Supplementary Appendix

List of Investigators

Name	Institution	Location
Albertson, Timothy	UC Davis Health Investigational Drug Pharmacy	Sacramento, CA, USA
	UC Davis Medical Center	Sacramento, CA, USA
Cisneros Herreros, José Miguel	Hospital Universitario Virgen Del Rocío	Sevilla, Spain
Clevenbergh, Philippe	University Hospital Brugmann	Brussels, Belgium
Comellas, Alejandro*	University of Iowa	Iowa City, IA, USA
Dougan, Michael	Massachusetts General Hospital	Boston, MA, USA
	Massachusetts General Hospital Translational and Clinical Research Center	Boston, MA, USA
	Massachusetts General Hospital, Clinical Trials Pharmacy	Boston, MA, USA
Fulton, Jennifer*	Baptist Health Research Institute	Jacksonville, FL, USA
	Baptist Medical Center	Jacksonville, FL, USA
	Baptist Medical Center Beaches	Jacksonville, FL, USA
	Baptist Medical Center South	Jacksonville, FL, USA
Górgolas Hernández-Mora, Miguel	Hospital Universitario Fundación Jiménez Díaz	Madrid, Spain
Krishna, Ganesh	El Camino Health	Mountain View, CA, USA
	Palo Alto Medical Foundation	Mountain View, CA, USA
Meehan, Patrick*	MultiCare Health System-Tacoma General Hospital	Tacoma, WA, USA
Ostrosky-Zeichner, Luis*	Memorial Hermann Hospital – Texas Medical Center	Houston, TX, USA
	University of Texas Physicians – Texas Medical Center	Houston, TX, USA
Robinson, Philip	Hoag Memorial Hospital Presbyterian	Newport Beach, CA, USA
Tarcisio de Faria Freire, Antonio	Santa Casa De Misericórdia de Belo Horizonte	Belo Horizonte, Brazil
Thacker, Amber	Regional One Health	Memphis, TN, USA

*Did not screen any participants for inclusion.

Clinical Status

Clinical status was measured by site staff on an 8-point ordinal scale:

1) Death
2) Hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO)
3) Hospitalized, on noninvasive ventilation or high-flow oxygen devices
4) Hospitalized, requiring supplemental oxygen
5) Hospitalized, not requiring supplemental oxygen but requiring ongoing medical care (COVID-19-related or otherwise)
6) Hospitalized, not requiring supplemental oxygen and no longer requiring ongoing medical care for COVID-19 (to be used if hospitalization is extended for infection control reasons)
7) Not hospitalized, limitation on activities and/or requiring home oxygen
8) Not hospitalized, no limitation on activities

Inclusion and Exclusion Criteria

Eligible male or female participants were between 18 and 79 years of age at screening and had confirmed presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection as determined by reverse transcription polymerase chain reaction or another method that measures SARS-CoV-2 viral genetic material, or by specific SARS-CoV-2 antigen tests in any specimen (eg, respiratory fluid sample, saliva, or blood). For MAD, confirmed presence of SARS-CoV-2 viral genetic material in a sample must have been obtained ≤72 hours before screening. Eligible participants had symptoms consistent with COVID-19 indicated by ≥1 of the following: fever, cough, sore throat, malaise, headache, muscle pain, shortness of breath, new loss of taste or smell, nausea, chills, fatigue, rhinorrhea, diarrhea, vomiting, or radiographic infiltrates by imaging consistent with COVID-19 (if performed as part of a participant's standard medical care). For both SAD and MAD,

onset of symptoms must have occurred within 15 days of screening. Participants were to have total body weight ≥50 kg (110 lb) and body mass index (BMI) <40 kg/m^2 (participants 76–79 years of age were to have BMI <35 kg/m^2).

Key exclusion criteria included evidence of critical illness at the time of screening and randomization, defined by ≥1 of the following: respiratory failure (need for mechanical ventilation or ECMO) or clinical diagnosis of respiratory failure (ie, clinical need for mechanical ventilation or ECMO, even if not able to be administered in a setting of resource limitation); multi-organ dysfunction/failure, cardiac failure, or septic shock; anticipation by the study investigator of progression to critical disease, including need for mechanical ventilation, within 24 hours of enrollment; preexisting moderate to severe or poorly controlled cardiovascular disease, including hypertension or diabetes, or moderate to severe or poorly controlled chronic lung diseases, including asthma or chronic obstructive pulmonary disease; known medical history of ischemic heart disease, heart failure, dysrhythmia, or other preexisting cardiac condition that, in the opinion of the investigator, might confuse interpretation of electrocardiogram (ECG) or cardiovascular findings; known medical history of recent acute or chronic liver disease (other than nonalcoholic hepatic steatosis), including chronic or active hepatitis B or C infection or primary biliary cirrhosis; history of venous thromboembolic events; known HIV infection with a $CD4^+$ cell count <500/mm^3 and/or resulted in the participant receiving a boosted antiretroviral treatment regimen; known medical history of recurrent seizures; confirmed concurrent active systemic infection other than COVID-19; current diagnosis of cancer, unless in remission and untreated; receipt of any medications or substances that are strong inhibitors or inducers of CYP3A4; receipt of systemic oral, intravenous, or intramuscular corticosteroid therapy (>40 mg/day equivalent prednisolone) within the previous 28 days, except as required for COVID-19 treatment; female participants who were taking hormonal therapy for contraception or as estrogen replacement therapy, post menopause; previous administration of any investigational drug within 30 days or 5 half-lives preceding the first dose of investigational product used in this study (whichever was longer); and any of the following abnormalities in clinical laboratory tests at screening, confirmed

