First-in-Human Study to Evaluate Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of a Rapidly Developed SARS-CoV-2 Therapeutic Antibody, AOD01, in Healthy Adults

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ABSTRACT

Introduction: AOD01 is a novel, fully human immunoglobulin (Ig) G1 neutralizing monoclonal antibody that was developed as a therapeutic against severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). This first-in-human study assessed safety, tolerability, pharmacokinetics (PK), and pharmacodynamics of AOD01 in healthy volunteers. *Methods*: Intravenous doses of AOD01 were evaluated in escalating cohorts [four single-dose cohorts (2, 5, 10, and 20 mg/kg) and one two-dose cohort (two doses of 20 mg/kg, 24 h apart)]. *Results*: Twenty-three subjects were randomized to receive AOD01 or a placebo in blinded fashion. A total of 34 treatment-emergent adverse events (TEAEs) were reported; all were mild in severity. Related events (headache and diarrhea) were reported in one subject each. No event of infusion reactions, serious adverse

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event (SAE), or discontinuation due to AE were reported. The changes in laboratory parameters, vital signs, and electrocardiograms were minimal. Dose-related exposure was seen from doses 2 to 20 mg/kg as confirmed by C_{max} and $AUC_{0-tlast}$. The median T_{max} was 1.5–3 h. Clearance was dose independent. Study results revealed long half-lives (163–465 h). Antidrug antibodies (ADA) to AOD01 were not detected among subjects, except in one subject of the two-dose cohort on day 92. Sustained ex vivo neutralization of SARS-CoV-2 was recorded until day 29 with single doses from 2 to 20 mg/kg and until day 43 with two doses of 20 mg/kg. *Conclusions*: AOD01 was safe and well tolerated, demonstrated dose-related PK, non-immunogenic status, and sustained ex vivo neutralization of SARS-CoV-2 after single intravenous dose ranging from 2 to 20 mg/kg and two doses of 20 mg/kg and show good potential for treatment of SARS-CoV-2 infection.(Health Sciences Authority identifier number CTA2000119).

Keywords: Antidrug antibodies; AOD01; Neutralization antibody; SARS-CoV-2

Key Summary Points

This study was planned to assessed safety, tolerability, pharmacokinetics, and pharmacodynamics of AOD01.

AOD01 is a novel, fully human immunoglobulin (Ig) G1 neutralizing monoclonal antibody with Fc-mediated antiviral activity, developed as a therapeutic treatment against SARS-CoV-2.

After single IV dose ranging from 2 to 20 mg/kg and two doses of 20 mg/kg, AOD01 demonstrated dose-related PK, non-immunogenic, and sustained ex vivo neutralization of SARS-CoV-2.

AOD01 was found to be safe and well tolerated in this study.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic caused by the novel severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) has affected public health for the last 2 years and it continues to spread on a global scale [1]. Its manifestation ranges from respiratory tract infection to severe disease and death. Though the vaccination of the global population is underway, other options to protect the susceptible population and subjects who develop breakthrough infections should be considered. This has underlined the importance of therapeutic strategies which can provide protective immunity and/or treatment against the SARS-CoV virus [2, 3].

Monoclonal antibodies (mAb) are proven effective early treatment options for COVID-19 [4]. Immediate passive humoral immunotherapy with neutralizing monoclonal antibodies plays a very important role in treating infections. Neutralizing monoclonal antibodies bind to the viral spike (S) protein, which mediates virus entry into the susceptible cells, and prevent viral entry into cells by blocking the interaction between S protein and angiotensin-converting enzyme 2 (ACE2) receptor on the host cell surface [5–8]. Efforts are underway to develop therapeutic antibodies for SARS-CoV-2. To date, a few therapeutic antibodies have been authorized for emergency clinical use, while several antibodies are in clinical development [9–13].

AOD01 is a novel, fully human immunoglobulin (Ig) G1 SARS-CoV-2 neutralizing monoclonal antibody with Fc-mediated antiviral activity. Its functionality is similar to other therapeutic antibodies [2, 14, 15]. AOD01 was identified in a patient infected with the virus during the initial outbreak in Singapore involving the wild-type virus. AOD01 was identified following isolation and screening of a panel of memory B cells 2 months after the start of the outbreak, with clinical-grade drug product manufactured within 6 months of identification [2]. It has been shown to be efficacious in vitro against both the Delta variant and the wild-type virus. In vitro studies demonstrated

that AOD01 inhibits virus interaction with ACE2 receptors. In a direct SARS-CoV-2 neutralization assay, AOD01 had a half-maximal inhibitory concentration of 0.3 μg/mL. The in vivo efficacy of AOD01 was demonstrated in three animal models of COVID-19. In the golden Syrian hamster and Indian rhesus macaque SARS-CoV-2 models, AOD01, at a single dose of 20 mg/kg given 4 to 6 h after the virus challenge, was efficacious in minimizing or preventing disease progression. Reduction in viral load was measured by median tissue culture infectious dose ($TCID_{50}$) and real-time quantitative polymerase chain reaction (RT-qPCR) analysis in the Indian rhesus macaque SARS-CoV-2 model. Doses of 5 and 10 mg/kg were also found to be equally efficacious based on $TCID_{50}$ data. Similarly, AOD01 treatment in SARS-CoV-2-challenged k18-human ACE2 (hACE2) transgenic mice was found efficacious at doses ranging from 5 to 20 mg/kg given once, 6 h after the virus challenge. It was seen that the optimal therapeutic efficacy of AOD01 requires Fc-mediated effector functions that promote an IFNγ-driven antiviral immune response along with its neutralization ability. Antibody-dependent enhancement (ADE) was not observed in the mouse model when treated with subtherapeutic doses of AOD01 [2].

On the basis of the favorable data derived from these preclinical experiments, AOD01 has clear potential for treating COVID-19 (AOD01, data on file). Hence, the first-in-human (FIH) study of AOD01 was undertaken in healthy volunteers to study safety, tolerability, pharmacokinetics (PK), and immunogenicity.

