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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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See Comment page 361

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Summary

Background Middle East respiratory syndrome (MERS) is a severe respiratory illness with an overall mortality of 35%. There is no licensed or proven treatment. Passive immunotherapy approaches are being developed to prevent and treat several human medical conditions where alternative therapeutic options are absent. We report the safety of a fully human polyclonal IgG antibody (SAB-301) produced from the hyperimmune plasma of transchromosomic cattle immunised with a MERS coronavirus vaccine.

Methods We did a phase 1 double-blind, placebo-controlled, single-dose escalation trial at the National Institutes of Health Clinical Center. We recruited healthy participants aged 18–60 years who had normal laboratory parameters at enrolment, a body-mass index of 19–32 kg/m², and a creatinine clearance of 70 mL/min or more, and who did not have any chronic medical problems that required daily oral medications, a positive rheumatoid factor (≥15 IU/mL), IgA deficiency (<7 mg/dL), or history of allergy to intravenous immunoglobulin or human blood products. Participants were randomly assigned by a computer-generated table, made by a masked pharmacist, to one of six cohorts (containing between three and ten participants each). Cohorts 1 and 2 had three participants, randomly assigned 2:1 to receive active drug SAB-301 versus normal saline placebo; cohorts 3 and 4 had six participants randomised 2:1; and cohorts 5 and 6 had ten participants, randomised 4:1. Participants received 1 mg/kg, 2·5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, or 50 mg/kg of SAB-301, or equivalent volume placebo (saline control), on day 0, and were followed up by clinical, laboratory, and pharmacokinetic assessments on days 1, 3, 7, 21, 42, and 90. The primary outcome was safety, and immunogenicity was a secondary outcome. We analysed the intention-to-treat population. This trial is registered with ClinicalTrials.gov, number NCT02788188.

Findings Between June 2, 2016, and Jan 4, 2017, we screened 43 participants, of whom 38 were eligible and randomly assigned to receive SAB-301 (n=28) or placebo (n=10). 97 adverse events were reported: 64 adverse events occurred in 23 (82%) of 28 participants receiving SAB-301 (mean 2·3 adverse events per participant). 33 adverse events occurred in all ten participants receiving placebo (mean 3·3 adverse events per participant). The most common adverse events were headache (n=6 [21%] in participants who received SAB-301 and n=2 [20%] in those receiving placebo), albuminuria (n=5 [18%] *vs* n=2 [20%]), myalgia (n=3 [11%] *vs* n=1 [10%]), increased creatine kinase (n=3 [11%] *vs* 1 [10%]), and common cold (n=3 [11%] *vs* n=2 [20%]). There was one serious adverse event (hospital admission for suicide attempt) in one participant who received 50 mg/kg of SAB-301. The area under the concentration–time curve (AUC) in the 50 mg/kg dose (27 498 μg×days per mL) is comparable to the AUC that was associated with efficacy in a preclinical model.

Interpretation Single infusions of SAB-301 up to 50 mg/kg appear to be safe and well tolerated in healthy participants. Human immunoglobulin derived from transchromosomic cattle could offer a new platform technology to produce fully human polyclonal IgG antibodies for other medical conditions.

Funding National Institute of Allergy and Infectious Diseases, National Institutes of Health, and Biomedical Advanced Research and Development Authority.

Introduction

Middle East respiratory syndrome (MERS) is a severe respiratory illness caused by a novel coronavirus (CoV). The spectrum of clinical illness ranges from asymptomatic illness to a severe acute respiratory disease requiring intensive care and mechanical ventilation. MERS has an overall mortality of 35% and there is no licensed or proven treatment.[1] The use of immune anti-MERS coronavirus (MERS-CoV) plasma has been suggested as one potential therapeutic approach.[2] However, it is difficult to collect sufficient anti-MERS-CoV plasma from infected patients to implement this strategy.[3] The use of passive immune therapy, either in the form of direct plasma infusion or intravenous immunoglobulin, is often invoked both for emerging infectious diseases, such as Ebola virus disease,[4] as well as more common diseases

Research in context

Evidence before this study

We searched PubMed on Oct 4, 2017, for studies using these search terms in the title or abstract: ("Middle East respiratory syndrome" OR "MERS") AND ("antivirals" OR "treatment"). We restricted the article type to clinical trials and the species to human, and there were no language restrictions. We found no articles. After removing the search restriction of title or abstract for these terms, removing the species restrictions to human beings, and excluding studies that did not assess MERS therapeutics (rather they mentioned it in the background or discussion), we found no previous publications of MERS therapeutics in human beings. Additionally, we searched for studies using the search terms ("intravenous immunoglobulin" OR "IGIV"), AND ("bovine" or "cow"), and restricted the article type to clinical trials and the species to human. After excluding studies that did not assess bovine intravenous immunoglobulin, we identified no previous publications of MERS therapeutics in humans.

Added value of this study

To our knowledge, this is the first study to show the safety, tolerability, and pharmacokinetics of intravenous immunoglobulin derived from transchromosomic cattle, and the first study to describe the safety of a putative therapeutic specifically for MERS.