by a single repeat test if deemed necessary: aspartate aminotransferase or alanine transaminase level ≥3 × upper limit of normal (ULN); total bilirubin level ≥1.5 × ULN, unless due to known Gilbert's syndrome; CL_{cr} <60 mL/min using the Cockcroft-Gault equation; or absolute neutrophil count <1000/mm^3.

Participants were allowed to participate if receiving local standard of care for COVID-19, including remdesivir, convalescent serum/plasma, and monoclonal antibody treatments. Participation in an open-label, investigational/observational study for convalescent serum/plasma was also allowed so long as any associated study procedures did not, in the opinion of the investigator, interfere with the current study. Other therapies with emergency use authorization status were allowed after discussion with the sponsor.

In Vivo Nonclinical Safety Studies

Rat and monkey were selected as the toxicology species based on similarities in metabolism of lufotrelvir and PF-00835231 in rats, monkeys, and humans and the lack of pharmacologically relevant species since the target for lufotrelvir and PF-00835231 is a virus-specific protein and off-target potential is low based on conducted assessments. Continuous intravenous infusion was selected for in vivo studies because it is the intended route of clinical administration. The highest dose was selected based on the limit dose of 1000 mg/kg for lufotrelvir and the maximum feasible dose based on solubility for PF-00835231. Study designs and parameters evaluated in toxicology studies were consistent with accepted principles and practices as outlined in ICH, Organization for Economic Co-operation and Development (OECD) guidelines, and national regulations (US Food and Drug Administration [FDA], European Community Directives, and Japan regulations). All definitive studies were conducted in accordance with FDA Good Laboratory Practice regulations in an OECD Mutual Acceptance of Data member state.

24-Hour Continuous Intravenous Infusion Toxicity Study of Lufotrelvir in Rats

Briefly, 8-week-old male and female Sprague-Dawley rats (n=15/sex/group) implanted with femoral catheters exteriorized between the scapulae were administered as continuous intravenous infusions of control (citrate buffered saline) or lufotrelvir at doses of 80, 360, and 1000 mg/kg. Ten animals per sex per group were necropsied on Day 2, and the remaining animals (n=5/sex/group) were necropsied on Day 14 to evaluate potential delayed toxicity and reversibility. Study evaluations included detailed clinical observations, body weights, food consumption, functional observation battery (including activity, posture, gait, righting reflex, stimulus response, pupil response, grip strengths, pain perception, body temperature), clinical pathology (hematology, coagulation, clinical chemistry, urinalysis), toxicokinetics, and macroscopic and microscopic pathology.

24-Hour Continuous Intravenous Infusion Toxicity Study of PF-00835231 in Rats

Briefly, 8-week-old Sprague-Dawley rats (n=15/sex/group) implanted with femoral catheters exteriorized between the scapulae were administered control (citrate buffered saline), Vehicle 1 (2.96 mg/mL ethanol, 12.6 mg/mL polyethylene glycol [PEG] 400, 16 mg/mL sulfobutylether-β-cyclodextrin [SBE-β-CD] in citrate buffered saline), Vehicle 2 (14.8 mg/mL ethanol, 63 mg/mL PEG 400, 80 mg/mL SBE-β-CD in citrate buffer saline), or PF-00835231 at doses of 30, 60, and 120 mg/kg. The 30- and 60-mg/kg doses were formulated in Vehicle 1, and the 120-mg/kg dose was formulated in Vehicle 2. Ten animals per sex per group were necropsied on Day 2, and the remaining animals (n=5/sex/group) were necropsied on Day 14 to evaluate potential delayed toxicity and reversibility. Study evaluations included detailed clinical observations, body weights, food consumption, ophthalmic examinations, pulmonary function (respiratory rate, tidal volume, minute volume), clinical pathology (hematology, coagulation, clinical chemistry, urinalysis), toxicokinetics, and macroscopic and microscopic pathology.

