lower limit of quantification (LLOQ) of 15·625 µg/mL in human serum. In this assay, the recombinant MERS-CoV spike protein, the antigen used for vaccinations of Tc bovines for generating anti-MERS-CoV therapeutic antibody SAB-301, was used as the coating antigen. SAB-301, spiked in naïve human serum, with known concentrations were used as the standard reference and positive control. Naive human serum was used as negative control. Briefly, Maxisorp Nunc ELISA plates were coated with 2 µg/ml recombinant MERS-CoV spike protein overnight at 4 °C. The following day, the coated plates were washed with PBST (PBS with 0·05% Tween 20) and blocked with 10% goat serum in PBS at room temperature (RT) for 1 hour. After washing with PBST, the standard, controls, and participant serum samples were added to the plates with serial dilution in PBST and incubated for 1 hour at RT. Following washing with PBST, goat anti-human IgG-Fc conjugated with horseradish peroxidase (HRP) (from Bethyl Labs) was added to plates and incubated for 1 hour at RT. The bound SAB-301 were detected colorimetrically by using a TMB substrate kit. The absorbance was read in a microplate reader at 450nm. A four-parameter standard curve was generated using seven serial dilution values and SAB-301 concentrations in serum samples were calculated by interpolation on the curve by Gen5 software. The accuracy of the ELISA is verified by the concentration value produced by the positive control and lack of value in the negative control.

MERS-CoV microneutralization test

MERS-CoV neutralizing antibody titers were determined by a micro-neutralization assay. Briefly, approximately 200 TCID-50 units of Jordan-N3/2012 MERS-CoV virus were incubated for 60 minutes with two-fold serial dilutions of serum samples and used to infect Vero cells

plated a day before in a 96-well microtiter plate ($2x10^4$ cells/well). After 72 hours of incubation, cells were fixed and MERS-CoV specific antigen was measured using anti-S1 rabbit polyclonal antibody and a peroxidase labeled anti-rabbit Ig secondary antibody using a standard ELISA format. Highest serum dilution that resulted in ≥ 50% reduction in absorbance compared to control was taken as 50% neutralization titer.

Anti-drug antibodies (ADAs) for SAB-301

The presence of anti-drug antibodies (ADAs) was determined using a qualified bridging ELISA by formation of a complex with SAB-301 and biotin-labeled SAB-301. Mouse anti-Tc bovine-derived human IgG sera was used as positive controls. Naïve human serum was used as a negative control. Briefly, Maxisorp Nunc ELISA plates were coated with 10 µg/mL SAB-301 overnight at 4 °C. The following day, the diluted controls and serum samples were incubated with biotin-labeled SAB-301 on a rocker for 1·5 hour at RT. After the incubation with biotin-labeled SAB-301, the controls and samples were loaded onto SAB-301 pre-coated plates with serial dilution in PBS and incubated for 1·5 hour at RT on a rocker. Following washing with PBST, the streptavidin HRP conjugate (Thermo Scientific Pierce) was added to the assay plates containing biotinylated drug–ADA complexes and incubated for 1 hour at RT. After final washing, the ADAs were detected colorimetrically by using a TMB substrate kit. The absorbance was read in a microplate reader at 450nm. The ADA titer value (units/ml) is defined as the highest dilution of serum sample where the OD450 reading was 3-fold higher than blank.

Anti-bovine kappa ELISA

The presence of anti-bovine kappa human IgG and IgM antibodies was determined using indirect ELISA. In this assay, bovine IgG Fab fragment (Rockland Immunochemicals), which has previously been verified to contain bovine kappa chain, was used as a coating agent. Sheep anti-bovine Fab antibody (Immunology Consultants Laboratory) was used as a positive control. Naïve human serum was used as a negative control. Briefly, Maxisorp Nunc ELISA plates were coated with 10 µg/mL bovine IgG Fab fragment overnight at 4 °C. The following day, the coated plates were washed with PBST and blocked with 1% Casein solution at RT for 0·5 hour. After washing with PBST, the controls, and participant serum samples were added to the plates with serial dilution in 1% Casein solution and incubated for 1·5 hour at RT. After washing, the primary detection antibodies [biotinylated rabbit anti-sheep IgG (Jackson ImmunoResearch) to bind for the positive control and biotinylated goat anti-human IgG + IgM (Jackson ImmunoResearch) to bind for the negative control and the testing human serum samples] were added to the appropriate wells and incubated at room temperature for 1·5 hours. The Streptavidin-HRP (Thermo Scientific Pierce) was then added to the wells and incubated at room temperature for 1 hour. The anti-bovine kappa antibodies were detected colorimetrically by using a TMB substrate kit. The absorbance was read in a microplate reader at 450 nm. The anti-bovine kappa antibody titer value (units/ml) was defined as the highest dilution of serum sample where the OD450 reading was 3·5-fold higher than blank.