Implications of all the available evidence

The evidence from this study shows that single infusions of SAB-301 up to 50 mg/kg appear to be safe and well tolerated in healthy participants, and should be considered for further investigation in the treatment of MERS.

with high morbidity (despite the availability of antiviral therapeutics), such as severe influenza.[5] However, the use of immune plasma in all of these conditions has similar constraints. Consistent production of large quantities of anti-pathogen-neutralising human plasma or immunoglobulin requires collection of plasma from convalescent or vaccinated human volunteers. The limited plasma supply restricts wide implementation of these therapeutics. One novel alternative method of manufacturing neutralising intravenous antibodies of consistently high affinity and avidity is to use the hyperimmune plasma of transchromosomic cattle, which produce highly potent and antigen-specific, fully human polyclonal IgG de novo, and which mount a robust antibody immune response after vaccination.

To develop the transchromosomic cattle, a human artificial chromosome was constructed that comprised the entire human immunoglobulin gene repertoire (human immunoglobulin heavy chain [*IGH*] and human κ light chain [*IGK*]), which resides on two different chromosomes in human beings, specifically the *IGH* locus from chromosome 14 and the *IGK* locus from chromosome 2.[6] The immunoglobulin gene repertoires from the human artificial chromosome provide the large diversity of human polyclonal antibodies. To avoid possible human–bovine interspecies incompatibility between the human immunoglobulin-μ-chain protein (hIgM) and bovine transmembrane α and β immunoglobulins (bIgα and bIgβ) in the pre-B-cell-receptor complex, the hIgM constant domain was partly replaced with the counterpart of bovine IgM (bIgM). Furthermore, the DNA regulatory elements Iγ1 and Sγ1 were bovinised on the human artificial chromosome to facilitate DNA–protein interactions in the bovine B cell. This human artificial chromosome is designated as isKcHACΔ, and is transferred to bovine fibroblasts that are homozygous triple knockouts of the two bovine immunoglobulin-μ heavy-chain loci (*bIGHM* and *bIGHML1*) and the bovine λ light-chain locus (*bIGL*). The triple-knockout bovine fibroblasts carrying the human artificial chromosome were used as nuclear donors to clone transchromosomic cattle.

The MERS-CoV spike protein attaches to the host-cell receptor CD26 (also known as dipeptidyl peptidase 4 or DPP4), and mediates subsequent cell fusion.[7] Vaccination of mice with the plasmid vaccines expressing the spike glycoprotein showed the generation of neutralising antibodies through blocking both the receptor binding and non-receptor-binding domains.[8] Seroconversion to the spike protein (measured by IgG) was associated with resolution of clinical illness in patients with MERS-CoV infection, and the levels of anti-spike IgG were inversely correlated with lower respiratory tract viral loads.[9] For these reasons, purified Al-Hasa strain MERS-CoV spike protein nanoparticles[10] (a clade B strain, manufactured by Novavax Inc, Gaithersburg, MD, USA) were chosen as the immunogen to generate an anti-MERS-CoV intravenous immunoglobulin in this transchromosomic bovine system (thereafter known as SAB-301).[11]

The purpose of this phase 1 study was to evaluate the safety, tolerability, and pharmacokinetics of SAB-301 after a single infusion of SAB-301 in healthy adults. This trial represents the first-in-human clinical study using a fully human antibody derived from transchromosomic cattle.

Methods

Study design and participants

We did this double-blind, placebo-controlled, single-dose escalation, phase 1 randomised controlled trial at the National Institutes of Health (NIH) Clinical Center (Bethesda, MD). The study was conducted in accordance with the applicable regulatory and International Conference on Harmonization–Good Clinical Practice requirements. The study protocol was approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board (Protocol 16-I-0119).

Eligible participants were healthy adult men or women aged 18–60 years, with a body-mass index of 19–32 kg/m² and a creatinine clearance of 70 mL/min or more (calculated using the Chronic Kidney Disease Epidemiology Collaboration formula). All participants were required to have normal laboratory parameters at enrolment, and could not have any chronic medical problem that required daily oral medications, a positive rheumatoid factor (defined as a rheumatoid factor ≥15 IU/mL), or IgA deficiency (defined as IgA <7 mg/dL), nor any history of an allergy to intravenous immunoglobulin or human blood products. All participants were required to use two effective forms of contraception, at least one of which had to be a barrier method. A full list of exclusion criteria is included in the appendix.

See Online for appendix

Participants provided written informed consent before screening.

Randomisation and masking

An unmasked pharmacist at the NIH Clinical Center Pharmacy generated a blinded randomisation scheme before study initiation. After screening, study participants were enrolled sequentially in one of six cohorts. On the day of study drug dosing, the unmasked pharmacist determined treatment allocation by referring to the next position on the randomisation scheme. Cohorts 1 and 2 had three participants, randomly assigned 2:1 to receive active drug SAB-301 versus normal saline placebo; cohorts 3 and 4 had six participants randomly assigned 2:1; and cohorts 5 and 6 had ten participants, randomly assigned 4:1. Study participants and study team members remained masked throughout the entire study. The study treatment was provided in an infusion bag, the contents of which were obscured by an opaque covering over the bag and tubing.

Procedures

SAB-301 was manufactured by SAB Biotherapeutics Inc (Sioux Falls, SD, USA). Two transchromosomic bovines were hyperimmunised with purified Al-Hasa strain MERS-CoV S protein nanoparticles (Novavax Inc; 2–5 mg/kg) formulated with SAB's proprietary adjuvant formulation (SAB-adj-1). The transchromosomic cattle were vaccinated five times at 3–4-week intervals. Hyperimmune plasma (up to 2·1% of bodyweight) was collected from each transchromosomic cow on days 8, 11, and 14 days after the third, fourth, and fifth vaccination. Plasma was stored at –20°C until use. Additional SAB-301 manufacturing methods are detailed in the appendix.