METHODS

Study Design

This phase I, FIH, ascending dose study was conducted at Tan Tock Seng Hospital, Singapore from October 2020 to April 2021. The primary objective was to evaluate the safety and tolerability of ascending doses of AOD01. Secondary objectives were to characterize the PK profile and to assess the immunogenicity of ascending doses of AOD01. Ex vivo bioassay and immunomonitoring of clinical samples were exploratory objectives of the study.

Ethics

The study was approved by the institutional review board and was conducted in accordance with the Declaration of Helsinki, in compliance with the Council for International Organizations of Medical Sciences International Ethical Guidelines, applicable ICH Good Clinical Practice (GCP) Guidelines, and applicable laws and regulations. Written informed consents were obtained from all participants prior to enrolment in the study.

Study Participants

Healthy male and female subjects aged 21 to 55 years, body weight within 50 to 100 kg, body mass index (BMI) within 18.0 to 35.0 kg/m^2, who agreed to use contraceptive methods and women of non-childbearing potential were enrolled in the study.

Subjects with a history of any systemic disorders that may interfere with administration of the drug, laboratory-confirmed SARS-CoV-2, evidence of active or latent tuberculosis (TB), allergies to humanized mAbs, malignancy within the past 5 years, current or chronic history of liver diseases, alanine transaminase (ALT), and bilirubin greater than 1.5 times the upper limit of normal (ULN), corrected QT interval (QTc) greater than 450 ms for men or greater than 470 ms for women, human immunodeficiency virus (HIV), hepatitis B surface antigen (HbsAg), and hepatitis C virus (HCV) infections were excluded from the study. Individuals who had used over the counter or prescription medication within 7 days, live vaccine(s) within 1 month, treatment with biologic agents within 3 months or 5 half-lives prior to dosing, exposure to more than four new chemical entities within 12 months, alcohol consumption, or use of drugs of abuse were also excluded from the study.

Study Procedure

The study investigated four single-dose cohorts at dose levels of 2, 5, 10, and 20 mg/kg and one two-dose cohort at the highest safe dose level of 20 mg/kg as determined from the single ascending dose cohorts. A sentinel group of two subjects (one AOD01 and one placebo) was dosed as planned within each of the first, fourth, and fifth cohorts. The remainder of the cohort was dosed 24 h after the dosing of the last subject in the sentinel group. Dosing in the two-dose cohort was done 72 h after the start of dosing of the last subject in the safe single-dose cohort.

The duration of the study was 92 days which includes a drug administration period (1 day for single-dose cohorts and 2 days for the two-dose cohort), an inpatient observation period (5 days, including the day of study drug administrations), and a follow-up period (92 days from day 1).

Treatment

AOD01 was administered as a continuous slow intravenous (IV) infusion over 60 min. A 5-mL vial was used which contained AOD01 at a concentration of 30 mg/mL. AOD01 was diluted with commercially available 500 mL of 0.9% sodium chloride solution to achieve the desired dose level. Commercially available 0.9% sodium chloride was used as the placebo. Treatment duration was 1 day for single-dose cohorts and 2 days for the two-dose cohort. For the two-dose cohort, the second dose was administered 24 h after the first dose.

Randomization and Blinding

The subjects were randomly allocated to receive either AOD01 or a placebo. The randomization list was generated using SAS® Version 9.4. An interactive response technology was used to administer the randomization schedule. This was a blinded study where subjects, investigator staff, persons performing the assessments, and data analysts remained blinded to the identity of study treatments.

Study Endpoints

The primary endpoint was the incidence and severity of treatment-emergent adverse events (TEAEs) in subjects treated with AOD01 or placebo. Secondary endpoints were the assessment of PK parameters (C_{max}, AUC_{0-t}, AUC_{0-inf}, T_{max}, $t_{1/2}$) and antidrug antibody (ADA) to AOD01. Ex vivo bioassay for neutralization of SARS-CoV-2 and immunomonitoring of clinical samples for changes in cytokines, immune cells, and RNA sequencing of whole blood were exploratory endpoints.

Safety Assessment

Safety assessments were performed starting from the signing of the informed consent form until the last follow-up visit.

Safety parameters included TEAEs, related TEAEs, TEAEs by severity, TEAEs leading to discontinuation, serious TEAEs, and death. Parameters also included acute or delayed infusion reaction, vital signs, physical examination, electrocardiogram (ECG) changes, and laboratory parameters. Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 was used for the assessment of severity. Subjects were also evaluated for symptoms of COVID-19 at each follow-up visit.

PK Assessment

Blood collected in BD Vacutainer® serum tubes with silica was used to obtain serum as per the manufacturer's instruction for PK assessment. For single-dose cohorts, PK sampling was performed on day 1 and through day 5 discharge at the following time points: pre-infusion, at 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 h after the start of the infusion, at the follow-up visits on days 6, 8, 15, 22, 29, 43, 57, 71, and 92.

For the two-dose cohort, PK sampling was performed on day 1 at the following time points: pre-infusion, at 0.5, 1, 2, 4, 6, 8, 10, and 12 h after the start of infusion. On day 2, sampling was performed pre-infusion, at 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48, 60, 72, 96, and 120 h after the start of infusion. Sampling was done on

follow-up visits on days 8, 9, 15, 22, 29, 43, 57, 71, and 92. PK parameters ($AUC_{0-tlast}$, AUC_{0-inf}, AUC_{0-tau}, $t_{1/2}$, CL/F, V/F, C_{max}, C_{trough}, and T_{max}) were estimated.

