Pilot Study on Challenge of SARS-CoV-2 in Cynomolgus Macaques

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Introduction

In 2019, a novel coronavirus (SARS-CoV-2) was detected in Wuhan City, Hubei Province, China. As of May 2021, the disease has been affecting countries all over the world, including in the United States. Coronaviruses cause mild to severe upper-respiratory illness in humans. Coronaviruses are zoonotic, meaning they can spread from animals to humans. Severe coronavirus infections caused by SARS-CoV, MERS-CoV, and SARS-CoV-2 can cause pneumonia, respiratory failure, and death (WHO Int., February 2020). We infected cynomolgus macaques with SARS-CoV-2 by intratracheal instillation. We measured bronchioalveolar lavage fluid (BALF) viral titers, nasal swab viral titers, blood gas parameters, pathology and body weight changes to demonstrate proficiency in the methods used for SARS-CoV-2 infections in non-human primates. We successfully administered SARS-CoV-2 to cynomolgus macaques and detected virus in BALF. We also observed a variety of inflammatory changes in bronchial and alveolar tissues.

Study Design

Six cynomolgus macaques were used in this study. There was a single group of six animals. A physical exam was performed by an MRIGlobal veterinarian prior to study start. Animals were unremarkable with rectal temperatures ranging from 100.6° to 102.7° F, heart rates ranging from 90 to 132 beats per minute, breaths ranging from 30 to 54 breaths per minute, normal appearance, including the eyes, ears, throat, skin, coat, heart, lungs, abdomen, and lymph nodes all being normal upon inspection.

All six NHPs were exposed by both the intranasal (IN) and intratracheal (IT) routes on Study Day 0. The challenge exposure was performed under sedation with Ketamine (15 mg/kg, IM) and Dexmedetomidine (0.05 mg/kg, IM. Following sedation for procedures, the animal were placed into their home cage and allowed to recover from the sedation. Antisedan® (atipamezole hydrochloride; in equal volume to dexmedetomidine) was administered IM to reverse the sedation.

After SARS-CoV-2 challenge, animals were singly housed. Animals were observed twice daily for clinical symptoms of disease by approved staff and/or authorized investigators with experience in assessing distress in macaques. One male (C94374) and one female (C96099) were euthanized on Study Day 3. All other animals were euthanized on Study Day 9. Necropsy and histopathology was performed.

BALF was analyzed for viral titers by $TCID_{50}$ and PCR. BALF was not analyzed for T cell percentages by flow cytometry. Whole blood was collected from every live animal on Study Days 0, 1, 3, 4, 7, and 9. On Study Day 0, 1, and 3 (4 of 6 animals), blood was analyzed for blood gases. Excess blood was processed to plasma and stored for potential future analysis.

Main endpoints:

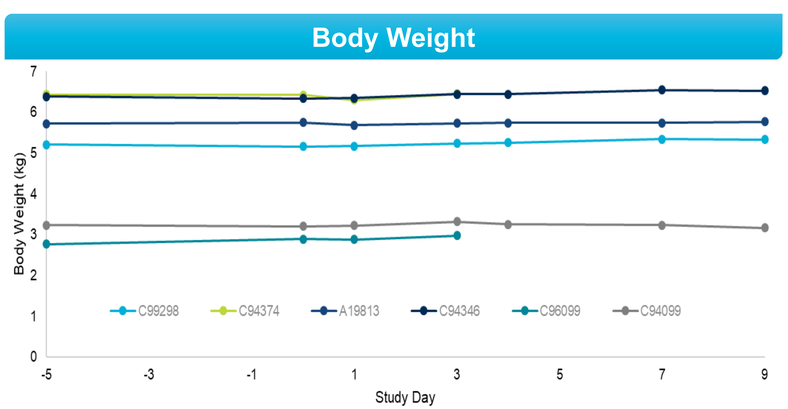
- Clinical observations twice daily through the end of the study
- Body weights (BW) (under sedation) collected on Study days 0, 1, 3, 4, 7, and 9
- BALF collection for $TCID_{50}$ and PCR
- Lungs and nasal turbinates collected at necropsy, fixed, stained, and analyzed for microscopic pathology

Animals Used on Study

Cynomolgus macaques and all non-human primates became highly in demand immediately following the public health emergency declaration of the novel coronavirus. However, the Charles River Laboratories facility in Stillwell, KS was being closed and had a colony of cynomolgus macaques that were going to be euthanized for disposal. We offered to take these animals and use them on this SARS-CoV-2 study. The table below shows how these animals had been used on previous studies. The animals were 4.5 to 5.5 years of age and ranged from 2.778 to 6.434 kg at the time of dosing.

Sex	ID Number	DOB	Times Dosed at CRL	Origin	Large Molecule Dosed?
M	A19813	17-May-15	25	Vietnam	No
M	C94346	19-Apr-16	5	China	Yes
M	C94374	26-May-16	3	China	No
M	C99298	17-May-15	20	China	Yes
F	C94099	11-Feb-16	17	China	Yes
F	C96099	8-Nov-15	16	China	No

Body Weight



Blood Gas

Animal IDs	Sex	Study Day		
		0	1	3
		11/30/2020	12/1/2020	12/3/2020
C99298	M	43.1	36.4	48.6
C94374	M	32.2	34.6	ND†
A19813	M	38.5	45.9	61.8
C94346	M	22.2	30.1	33.5
C96099	F	35.1	30.4	ND†
C94099	F	37.5	40.8	36.7

Virus Titer

Collection Day	Sample ID	BALF Copies/mL	Nasal Swabs Copies/mL
Pre	A19813	N/A	N/A
Pre	C94099	N/A	N/A
Pre	C94346	N/A	N/A
Pre	C94374	N/A	N/A
Pre	C96099	N/A	N/A
Pre	C99298	N/A	N/A
D1	C99298	N/A	4.10E+05
D1	C94374	N/A	5.40E+08
D1	A19813	N/A	2.10E+06
D1	C94346	N/A	3.80E+06
D1	C96099	N/A	3.80E+06
D1	C94099	N/A	4.50E+05
D3	A19813	1.80E+04	7.60E+05
D3	C94099	2.20E+04	3.20E+04
D3	C94346	N/A	5.10E+06
D3	C99298	3.70E+03	2.20E+06
D4	A19813	3.70E+04	3.10E+05
D4	C94099	6.70E+02	2.90E+04
D4	C94346	undetectable†	8.90E+04
D4	C99298	4.90E+03	2.10E+06
D7	A19813	N/A	1.60E+08
D7	C94099	undetectable†	4.30E+03
D7	C94346	N/A	5.20E+05
D7	C99298	N/A	1.20E+07
D9	C94346	N/A	3.70E+03
D9	C99298	undetectable†	2.20E+05

Conclusions

- Infecting Cynomolgus macaques with SARS-CoV-2 resulted in a mild disease
- Virus could be detected largely in the nasal passages but also the BALF

Contact Information

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