SAB-301 was supplied in vials containing 672 mg per 9 mL. The lot of SAB-301 we used had an ELISA binding titre of 109 236 U/mg. We assayed SAB-301 for its ability to neutralise MERS-CoV in vitro using a fluorescence-reduction neutralising assay in Vero E6 cells. The concentration of SAB-301 at which 50% inhibition of relative fluorescence intensity was observed was 2·69 µg/mL.

The doses used in the six cohorts were: 1 mg/kg (cohort 1; prepared at a concentration of 1 mg/mL in normal saline), 2·5 mg/kg (cohort 2; prepared at 1 mg/mL), 5 mg/kg (cohort 3; prepared at 4 mg/mL), 10 mg/kg (cohort 4; prepared at 4 mg/mL), 20 mg/kg (cohort 5; prepared at 20 mg/mL), and 50 mg/kg (cohort 6; prepared at 20 mg/mL). Participants randomly assigned to placebo received a normal saline control at a volume equivalent to receiving active drug above. The infusions were started at a rate of 0·5 mL/kg per h, escalating by 0·5 mL/kg per h increments every 15 min to a maximum rate of 1–3 mL/kg per h, depending on cohort. In the highest dose cohort at the maximum infusion rate of 40 mg/kg per h, this infusion was less than a tenth of the maximum rate of standard intravenous immunoglobulin as per administration guidelines at the NIH Clinical Center (maximum 480 mg/kg per h).

The starting dose of 1 mg/kg was 370-times lower than the maximum preclinical animal toxicity study dose of 370 mg/kg. The target dose of 50 mg/kg exceeds the effective doses in preclinical models,[11] and is 7·4-times lower than the maximum nonclinical dose (SAB Biotherapeutics Inc, unpublished).

Participants were screened up to 4 weeks before dosing. Vital signs were obtained before the start of infusion, and then approximately 15 and 30 min after the start of the infusion, every 30 min thereafter until the end of the infusion, and then every 1 h for 6 h after infusion. Participants were seen on days 1, 3, 7, 21, 42, and 90. Blood samples for the pharmacokinetic analysis were obtained at baseline, 1 h after the end of infusion, 6 h after the end of infusion, and on days 1, 3, 7, 21, 42, and 90. At each study visit, participants were questioned about new symptoms, their medical condition, and new medications. All new symptoms or abnormal laboratory results were captured as adverse events.

We tested serum for SAB-301 pharmacokinetics using an ELISA assay and functional inhibition (pharmacodynamics) using a MERS micro-neutralisation assay. We assessed immunogenicity by testing for anti-IgG antibodies (using rheumatoid factor), SAB-301 antidrug antibodies, anti-bovine κ antibodies, and anti-camelid antibodies (an antibody used in the manufacturing process; appendix).

Outcomes

The primary outcome was safety—ie, the type and frequency of adverse events experienced by participants receiving SAB-301 compared with placebo. Secondary outcomes were the pharmacokinetic profile, MERS virus neutralisation assay over time (pharmacodynamics), and immunogenicity.

Statistical analysis

We determined the study sample size to detect an adverse event with a true rate of 20% and a probability of 0·83 in the cohorts with larger doses (cohort 5 and 6), whereas

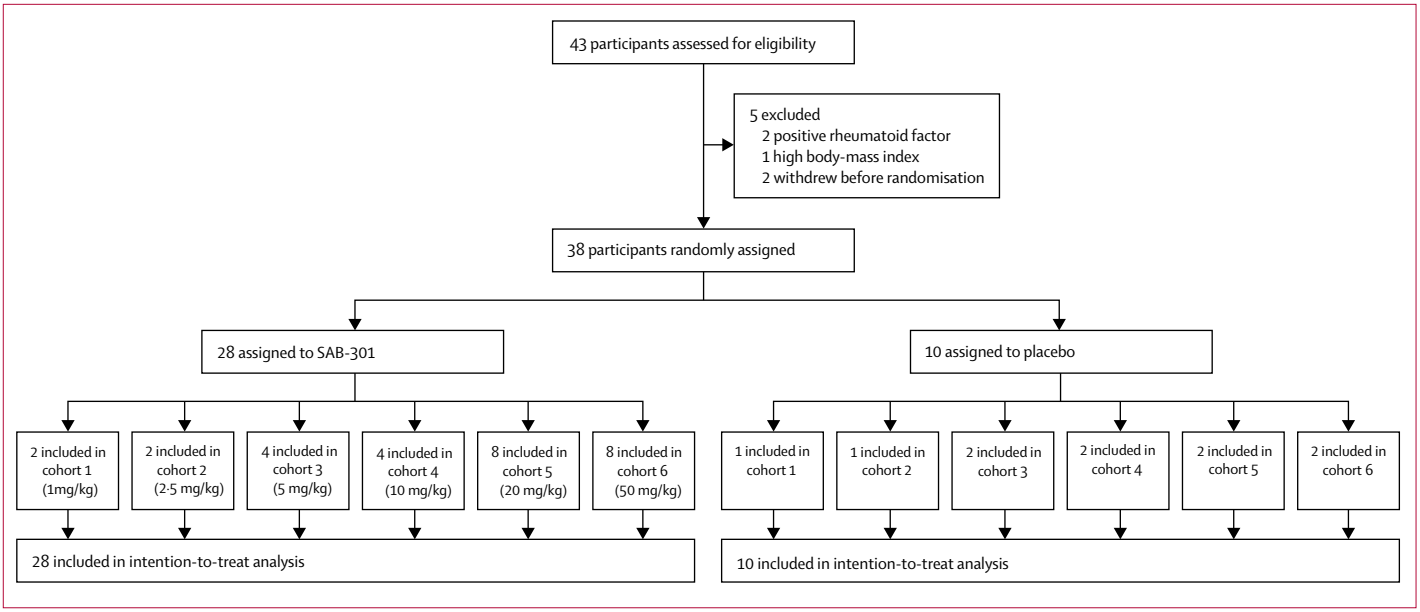

Figure 1: **Trial profile**

rarer events with a true rate of 1% could be detected with a probability of 0·06.