A precise, sensitive, and reproducible method was validated for the quantification of AOD01 in human serum used in an enzyme-linked immunosorbent assay (ELISA). In brief, isolated spike protein from COVID-19 virus was used as the coating protein; 1 µg/mL spike protein was made up in $1\times$ phosphate buffered saline (PBS) and 30 µL per well was left overnight at 2–8 °C. The plates were then blocked with 1% BSA in PBS for 1.5 h. Serum samples were then diluted to minimum required dilution (MRD) (1:100 and subsequently 1:50 to get a total dilution of 1:5000) and added to the wells. The wells were then incubated at room temperature for 1 h and anti-horse reddish peroxidase (HRP) was used as a secondary antibody. 3,3′,5,5′-Tetramethylbenzidine (TMB) is then added 1 h later for 10 min and quenched with 1 M HCl solution. The plate is read with the HIDEX Sense reader for OD (450 nm) values.

Immunogenicity Assessment

Blood collected in BD Vacutainer® serum tubes with silica was used to obtain serum as per the manufacturer's instruction. For immunogenicity analysis, blood samples were collected pre-infusion (day 1) and on days 15, 29, 57, and 92 from subjects in single-dose cohorts. For subjects in a two-dose cohort, samples were collected pre-infusion (day 1) and on days 9, 15, 29, 57, and 92. Antidrug antibodies were measured with an assay developed and validated in-house utilizing the receptor-binding domain of the SARS-CoV-2 virus's spike protein as a surrogate antigen.

Biomarker Assessment

Immunomonitoring Assessment
Blood collected in BD Vacutainer® CPT™ with sodium citrate was used to obtain plasma as per the manufacturer's instruction for cytokine analysis. Blood samples for measurement of plasma cytokines, chemokines, acute phase reactants, and RNA sequencing were collected pre-infusion, and 1 and 4 h after the start of infusion on day 1, and on days 2, 3, and 8 for subjects in single-dose cohorts. For the two-dose cohort, samples were collected pre-infusion (day 1), at 1 and 4 h after the start of infusion on days 1 and 2 and on days 3, 4, and 9.

Ex Vivo Bioassay Assessment
Blood samples to evaluate ex vivo neutralization of SARS-CoV-2 in single-dose cohorts were collected pre-infusion (day 1), and on day 2, 5, 8, 15, 29, 43, and 92. Samples were collected pre-infusion (day 1), and on day 3, 5, 9, 15, 29, 43, and 92 in subjects who received two doses of AOD01.

Statistical Analysis

All statistical analyses were conducted using SAS version 9.4. Descriptive statistics were provided in the summary tables as per the dose cohorts. Continuous variables were summarized using N, mean, standard deviation (SD), median, and range. Categorical variables were summarized using frequency counts and percentages. Dose proportionality for AOD01 in PK analysis was evaluated from single-dose cohorts for C_{max}, $AUC_{0-tlast}$, and AUC_{0-inf}. PK calculations were performed using Phoenix® WinNonlin® (Certara L.P., Princeton, NJ) Version 8.0 or SAS Version 9.4.

No formal sample size calculation was performed for this study. The sample size was chosen on the basis of experience from previous studies of a similar nature. All subjects who received at least one dose of the study drug were included in the safety analysis. Subjects in whom serum concentration data facilitate the derivation of at least one PK parameter and in whom the time of drug administration on the day of sampling was known were included in PK analysis.

RESULTS

Subjects Disposition and Baseline Characteristics

A total of 24 subjects were randomly assigned to five study cohorts; of these, 23 subjects (95.8%) received the study drug (AOD01 or placebo). One subject randomized to a 5 mg/kg single-dose cohort withdrew his consent and was replaced by another subject. All 23 subjects completed the study (see Table S1 in the electronic supplementary material for details). The demographic characteristics were similar across the study cohorts. Nineteen subjects (82.6%) in the study were male. Twenty-two subjects (95.7%) were not Hispanic or Latino by ethnicity. All subjects were Asian, mostly Chinese (16 subjects [69.6%]) or Malay (5 subjects [21.7%]). The mean age was 32.9 years, the mean weight was 69.7 kg, and the mean BMI was 24.0 kg/m^2. All subjects had negative viral serology, no findings on chest X-ray, and negative RT-PCR results on screening. For all study cohorts, the median infusion time was 60 min (range 60–67 min). The infusion was not interrupted in any subject (Table 1).

Safety

A total of 34 TEAEs were reported in 17 subjects (73.9%) in the study. The most common TEAE was catheter site rash which was reported in four subjects (17.4%). Other commonly reported TEAEs included diarrhea, rhinorrhea, and headache reported in three subjects (13.0%) each. Vessel puncture site bruises, musculoskeletal chest pain, and rash were reported in two subjects (8.7%) each.

All TEAEs were mild in severity. Two subjects (8.7%) reported two TEAEs that were related to study drugs: headache in one subject (33.3%) in AOD01 20 mg/kg (single-dose cohort) and diarrhea in one subject (33.3%) in AOD01 20 mg/kg (two-dose cohort). Both TEAEs were mild in severity and resolved in a day. Headache was treated with paracetamol and no treatment was given for diarrhea. None of the subjects reported TEAEs leading to study treatment discontinuation, acute or delayed infusion reactions, serious adverse events (SAEs), or AEs leading to death (Table 2).

Eight subjects reported COVID-19 signs and symptoms during the study. Of these eight subjects, the coronavirus PCR test was performed in only one subject in the placebo group with a headache on day 57 and the test result was negative. All other subjects were judged as not requiring COVID-19 testing by the investigator (see Table S2 in the electronic supplementary material for details). For the laboratory parameters, vital signs, and ECG parameters, the mean changes from baseline were minimal and not clinically significant. Physical examination findings were also normal in most of the subjects.

Pharmacokinetics

The mean serum concentration–time profiles for all single-dose cohorts followed a similar trend. Serum concentrations peaked rapidly with median T_{max} values observed at 1.9, 1.5, 1.5, and 2.9 h relative to the start of the infusion for 2, 5, 10, and 20 mg/kg dose levels, respectively. Thereafter, concentrations declined gradually reaching below the limit of quantification (BLQ) by 43 days for the 2 and 5 mg/kg dose levels and to concentrations of about 2% (and below) of the respective peak concentrations observed for the 10 and 20 mg/kg dose levels by day 92 (end of study, EOS) (Fig. 1a).