Anti-camelid antibody fragment ELISA

The presence of anti-camelid chain antibody fragment human IgG and IgM antibodies was determined using indirect ELISA. Goat IgG anti-camelid chain antibody fragment (Life Technologies) was used as a positive control. Naïve human serum was used as a negative control. Briefly, Maxisorp Nunc ELISA plates were coated with 2 µg/mL camelid chain antibody fragment (Thermo Fisher Scientific) overnight at 4 °C. The following day, the coated plates were washed with PBST and blocked with 1% Casein solution at RT for 0·5 hour. After washing with PBST, the controls, and participant serum samples were added to the plates with serial dilution in 1% Casein solution and incubated for 1·5 hour at RT. After washing, the primary detection antibodies [biotinylated rabbit anti-goat IgG (Jackson ImmunoResearch) to bind for the positive control, and biotinylated goat anti-human IgG + IgM (Jackson ImmunoResearch) to bind for the negative control and the testing human serum samples] were added to the appropriate wells and incubated at room temperature for 1·5 hours. The Streptavidin-HRP (Thermo Scientific Pierce) was then added to the wells and incubated at RT for 1 hour. The anti-bovine kappa antibodies were detected colorimetrically by using a TMB substrate kit. The absorbance was read in a microplate reader at 450 nm. The anti-camelid chain antibody fragment antibody titer value (units/ml) is defined as the highest dilution of serum sample where the OD450 reading was 3-fold higher than blank.

Additional Results

Supplemental Table 1: Geometric Mean (CV%) PK Parameters from Two-Compartmental Model of Single Ascending and Multiple Dose Cohorts

CL_1 (mL/day/kg)	CL_2 (mL/day/kg)	V_1 (mL/kg)	V_2 (mL/kg)	α (days^{-1})	β (days^{-1})	α $T_{1/2}$ (days)	β $T_{1/2}$ (days)
1·66 (11·40)	11·52 (21·30)	37·29 (2·76)	27·71 (16·44)	0·76 (25·28)	0·02 (17·16)	0·91 (25·26)	28·43 (17·14)

Key: CV% = coefficient of variation, α = distribution phase rate constant, β = terminal elimination rate constant, CL1 = clearance from the central compartment, CL2 = clearance from the peripheral compartment, t1/2α = initial distributional elimination half-life, t1/2β = terminal elimination half-life, V1 = volume of distribution of the central compartment, V2 = volume of distribution of the peripheral compartment

Supplemental Table 2: Major impurity levels in SAB-301 final drug product (QC release test results)

Test	Results	Test sensitivity
HPLC size exclusion chromatography	99·28% IgG monomer, 0·72% IgG aggregates	NA
Residual Bovine Serum Albumin	Below detection limit	0·5 ng/mL
Residual Bovine Plasma Proteins	4·91ppm	2·0 ng/mL
Chimeric IgG	139·12µg/mL (0·19% total IgG)	7·81 ng/mL
Residual Affinity Ligand	Below detection limit	1·56 ng/mL
Anti-Complementary Activity (ACA)	Not detectable	NA
Hemagglutination Assay	Negative	NA

Supplemental Table 3. Human IGIV and a sample from the clinical lot SAB-301 were analyzed for the IgG subclass proportions by ELISA. Percentage of each subclass is presented as compared to the positive control.

Antibody	IgG1 (%)	IgG2 (%)	IgG3 (%)	IgG4 (%)
Human IGIV*	61·09	35·39	2·75	0·77
clinical lot SAB-301	88·63	11·35	0·00	0·02

*Human-derived IGIV served as the positive control and was run on the same IgG subclass ELISA plates with the other antibodies.

Supplemental Table 4. MERS-CoV microneutralization titers, by treatment arm, over time. Data presented in dilutions (1:X).

Cohort	Baseline	1 Hour	6 Hour	Day 1	Day 3	Day 7	Day 21	Day 42	Day 90
1 mg/kg	0	15	15	15	10	0	0	0	0
2.5 mg/kg	0	60	60	60	40	20	20	5	0
5 mg/kg	0	160	187	130	80	50	30	22.5	2.5
10 mg/kg	0	320	360	360	220	120	90	50	12.5
20 mg/kg	0	920	1200	600	520	400	280	155	27.5
50 mg/kg	0	2400	2160	1360	1440	1240	600	240	75

Supplemental Figure 1. MERS-CoV microneutralization titers over time, by treatment arm.