We did the analysis in the intention-to-treat population. We did not impute missing data.

We analysed pharmacokinetic data using Phoenix WinNonlin software (version 7.0; Cetara, St Louis, MO) for both non-compartmental analysis and compartmental modelling. For the drug in the serum, key non-compartmental analysis pharmacokinetic parameters of interest included the maximum observed concentration (C_{max}); apparent elimination rate constant (λ_z, determined by calculating the absolute value of the slope of the log–linear regression plasma concentration–time plot, with a minimum of three concentrations along the terminal phase); the elimination half-life ($T_{½}$, calculated as $0·693/\lambda_z$); the area under the concentration–time curve (AUC), from 0 h to the last pharmacokinetic sample post-dose (AUC_{last}) and to infinity ($AUC_{0-\infty}$), calculated with the linear-up, log-down trapezoidal rule; clearance calculated as dose/$AUC_{0-\infty}$; and volume of distribution, calculated as dose/($AUC_{last}\times\lambda_z$). We calculated geometric mean ratios and 90% CIs to compare pharmacokinetic parameters between cohorts for assessment of dose linearity and proportionality.

We tested structural models of one, two, and three compartments. To determine goodness of fit of the compartmental models, we visually inspected plots of observed and predicted concentrations versus time, and weighted residuals versus predicted concentrations, pharmacokinetic parameter coefficient of variation, and Akaike information criteria. The two-compartment model that we selected was parameterised in terms of central compartment clearance, central compartment volume of distribution, inter-compartmental clearance, peripheral volume of distribution, and secondary parameters of distribution elimination rate and distributional half-life.

We plotted the reciprocal of the highest 50% neutralisation titres over time and used them to calculate the AUC_{last}. We assessed the correlation of neutralisation titre AUC_{last} with SAB-301 AUC_{last} using linear regression.

This trial is registered with ClinicalTrials.gov, number NCT02788188.

Role of the funding source

Employees of the sponsor of the study were involved with study design, data collection, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between June 2, 2016, and Jan 4, 2017, we screened 43 participants for this study, of whom 38 were sequentially randomly assigned to six cohorts (figure 1). The study ended after being fully enrolled. After allocation to study groups, all participants completed the planned follow-up. Baseline characteristics of the randomly assigned participants are shown in table 1.

97 adverse events were reported: 64 occurred in 23 (82%) of 28 participants receiving SAB-301 (mean 2·3 adverse events per participant), and 33 adverse events occurred in all ten participants receiving placebo (mean 3·3 adverse events per participant; table 2). There was one grade 3 adverse event of depression, and one grade 4 adverse event of suicide attempt, both occurring in the same participant. There was one serious adverse

event of hospital admission for a suicide attempt by the same participant who had received 50 mg/kg of SAB-301, which, as the other two adverse events in this participant, was judged to be unrelated to study intervention. This participant had a history of depression for several years that was not disclosed at the time of screening, and the participant was not receiving medical care or treatment at the time of enrolment.

The most common adverse events were headache, albuminuria, increased creatine kinase, common cold, myalgia, and low serum bicarbonate. Most adverse events were noted in similar proportions in those receiving SAB-301 and placebo (table 2). There were two hypotensive events that occurred during study drug administration, both in cohort 6 (50 mg/kg of SAB-301): one in a participant who received SAB-301 and the other in one who received placebo. Regarding the hypotensive event in the participant receiving SAB-301, 30 min after the start of the infusion, the participant complained of feeling warm and slightly lethargic. Blood pressure at the time was 96/58 (about 20 mm Hg lower than pre-infusion), and there was no increase in heart rate. The infusion was stopped for 15 min with no other intervention, and the hypotension resolved. The infusion was restarted with no further episodes of hypotension. Low serum bicarbonate was seen only in participants receiving SAB-301 (three [11%] of 28), and were only noted in cohorts 5 (20 mg/kg of SAB-301) and 6 (50 mg/kg of SAB-301). Two vasovagal reactions occurred in participants assigned to receive SAB-301,

	SAB-301 dose						Total SAB-301 (n=28)	Placebo (n=10)
	1 mg/kg (n=2)	2·5 mg/kg (n=2)	5 mg/kg (n=4)	10 mg/kg (n=4)	20 mg/kg (n=8)	50 mg/kg (n=8)		
Age (years)								
Mean (SD)	48 (12·7)	32·5 (10·6)	40·3 (9·3)	34·3 (10·8)	27 (4·5)	29·6 (10·5)	32·5 (10·2)	35·7 (11·3)
Median (range)	48 (39–57)	32·5 (25–40)	38 (32–53)	35 (21–46)	27 (21–33)	26 (21–52)	31·5 (21–57)	36 (20–53)
Race								
Asian	0	0	0	0	0	1 (13%)	1 (4%)	2 (20%)
Black	0	0	2 (50%)	0	2 (25%)	0	4 (14%)	2 (20%)
Not specified	0	0	0	0	0	0	0	1 (10%)
White	2 (100%)	2 (100%)	2 (50%)	4 (100%)	6 (75%)	7 (88%)	23 (82%)	5 (50%)
Sex								
Female	0	2 (100%)	3 (75%)	3 (75%)	4 (50%)	3 (38%)	15 (54%)	3 (30%)
Male	2 (100%)	0	1 (25%)	1 (25%)	4 (50%)	5 (63%)	13 (46%)	7 (70%)
BMI (kg/m^2)	23·5 (0·2)	27·5 (3·2)	25·6 (4·4)	25·5 (2·5)	25·1 (3·5)	25·1 (4·0)	25·3 (3·3)	26·1 (2·6)