The mean serum concentration–time profiles for the two-dose cohort were similar on both day 1 and day 2 of dosing. Serum concentrations peaked at a median T_{max} value of about 2 h on both days. After the median T_{max} value, the concentration dropped gradually across the sampling period and finally reached a concentration that was about 0.5% of the peak by day 92 (EOS) (Fig. 1b).

The geometric mean peak (C_{max}) and total ($AUC_{0-tlast}$) exposures increased with increasing the dose from 2 to 20 mg/kg. The mean $t_{1/2}$ values for the 2, 5, 10, and 20 mg/kg dose levels were 163, 126, 199, and 465 h, respectively. The mean clearance was dose independent

Table 1 Demographics and baseline characteristics

Baseline characteristics	AOD01					Placebo (N = 8)	All (N = 23)
	One-dose cohorts				Two-dose cohort		
	2 mg/kg (N = 3)	5 mg/kg (N = 3)	10 mg/kg (N = 3)	20 mg/kg (N = 3)	20 mg/kg (N = 3)		
Age (years)							
Mean (SD)	32.7 (3.0)	32.7 (3.2)	30.3 (3.7)	37.7 (6.6)	30.3 (4.5)	33.3 (8.2)	32.9 (5.9)
Median	32.0	34.0	32.0	36.0	30.0	30.5	32.0
Range	30–36	29–35	26–33	32–45	26–35	27–52	26–52
Sex, n (%)							
Male	3 (100.0)	3 (100.0)	3 (100.0)	2 (66.7)	2 (66.7)	6 (75.0)	19 (82.6)
Female	0	0	0	1 (33.3)	1 (33.3)	2 (25.0)	4 (17.4)
Ethnicity, n (%)							
Hispanic or Latino	0	0	0	0	0	1 (12.5)	1 (4.3)
Not Hispanic or Latino	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	7 (87.5)	22 (95.7)
Race, n (%)							
Asian	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	8 (100.0)	23 (100.0)
Chinese	2 (66.7)	2 (66.7)	2 (66.7)	3 (100.0)	2 (66.7)	5 (62.5)	16 (69.6)
Malay	1 (33.3)	1 (33.3)	1 (33.3)	0	0	2 (25.0)	5 (21.7)
Other	0	0	0	0	1 (33.3)	1 (12.5)	2 (8.7)
Height (cm)							
Mean (SD)	167.5 (2.5)	170.8 (4.8)	166.0 (5.6)	166.2 (9.2)	174.6 (17.1)	171.9 (6.7)	170.0 (7.9)
Median	168.0	168.6	165.3	169.6	181.8	170.0	168.7
Range	164.8–169.8	167.4–176.4	160.8–172.0	155.8–173.4	155.1–187.0	164.0–183.8	155.1–187.0
Weight (kg)							
Mean (SD)	66.3 (5.4)	68.0 (9.5)	64.5 (9.3)	70.0 (12.7)	75.5 (22.2)	71.3 (8.3)	69.7 (10.5)
Median	65.6	68.0	68.1	74.2	85.1	73.6	71.6
Range	61.2–72.1	58.5–77.5	54.0–71.6	55.8–80.2	50.1–91.3	59.5–83.0	50.1–91.3
BMI (kg/m^2)							
Mean (SD)	23.6 (1.7)	23.2 (2.3)	23.5 (3.9)	25.1 (1.9)	24.2 (2.9)	24.1 (3.1)	24.0 (2.5)
Median	24.2	24.3	23.0	25.8	25.7	23.4	24.2
Range	21.7–25.0	20.6–24.9	19.8–27.7	23.0–26.7	20.8–26.1	19.7–29.7	19.7–29.7

*BMI* body mass index, *N* sample size for the cohort, *n* number of subjects, *SD* standard deviation

Table 2 Treatment-emergent adverse events by system organ class and preferred term

System Organ Class and Preferred Term	AOD01										Two-dose cohort		Placebo (N = 8)		All (N = 23)	
	One-dose cohorts										20 mg/kg (N = 3)					
	2 mg/kg (N = 3)		5 mg/kg (N = 3)		10 mg/kg (N = 3)		20 mg/kg (N = 3)									
	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E
Subjects with at least 1 TEAE	2 (66.7)	3	2 (66.7)	4	2 (66.7)	2	3 (100.0)	5	2 (66.7)	6	6 (75.0)	14	17 (73.9)	34
General disorders and administration site conditions	0	–	1 (33.3)	1	0	–	1 (33.3)	1	1 (33.3)	1	4 (50.0)	4	7 (30.4)	7
Catheter site dermatitis	0	–	0	–	0	–	0	–	1 (33.3)	1	0	–	1 (4.3)	1
Catheter site rash	0	–	0	–	0	–	1 (33.3)	1	0	–	3 (37.5)	3	4 (17.4)	4
Vessel puncture site bruise	0	–	1 (33.3)	1	0	–	0	–	0	–	1 (12.5)	1	2 (8.7)	2
Gastrointestinal disorders	1 (33.3)	1	1 (33.3)	1	0	–	0	–	1 (33.3)	2	1 (12.5)	1	4 (17.4)	5
Diarrhea	0	–	1 (33.3)	1	0	–	0	–	1 (33.3)	2	1 (12.5)	1	3 (13.0)	4
Tooth disorder	1 (33.3)	1	0	–	0	–	0	–	0	–	0	–	1 (4.3)	1
Respiratory, thoracic, and mediastinal disorders	1 (33.3)	1	0	–	1 (33.3)	1	1 (33.3)	1	1 (33.3)	1	0	–	4 (17.4)	4
Rhinorrhea	0	–	0	–	1 (33.3)	1	1 (33.3)	1	1 (33.3)	1	0	–	3 (13.0)	3
Throat irritation	1 (33.3)	1	0	–	0	–	0	–	0	–	0	–	1 (4.3)	1
Musculoskeletal and connective tissue disorders	0	–	0	–	1 (33.3)	1	0	–	2 (66.7)	2	0	–	3 (13.0)	3
Arthralgia	0	–	0	–	1 (33.3)	1	0	–	0	–	0	–	1 (4.3)	1
Musculoskeletal chest pain	0	–	0	–	0	–	0	–	2 (66.7)	2	0	–	2 (8.7)	2
Nervous system disorders	0	–	0	–	0	–	1 (33.3)	1	0	–	2 (25.0)	5	3 (13.0)	6
Headache	0	–	0	–	0	–	1 (33.3)	1	0	–	2 (25.0)	5	3 (13.0)	6
Skin and subcutaneous tissue disorders	0	–	1 (33.3)	1	0	–	1 (33.3)	1	0	–	1 (12.5)	2	3 (13.0)	4
Rash	0	–	0	–	0	–	1 (33.3)	1	0	–	1 (12.5)	1	2 (8.7)	2
Rash erythematous	0	–	0	–	0	–	0	–	0	–	1 (12.5)	1	1 (4.3)	1

Table 2 continued

System Organ Class and Preferred Term	AOD01										Two-dose cohort		Placebo (N = 8)		All (N = 23)	
	One-dose cohorts										20 mg/kg (N = 3)					
	2 mg/kg (N = 3)		5 mg/kg (N = 3)		10 mg/kg (N = 3)		20 mg/kg (N = 3)									
	n (%)	E	n (%)	E	n (%)	E	n (%)	E			n (%)	E	n (%)	E	n (%)	E
Rash maculo-papular	0	–	1 (33.3)	1	0	–	0	–			0	–	0	–	1 (4.3)	1
Infections and infestations	0	–	0	–	0	–	1 (33.3)	1			0	–	0	–	1 (4.3)	1
Upper respiratory tract infection	0	–	0	–	0	–	1 (33.3)	1			0	–	0	–	1 (4.3)	1
Injury, poisoning, and procedural complications	1 (33.3)	1	0	–	0	–	0	–			0	–	0	–	1 (4.3)	1
Muscle strain	1 (33.3)	1	0	–	0	–	0	–			0	–	0	–	1 (4.3)	1
Metabolism and nutrition disorders	0	–	1 (33.3)	1	0	–	0	–			0	–	0	–	1 (4.3)	1
Hypokalemia	0	–	1 (33.3)	1	0	–	0	–			0	–	0	–	1 (4.3)	1
Vascular disorders	0	–	0	–	0	–	0	–			0	–	1 (12.5)	2	1 (4.3)	2
Thrombophlebitis	0	–	0	–	0	–	0	–			0	–	1 (12.5)	1	1 (4.3)	1
Venous thrombosis	0	–	0	–	0	–	0	–			0	–	1 (12.5)	1	1 (4.3)	1

E number of events, N sample size for the cohort, n number of subjects, TEAE treatment-emergent adverse event

(a) Single-dose cohort
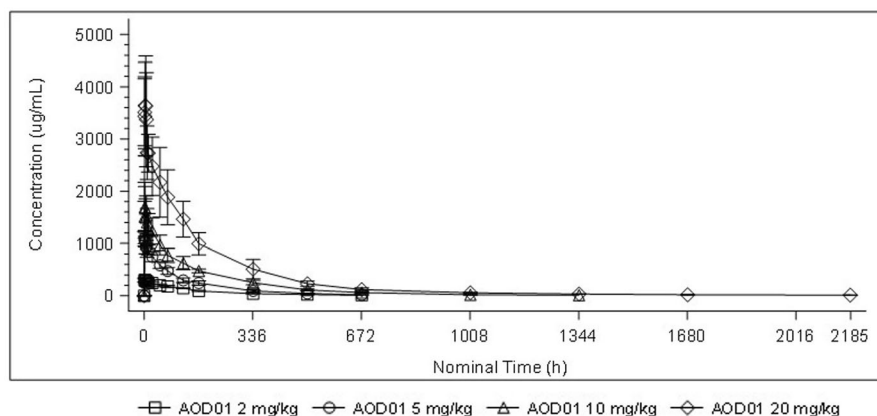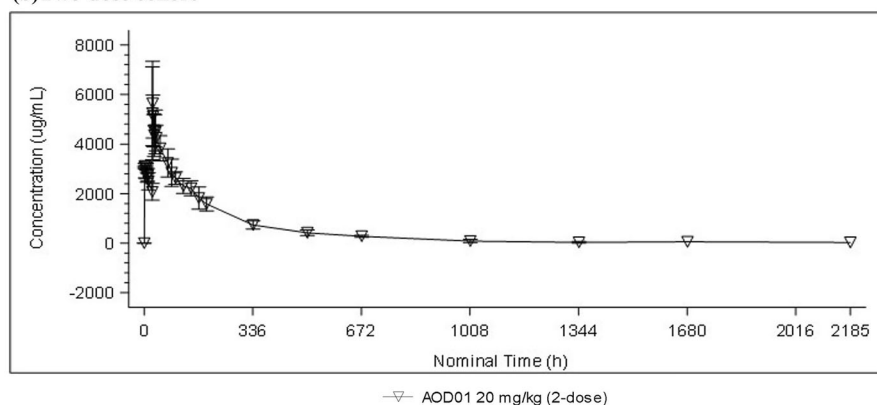
(b)Two-dose cohort

Fig. 1 Mean serum AOD01 concentration versus time profiles by dose. **a** Single-dose cohort. **b** Two-dose cohort

(approximately the same and between 0.035 and 0.04 mL/h/kg across all dose levels tested). The volume of distribution at 20 mg/kg was higher (23 mL/kg) when compared to lower dose levels that were between 7 and 11 mL/kg. Over the dose range of 2–10 mg/kg, AUC_{0-inf} was contained within the criteria for dose proportionality, but the other parameters, C_{max} and $AUC_{0-tlast}$ were not (Fig. 2; Table 3 and see Table S3 in the electronic supplementary material for details).