Data are number of patients (%), unless otherwise specified. BMI=body-mass index.

Table 1: **Baseline characteristics of the intention-to-treat population**

	SAB-301 dose						Total SAB-301 (n=28)	Placebo (n=10)
	1 mg/kg (n=2)	2·5 mg/kg (n=2)	5 mg/kg (n=4)	10 mg/kg (n=4)	20 mg/kg (n=8)	50 mg/kg (n=8)		
Any adverse event	1 (50%)	2 (100%)	2 (50%)	4 (100%)	6 (75%)	8 (100%)	23 (82%)	10 (100%)
Any adverse event of grade 2 or more	1 (50%)	0	1 (25%)	2 (50%)	2 (25%)	2 (25%)	8 (29%)	6 (60%)
All adverse events (grade 1–4)								
Headache	0	1 (50%)	1 (25%)	2 (50%)	1 (13%)	1 (13%)	6 (21%)	2 (20%)
Albuminuria	0	0	1 (25%)	0	2 (25%)	2 (25%)	5 (18%)	2 (20%)
Increased creatine kinase	0	0	0	1 (25%)	1 (13%)	1 (13%)	3 (11%)	1 (10%)
Common cold	0	0	1 (25%)	0	1 (13%)	1 (13%)	3 (11%)	2 (20%)
Myalgia	0	0	0	1 (25%)	2 (25%)	0	3 (11%)	1 (10%)
Low blood bicarbonate	0	0	0	0	1 (13%)	2 (25%)	3 (11%)	0
Sinus congestion	0	0	0	0	2 (25%)	0	2 (7%)	1 (10%)
Fatigue	0	0	0	1 (25%)	1 (13%)	0	2 (7%)	0
Loose stools	0	0	1 (25%)	1 (25%)	0	0	2 (7%)	0
Sore throat	0	0	0	0	0	2 (25)	2 (7%)	0
Vasovagal reaction	0	0	0	0	1 (13%)	1 (13%)	2 (7%)	0

(Table 2 continues on next page)

	SAB-301 dose						Total SAB-301 (n=28)	Placebo (n = 10)
	1 mg/kg (n=2)	2·5 mg/kg (n=2)	5 mg/kg (n=4)	10 mg/kg (n=4)	20 mg/kg (n=8)	50 mg/kg (n=8)		
(Continued from previous page)								
Increased aspartate aminotransferase	0	0	0	0	1 (13%)	0	1 (4%)	0
Blood glucose increased	0	0	0	0	0	1 (13%)	1 (4%)	2 (20%)
Rhinorrhoea	0	0	0	0	1 (13%)	0	1 (4%)	1 (10%)
Arthralgia	0	1 (50%)	0	0	0	0	1 (4%)	1 (10%)
Hypotension	0	0	0	0	0	1 (13%)	1 (4%)	1 (10%)
Indigestion	0	0	1 (25%)	0	0	0	1 (4%)	1 (10%)
Urinary tract infection	0	0	0	0	0	1 (13%)	1 (4%)	1 (10%)
Abdominal cramps	0	0	0	0	0	1 (13%)	1 (4%)	0
Increased alanine aminotransferase	0	0	0	0	0	1 (13%)	1 (4%)	0
Anxiety depression	0	0	0	0	0	1 (13%)	1 (4%)	0
Candidal intertrigo	1 (50%)	0	0	0	0	0	1 (4%)	0
Dry cough	0	0	0	0	0	1 (13%)	1 (4%)	0
Fever	0	0	0	1 (25%)	0	0	1 (4%)	0
Gastroenteritis	0	0	0	1 (25%)	0	0	1 (4%)	0
Haematuria	0	0	1 (25%)	0	0	0	1 (4%)	0
Impetigo	0	0	0	1 (25%)	0	0	1 (4%)	0
Lethargy	0	0	0	0	0	1 (13%)	1 (4%)	0
Otitis media	0	0	0	0	1 (13%)	0	1 (4%)	0
Proteinuria	0	0	0	0	0	1 (13%)	1 (4%)	0
Rash	0	0	0	0	0	1 (13%)	1 (4%)	0
Shoulder pain	0	0	0	0	1 (13%)	0	1 (4%)	0
Sodium decreased	0	0	1 (25%)	0	0	0	1 (4%)	0
Streptococcal sore throat	0	0	0	0	0	1 (13%)	1 (4%)	0
Suicide attempt	0	0	0	0	0	1 (13%)	1 (4%)	0
Tooth pain	0	0	1 (25%)	0	0	0	1 (4%)	0
High total bilirubin	0	0	0	0	0	0	0	1 (10%)
Decreased blood glucose	0	0	0	0	0	0	0	1 (10%)
Chlamydia trachomatis infection	0	0	0	0	0	0	0	1 (10%)
Contusion of elbow	0	0	0	0	0	0	0	1 (10%)
Increased fasting blood glucose	0	0	0	0	0	0	0	1 (10%)
Glycosuria	0	0	0	0	0	0	0	1 (10%)
Hyponatraemia	0	0	0	0	0	0	0	1 (10%)
Lower back pain	0	0	0	0	0	0	0	1 (10%)
Nausea	0	0	0	0	0	0	0	1 (10%)
Pharyngitis	0	0	0	0	0	0	0	1 (10%)
Decreased potassium	0	0	0	0	0	0	0	1 (10%)
Sneezing	0	0	0	0	0	0	0	1 (10%)
Tinea capitis	0	0	0	0	0	0	0	1 (10%)
Urine white blood cells increased	0	0	0	0	0	0	0	1 (10%)
Weight loss	0	0	0	0	0	0	0	1 (10%)