Immunogenicity

Antidrug antibodies to AOD01 were not detected among subjects, except in one subject (33.3%) in a two-dose cohort on day 92. There was no impact of the ADAs on AOD01 PK or

safety. No AEs that could be attributed to ADAs were reported in this subject.

Biomarker Analysis

The flow cytometry and RNA sequencing results showed that single-dose or two-dose infusion of AOD01 did not lead to immune activation or cytokine release. The derived immunoscores, from the mean of healthy donor reference cytokine concentration, were zero at most time points. A transient rise was seen in a few cytokines, in the placebo group and AOD01 20 mg/kg (single-dose and two-dose) cohorts, which recovered within a day. No AEs were reported that could be attributed to the minimal rise in cytokine levels.
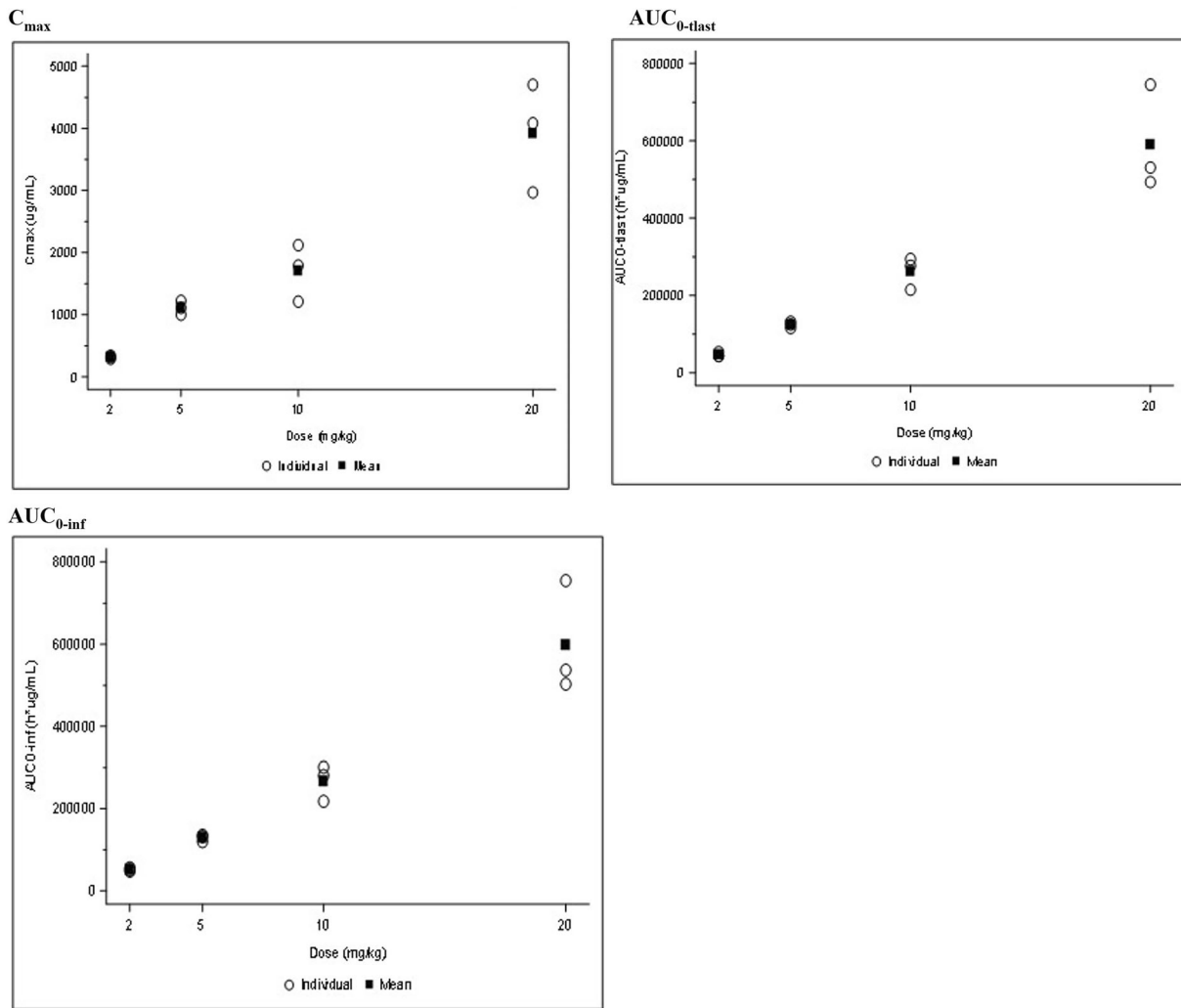
Fig. 2 Individual and mean AOD01 pharmacokinetic parameters versus dose

The scatter plots of normalized RNA Tempus count by time point showed that in both single-dose and two-dose AOD01 groups and the placebo group, the log2-CPM of normalized RNA Tempus counts at all post-infusion time points clustered around the diagonal line. This indicates that there was minimal difference in the gene expressions at pre-infusion and post-infusion time points (Fig. 3).

Ex Vivo Bioassay

In single-dose cohorts, ex vivo bioassay for neutralization of SARS-CoV-2 was recorded as positive from day 2 until day 8 in all 12 subjects who received AOD01. The neutralization capacity was sustained until day 15 in all three subjects (100.0%) of the AOD01 20 mg/kg cohort and one subject (33.3%) of the AOD01 2 mg/kg cohort and until day 29 in two subjects (66.7%) of the AOD01 10 mg/kg cohort. By day 43, neutralization was negative for all subjects receiving the single dose (Table 4).