Data are number of participants (%).

Table 2: **Number of participants with adverse events**

but they occurred with insertion of the intravenous cannula and before study drug administration. Other adverse events that occurred in more than one participant receiving SAB-301 and not in similar proportions to the placebo group were fatigue and loose stools (in participants receiving 5 mg/kg, 10 mg/kg, and 20 mg/kg), and sore throat (which occurred in the 50 mg/kg cohort).

Results from pharmacokinetic analysis show that SAB-301 has nearly linear, but slightly less than

	C_{max} (μg/mL)*	$AUC_{0-\infty}$ (μg × days per mL)*	Dose ratio *vs* 2·5 mg/kg	C_{max} ratio (90% CI)†	$AUC_{0-\infty}$ ratio (90% CI)†
1·0 mg/kg (n=2)	25·93	ND	ND	ND	ND
2·5 mg/kg (n=2)	66·99	1737·70	1	1	1
5 mg/kg (n=4)	104·28	2570·65	2	1·56 (0·70, 2·44)	1·48 (1·08–1·88)
10 mg/kg (n=4)	266·32	7492·40	4	3·98 (3·49–4·46)	4·31 (2·05–6·57)
20 mg/kg (n=8)	517·54	12703·65	8	7·73 (5·92–9·53)	7·31 (5·83–8·79)
50 mg/kg (n=8)	1279·67	27498·18	20	19·10 (17·03–21·17)	15·82 (13·79–17·86)

C_{max}=maximum concentration. $AUC_{0-\infty}$=area under the concentration–time curve from 0 h to infinity. ND=not determined. *Results are geometric mean. †Results are geometric mean ratio vs 2·5 mg/kg.

***Table 3*: Dose-linearity and proportionality of SAB-301**

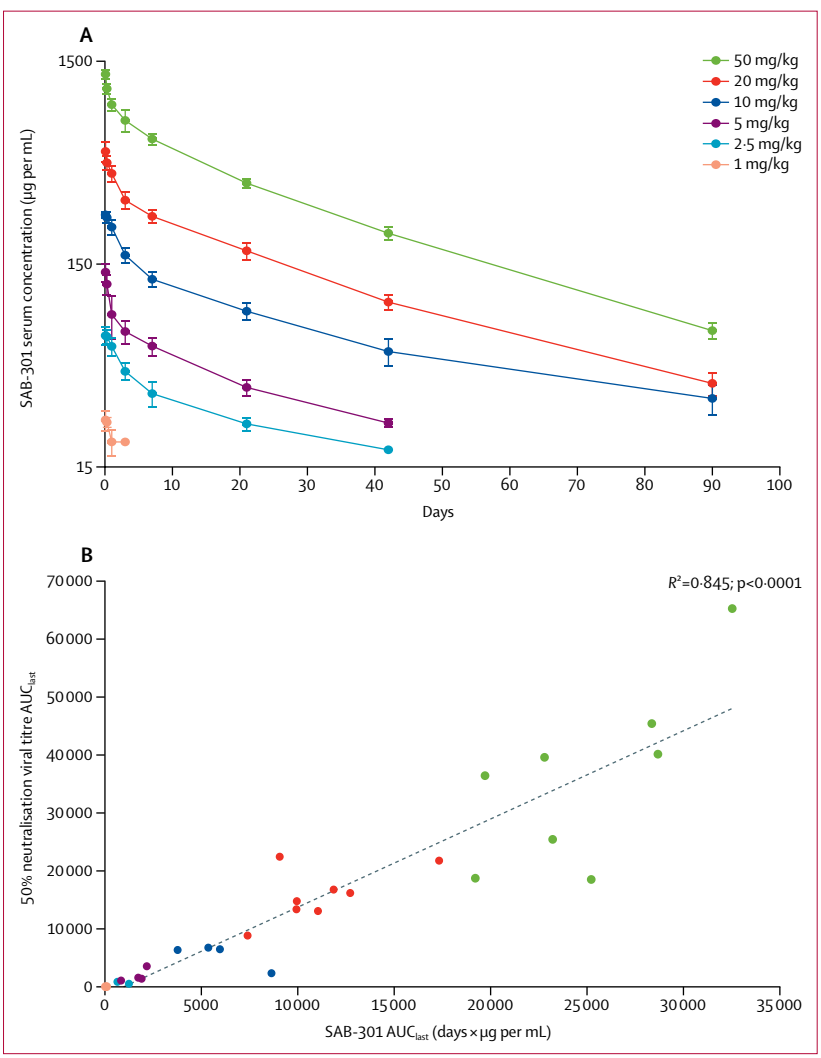

***Figure 2*: Pharmacokinetic analysis**
(A) Mean plasma concentrations of SAB-301 (μg/mL) over time in cohorts one to six. SEM shown as error bars. (B) SAB-301 AUC *vs* viral neutralisation titre AUC, in all cohorts. AUC_{last}=area under the concentration–time curve from 0 h to the last pharmacokinetic sample post-dose.

dose-proportional increases in the parameters of C_{max} and $AUC_{0-\infty}$ over the 20-fold range of doses from 2·5 mg/kg to 50 mg/kg (table 3). We did not use pharmacokinetic data from the 1 mg/kg cohort because a terminal phase could not be identified because of multiple concentrations that were below the limit of assay detection (15·63 μg/mL). Mean concentration versus time profiles of the SAB-301 cohorts at each dose are shown in figure 2A. SAB-301 concentrations after infusion appeared to follow a bi-exponential decline and best fit a two-compartmental pharmacokinetic model. Results for key pharmacokinetic parameters from non-compartmental analysis are shown in table 4 and parameters obtained from the two-compartmental model are presented in the appendix. The average terminal elimination half-life ($T_{1/2}$) was within the range of a typical antibody in human beings (approximately 28 days) across all dose cohorts, excluding the 1 mg/kg cohort. Both uncorrected and baseline-adjusted serum immunoglobulin concentrations over time did not appear to correlate with dose of SAB-301 administered or SAB-301 pharmacokinetic concentrations (data not shown).

We determined anti-MERS neutralising antibody titres using the Jordan-N3/2012 strain of MERS-CoV (a clade A strain). Microneutralisation titres correlated with SAB-301 concentrations in serum (figure 2B). The functional neutralisation of the MERS virus seems to follow the pharmacokinetic exposure of SAB-301 described previously (R^2=0·845, p<0·0001). Pharmacodynamic analysis using the microneutralisation titres showed similar results, with geometric mean titres in the 50 mg/kg cohort of 1/1240 at 7 days and 1/600 at 21 days (appendix).

We investigated the immunogenicity of SAB-301 in several ways. We assessed the presence of anti-IgG antibodies using rheumatoid factor. One participant in cohort 5 (20 mg/kg of SAB-301) became rheumatoid-factor positive on day 21, with a maximum rheumatoid factor of 17 IU/mL. This positive rhematoid factor persisted throughout the study, and there were no associated symptoms with this finding (the only adverse event reported in this participant was albuminuria).

We also assessed anti-drug antibody (anti-SAB-301). Three participants had anti-drug antibody present at baseline (before study drug administration), which persisted and did not change throughout the study (one participant in the 10 mg/kg of SAB-301 cohort and two in the 50 mg/kg of SAB-301 cohort). The pharmacokinetic parameters for these three participants were compared with the other participants in their respective cohorts, and there was no significant difference in $T_{½}$, C_{max}, or AUC. All participants with pre-existing anti-SAB-301 had higher results for $T_{½}$ and AUC than the cohort geometric mean. No new anti-drug antibodies were developed after administration of SAB-301.

We assessed the presence of anti-bovine κ light chain, representing residual bovine proteins that might be present in the final intravenous immunoglobulin product. Seven participants had antibodies to the bovine κ light chain detected at baseline. There was no development of new anti-bovine κ-light-chain antibodies after administration of SAB-301. We also assessed whether

	$T_{1/2}$ (days)	C_{max} (μg/mL)	C_{last} (μg/mL)	AUC_{last} (μg × days per mL)	$AUC_{0-\infty}$ (μg × days per mL)	Volume of distribution (mL/kg)	Clearance (mL/day per kg)
1·0 mg/kg (n=2)	ND	25·93 (13·87%)	18·27 (87·45%)	39·79(92·04%)	ND	ND	ND
2·5 mg/kg (n=2)	28·25 (16·73%)	66·99 (14·15%)	20·23 (14·85%)	891·79 (44·95%)	1737·70 (24·84%)	47·45 (8·29%)	58·64 (24·84%)
5 mg/kg (n=4)	26·10 (19·91%)	104·28 (44·05%)	25·28 (9·96%)	1555·93 (34·97%)	2570·65 (22·57%)	37·02 (24·28%)	73·23 (28·30%)
10 mg/kg (n=4)	38·45 (41·65%)	266·32 (58·31%)	31·90 (10·36%)	5673·41 (28·43%)	7492·40 (34·20%)	23·62 (17·94%)	74·05 (39·37%)
20 mg/kg (n=8)	33·82 (16·56%)	517·54 (33·41%)	35·88 (39·67%)	10 839·73 (26·79%)	12 703·65 (29·16%)	13·78 (23·04%)	76·81 (26·73%)
50 mg/kg (n=8)	28·69 (9·80%)	1279·67 (15·99%)	67·92 (27·52%)	24 585·58 (18·61%)	27 498·18 (18·89%)	10·22 (18·20%)	75·25 (19·50%)

Data are geometric mean (coefficient of variation). $T_{1/2}$=half-life. C_{max}=maximum concentration. C_{last}=concentration at last sample time point. AUC_{last}=area under the concentration time–curve from time of dose to last sample time point. $AUC_{0-\infty}$=area under the concentration time–curve from 0 h to infinity. ND=not determined.