In subjects who received two doses of AOD01 20 mg/kg, neutralization was recorded as positive from day 3 until day 15 in all three subjects (100.0%). The neutralization capacity was sustained until day 43 in one subject (33.3%), and by day 92 neutralization was negative for all subjects (Table 5).

Table 3 Pharmacokinetics following administration of AOD01

PK parameter (units)	AOD01					
	One-dose cohort				Two-dose cohort	
	2 mg/kg ($N = 3$)	5 mg/kg ($N = 3$)	10 mg/kg ($N = 3$)	20 mg/kg ($N = 3$)	20 mg/kg ($N = 3$) Day 1	20 mg/kg ($N = 3$) Day 2
C_{max} (µg/mL)[a]	316 (7.9)	1110 (10.1)	1660 (29.2)	3850 (23.9)	3170 (2.8)	5490 (28.7)
T_{max} (h)[b]	1.9 (1.5, 6.9)	1.5 (1.4, 1.9)	1.5 (1.4, 1.9)	2.9 (1.4, 5.03)	2.0 (1.6, 2.9)	1.9 (1.4, 2.2)
$AUC_{0-tlast}$ (h µg/ mL)[a]	46,700 (11.7)	124,000 (6.5)	260,000 (16.7)	580,000 (22.3)	58,900 (9.7)	864,000 (21.2)
AUC_{0-tau} (h µg/ mL)[a]	–	–	–	–	–	103,000 (14.8)
AUC_{0-inf} (h µg/ mL)	51,500 (7.7)	129,000 (6.4)	264,000 (17.1)	589,000 (22.0)	–	–
$t_{1/2}$ (h)[c,d]	163 (20.6)	126 (13.5)	199 (15.4)	465 (21.7)	25.4 (3.5)	236 (60.3)
C_{trough} (µg/mL)[a]	–	–	–	–	–	3840 (13.5)
CL (mL/h/kg)[d]	0.038 (7.7)	0.038 (6.4)	0.038 (17.5)	0.034 (20.4)	–	–
V (mL/kg)[d]	9.1 (20.4)	7.1 (20.2)	10.8 (6.5)	22.9 (25.8)	–	–

C_{max} peak drug concentration, T_{max} time to peak drug concentration, $AUC_{0-tlast}$ AUC up to the last measurable concentration, AUC_{0-tau} AUC to the end of the dosing period, AUC_{0-inf} AUC curve to infinite time, $t_{1/2}$ plasma half-life, C_{trough} concentration reached by a drug immediately before the next dose is administered, CL clearance, V volume of distribution

[a]For C_{max}, $AUC_{0-tlast}$, AUC_{0-tau}, AUC_{0-inf}, C_{trough}, geometric mean (coefficient of variation) is presented

[b]For T_{max}, the median (minimum, maximum) values are presented

[c]For $t_{1/2}$, $n = 2$ for AOD01 20 mg/kg (2 doses) day 1

[d]For $t_{1/2}$, CL, and V, arithmetic mean (coefficient of variation) is presented

DISCUSSION

In this randomized, placebo-controlled, FIH study, doses of AOD01 2, 5, 10, and 20 mg/kg and two doses of AOD01 20 mg/kg were found to be safe and well tolerated. The second dose was administered to subjects on day 2 to demonstrate the preliminary safety data necessary to justify the administration of a second dose to subjects with COVID-19 in the event where treating physicians needed an option to administer a second dose. Inclusion of the healthy subjects without any comorbid disease allowed for an unbiased assessment of the safety and tolerability of AOD01.

The safety profile in subjects who received AOD01 was similar to that in placebo-treated subjects. All the reported AEs were mild in severity and resolved with or without medication. There were no AEs leading to treatment discontinuation or treatment interruption, SAEs, and deaths, thereby assuring the safety of AOD01.

Though eight subjects reported COVID-19 signs and symptoms during the study, the PCR test was performed in only one subject. The other subjects had individual symptoms which were actively elicited from a COVID-19 questionnaire, but had no overall flu-like presentation, which the investigator evaluated as very
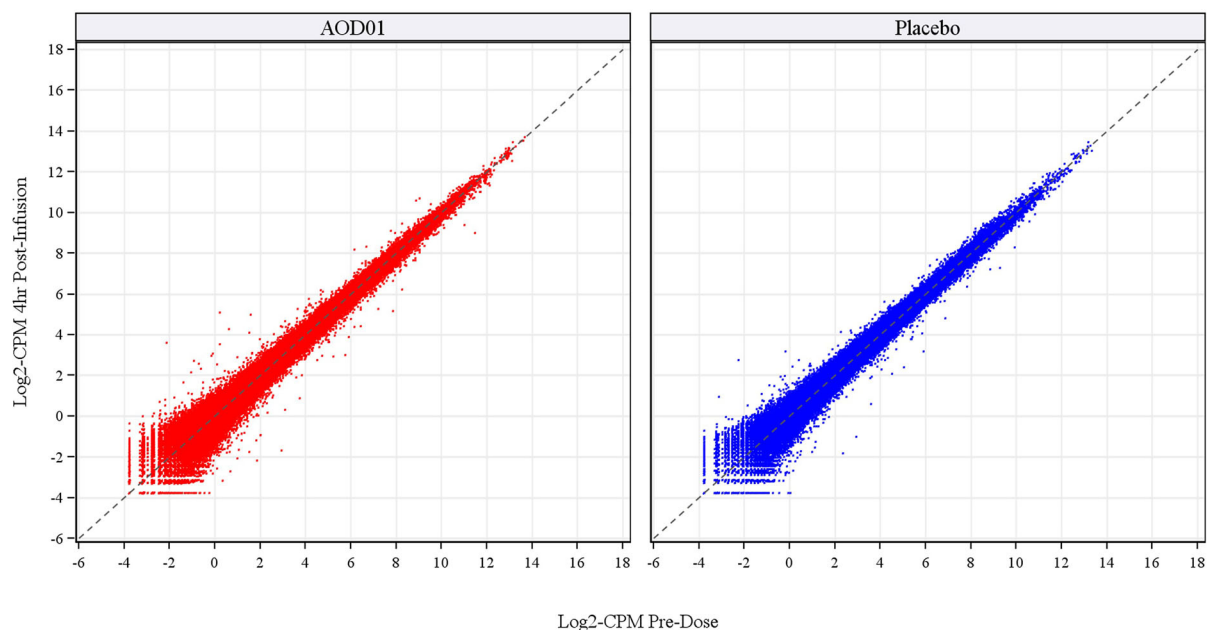
Scatterplots generated on the log2-CPM for each gene comparing pre-dose counts to each post-dose count within a cohort.