Table 4: **Pharmacokinetic parameters from the non-compartmental analysis**

anti-camelid antibody was present, representing immunogenicity towards a llama anti-human κ-light-chain antibody used in the manufacturing process to purify SAB-301. No participants had anti-camelid antibody at baseline, or developed these antibodies during the study.

Discussion

SAB-301 is a novel anti-MERS-CoV intravenous immunoglobulin manufactured from the hyperimmune plasma of transchromosomic cattle that produce fully human polyclonal IgG. The use of passive immunotherapy, either as immune plasma or intravenous immunoglobulin, has been recommended for multiple severe and emerging infectious diseases such as severe seasonal influenza,[5] pandemic influenza,[12] severe acute respiratory syndrome,[13] MERS,[2] and Ebola.[4] There are often limitations in collecting sufficient human plasma for production.[3,5] Albeit novel, the transchromosomic bovine production system is, more importantly, rapid and scalable. In transchromosomic cattle, new antigen-specific and high-titre human intravenous immunoglobulin can be generated within 3 months of a vaccine being available. Therefore, our study is noteworthy not only by advancing a potential therapeutic for MERS, but also by showing the potential safety of a novel production platform that can quickly generate a new putative drug candidate to an emerging infectious disease.

The results of this first-in-human phase 1 study suggest that SAB-301 appears to be safe and well tolerated at single doses up to 50 mg/kg. The adverse events seen in participants given SAB-301 were largely comparable to those given placebo. In events occurring in more than one participant given SAB-301, low bicarbonate, fatigue, loose stools, sore throat, vasovagal reaction, and aspartate aminotransferase increase did not have any corresponding events in the placebo group. Both vasovagal reactions occurred before study drug administration. Only low blood bicarbonate and sore throat seemed to occur more commonly in the higher dose cohort. With a small number of participants (as is typical in a phase 1 study), it is difficult to discern with certainty the attribution of adverse events to the study drug. These potential events will need to be investigated closely in any future studies.

The pharmacokinetic profile of SAB-301 is similar to what would be expected for human polyclonal or monoclonal antibodies with no human targets. In the critically ill population, the clinical illness of MERS (defined by duration of hospital stay) is a median 19 days (IQR 10–35), and MERS-CoV shedding (as detected by PCR) is median 20 days (95% CI 17–26) in survivors and can exceed 37 days in those that die from MERS.[14] The pharmacodynamic profile of SAB-301, with a neutralisation titre of 1/600 at 21 days and 1/240 at 35 days for 50 mg/kg, suggests good viral neutralisation activity for a period that exceeds the clinical illness and viral shedding after administration of a single dose. The maximum concentration occurred in the initial sample (1 h after the end of infusion), and SAB-301 concentrations remained detectable at the end of 90 days following single 10 mg/kg, 20 mg/kg, and 50 mg/kg doses. Of course, these might be different in an infected patient, in light of antibody–virus binding and subsequent alteration in pharmacokinetics or pharmacodynamics.

The efficacy of SAB-301 has previously been shown in an adenovirus human dipeptidyl peptidase 4 (Ad5-hDPP4)-transduced BALB/c mouse model.[11] Ad5-hDPP4 transduced BALB/c mice were intranasally infected with MERS-CoV and given SAB-301 at 24 or 48 h after infection. For mice given 500 μg (25 mg/kg, assuming 20 g body weight for mice) of SAB-301 at 24 h post infection, MERS-CoV titres were below the level of detection at day 5 ($p<0·0001$). When SAB-301 was administered 48 h after infection, viral titres were detectable but reduced by about 1000-fold by day 5 compared with the untreated control ($p<0·0001$). Given that animal models of MERS-CoV challenge do not replicate human disease effectively, the relevance of this model to extrapolate human efficacy is not known. The mouse dose of 500 μg (equivalent to 25 mg/kg) equates to a human dose of approximately 2 mg/kg based on body surface area conversion. In view of the high mortality rate in people with MERS-CoV infection and the difficulties extrapolating preclinical models to human disease, we suggest the highest tolerated dose in this phase 1 trial (50 mg/kg) should be used initially in human efficacy trials.

The vaccine used for immunising transchromosomic cattle to generate SAB-301 is the spike protein nanoparticle vaccine of the Al-Hasa strain, which belongs to a clade B of MERS-CoV, and the pharmacokinetic data show high antibody titres towards this clade B strain. SAB-301 was previously shown to neutralise the Jordan (Jordan-N3/2012) and Erasmus Medical Center 2012 (EMC/2012) strains of MERS-CoV, both of which are clade A.[11] Therefore SAB-301 is anticipated to have naturalising capacity to current clade A and B strains of MER–CoV.

To our knowledge, this is the first study to show the safety, tolerability, and pharmacokinetics of a novel therapeutic for MERS, as well as for this new source of fully human IgG produced in transchromosomic cattle. The data attained in this study suggest that SAB-301 is safe and well tolerated at potentially therapeutic exposures. Further clinical investigation of SAB-301 for the treatment of MERS is warranted.

Contributors

JHB, JV, ES, TL, and RTD were responsible for initial study design. JHB, JV, and RTD were responsible for study implementation and enrolment of participants. KR was responsible for the micro-neutralisation assay. HW, J-AJ, and ES were responsible for the pharmacokinetic assay and the immunogenicity assays. JHB, JV, PK, and RTD analysed and interpreted the data and wrote the first draft of the report. All authors had opportunity to review the data and edited the final report.

Declaration of interests

HW, J-AJ, and ES have financial interests in SAB Biotherapeutics Inc. All other authors declare no competing interests.

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