Fig. 3 Scatter plot of normalized RNA Tempus counts by time point

unlikely to be related to a viral infection and hence the COVID-19 testing was not performed in these subjects.

The study showed that AOD01 was non-immunogenic as no antidrug antibodies were detected except in one subject which did not result in any adverse event and change in PK profile. This safety profile of AOD01 was in contrast to other monoclonal antibody products which have reported immune-related AEs like pruritis and rash. Immediate hypersensitivity reactions were reported in nearly 2% of subjects in a study of neutralizing antibodies LY-CoV555 and LY-CoV016 [10, 16]. Allergies, pruritus, and rash were also reported in the MW33 neutralizing antibody trial [17].

AOD01 demonstrated sustained ex vivo neutralization of SARS-CoV-2 until at least day 29 with single doses from 2 to 20 mg/kg and until day 43 with two doses of 20 mg/kg suggesting its potential in COVID-19 treatment. Preliminary evidence suggests that AOD01 retains activity against the Delta variant which was the predominant strain during the second wave of the pandemic (internal data).

The study also demonstrated favorable PK characteristics. Pharmacokinetic analysis showed that AOD01 geometric mean C_{max} and $AUC_{0-tlast}$ values increased with increasing doses from 2 to 20 mg/kg. The mean and individual concentration–time profiles for AOD01 increased with increasing doses and were consistent with linear PK for single IV doses. Over the dose range of 2–20 mg/kg, dose proportionality could not be concluded; however, this may be due to the small sample size ($n = 3$ per dose level) in this study. In the 2 mg/kg and 10 mg/kg cohorts prolonged T_{max} was observed. This was due to the long half-life of the drug and the lack of target-mediated drug clearance in healthy volunteers, the minimal differences that were observed in T_{max} in some subjects seem to be less clinically significant.

The mean $t_{1/2}$ values of 2, 5, 10, and 20 mg/kg doses of AOD01 were 163 h (6.7 days), 126 h (5.2 days), 199 h (8.2 days), and 465 h (19.3 days), respectively. The mean $t_{1/2}$ of bamlanivimab after a single IV dose of 700 mg in patients with COVID-19 was 17.6 days [18]. Similarly, the half-lives of REGN10933 (casirivimab) were 24 and 25 days and 21 and 18 days

Table 4 Ex vivo bioassay for neutralization of SARS-CoV-2 (single-dose cohorts)

Positive neutralization of SARS-CoV-2	AOD01				Placebo (N = 6) n (%)	All (N = 18) n (%)
	2 mg/kg (N = 3) n (%)	5 mg/kg (N = 3) n (%)	10 mg/kg (N = 3) n (%)	20 mg/kg (N = 3) n (%)		
Baseline, n (%)	0	0	0	0	0	0
Day 2, 24 h post-infusion, n (%)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	0	12 (66.7)
Day 5, 96 h post-infusion, n (%)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	0	12 (66.7)
Day 8, 168 h post-infusion, n (%)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	0	12 (66.7)
Day 15, 336 h post-infusion, n (%)	1 (33.3)	0	3 (100.0)	3 (100.0)	0	7 (38.9)
Day 29, 672 h post-infusion, n (%)	0	0	2 (66.7)	0	0	2 (11.1)
Day 43, 1008 h post-infusion, n (%)	0	0	0	0	0	0
Day 92, EOS, n (%)	0	0	0	0	0	0

EOS end of study, *n* number of subjects

Table 5 Ex vivo bioassay for neutralization of SARS-CoV-2 (two-dose cohort)

Positive neutralization of SARS-CoV-2	AOD01 20 mg/kg (N = 3), n (%)	Placebo (N = 2), n (%)	All (N = 5), n (%)
Baseline, n (%)	0	0	0
Day 3, 24 h post-infusion second dose, n (%)	3 (100.0)	0	3 (60.0)
Day 5, 72 h post-infusion second dose, n (%)	3 (100.0)	0	3 (60.0)
Day 9, 168 h post-infusion second dose, n (%)	3 (100.0)	0	3 (60.0)
Day 15, 312 h post-infusion second dose, n (%)	3 (100.0)	0	3 (60.0)
Day 29, 648 h post-infusion second dose, n (%)	1 (33.3)	0	1 (20.0)
Day 43, 984 h post-infusion second dose, n (%)	1 (33.3)	0	1 (20.0)
Day 92, EOS, n (%)	0	0	0

EOS end of study, *n* number of subjects

for REGN10987 (imdevimab) after a single IV dose of 1.2 and 4 g of the study drug [19]. Thus, the half-life of AOD01 is similar to other monoclonal antibodies.

Though AOD01 has been identified from a patient infected with the wild-type virus and has been found to be efficacious in vitro against

the Delta variant, its efficacy against Omicron and other newer variants is yet to be studied.

CONCLUSION

In this FIH study, single doses of AOD01 2, 5, 10, and 20 mg/kg and two doses of AOD01 20 mg/kg were safe and well tolerated and demonstrated dose-related PK, non-immunogenic status, and sustained ex vivo neutralization of SARS-CoV-2. Concentration–time profiles were consistent with linear PK for single IV doses. The data showed that AOD01 has good potential as a treatment for SARS-CoV-2 infection and has paved a path to elucidate the efficacy and safety of AOD01 in patients with COVID-19.

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