Two-Week Continuous Intravenous Infusion Toxicity Study of Lufotrelvir in Rats

Briefly, 8-week-old male and female Sprague-Dawley rats (n=15/sex/group) implanted with femoral catheters exteriorized between the scapulae were administered control (citrate buffered saline with

Trehalose, pH 4) or lufotrelvir at doses of 70, 360, and 1000 mg/kg/day as a continuous intravenous infusion. Ten animals per sex per group were necropsied on Day 15, and the remaining animals (n=5/sex/group) were necropsied on Day 29 to evaluate potential reversibility. In addition, the potential for aneugenicity or clastogenicity was evaluated via an in vivo micronucleus assessment, and a pulmonary function assessment was incorporated into this study. Study evaluations included detailed clinical observations, body weights, food consumption, ophthalmic examinations, pulmonary function (respiratory rate, tidal volume, minute volume), clinical pathology (hematology, coagulation, clinical chemistry, urinalysis), toxicokinetics, and macroscopic and microscopic pathology.

Two-Week Continuous IV Infusion Toxicity and Toxicokinetic Study of Lufotrelvir in Monkeys

Mauritian cynomolgus monkeys (*Macaca fascicularis*) >2.5 years of age at study start with a weight range of 2.5–4 kg were surgically implanted with a vascular access port/catheter implanted in the left femoral vein with the port located in the left dorsal/thoracic area. In addition, to enable evaluation of the potential effect of lufotrelvir on cardiovascular parameters and body temperature in the conscious and unrestrained state, all animals were instrumented with a radiotelemetry transmitter (model number PhysioTel M-11, Data Sciences International, St. Paul, MN, USA) for recording ECG waveforms, central arterial blood pressure, and body temperature. The transmitter was located intraperitoneally, secured to the body wall, in a position dorsal and lateral to the median plane below the ribs. The blood pressure catheter was placed in the left iliac artery with the tip of the catheter located in the descending aorta. The ECG lead tips were positioned at the left lateral diaphragm and right jugular vein. All surgical procedures were performed based on Institutional Animal Care and Use Committee approved protocols. Housing setup was as specified in the US Department of Agriculture Animal Welfare Act (Code of Federal Regulations, Title 9) and as described in the Guide for the Care and Use of Laboratory Animals (National Research Council, current edition). Catheterized and instrumented monkeys (n=3/sex/group) were administered vehicle control or lufotrelvir at doses of 70, 360, and 1000 mg/kg/day as a continuous intravenous infusion. Two additional animals per sex per group assigned to the control and 360-mg/kg dose groups entered a 2-week recovery phase following the conclusion of the dosing phase. Infusion was stopped on Day 7 or Day 8 for the 1000-mg/kg/day dose

group due to intolerability. Study evaluations included detailed clinical observations, body weights, food consumption, ophthalmic examinations, cardiovascular parameters (ECG, blood pressure, heart rate, body temperature), clinical pathology (hematology, coagulation, clinical chemistry, urinalysis), toxicokinetics, and macroscopic and microscopic pathology.

Supplementary Table 1. Definition of Pharmacokinetic Parameters

Single Ascending Dose[a]				
Parameter	**Dose**	**Analyte**	**Definition**	**Method of Determination**
C_{max}	500 mg	Lufotrelvir and PF-00835231	Maximum plasma concentration	Observed directly from data
t_{max}	500 mg	Lufotrelvir and PF-00835231	Time for C_{max}	Observed directly from data as time of first occurrence
$t_{1/2}$	500 mg	PF-00835231 only	Terminal half-life	$Log_e(2)/k_{el}$, where k_{el} is the terminal phase rate constant calculated by a linear regression of the log-linear concentration–time curve
AUC_{last}	500 mg	Lufotrelvir and PF-00835231	Area under the plasma concentration–time profile from time zero to the time of the last quantifiable concentration (C_{last})	Linear/log trapezoidal method
AUC_{inf}	500 mg	PF-00835231 only	Area under the plasma concentration–time profile from time zero extrapolated to infinite time	$AUC_{last} + (C_{last}*/k_{el})$, where $C_{last}*$ is the predicted plasma concentration at the last quantifiable time point estimated from the log-linear regression analysis
C_{ss}	500 mg	Lufotrelvir and PF-00835231	Concentration at steady state	Lufotrelvir: $AUC_{2–12}/10$ (where $AUC_{2–12}$ is the area under the plasma concentration–time profile from the 2-h time point to the 12-h time point) divided by the 10-h time interval over which C_{ss} was assessed PF-00835231: $AUC_{2–12}/10$ (where $AUC_{2–12}$ is the area under the plasma concentration–time profile from the 2-h time point to the 12-h time point) divided by the 10-h time interval over which C_{ss} was assessed
C_{24}	All doses	Lufotrelvir and PF-00835231	Concentration at the 24-h time point	Observed directly from data
C_{ss} (dn)	500 mg	Lufotrelvir and PF-00835231	Concentration at steady state normalized to lufotrelvir dose	C_{ss}/dose of lufotrelvir

Multiple Ascending Dose		
Parameter	**Definition**	**Method of Determination**
C_{max}	Maximum plasma concentration	Observed directly from data
t_{max}	Time for C_{max}	Observed directly from data as time of first occurrence

$t_{1/2}$[b]	Terminal half-life	$Log_e(2)/k_{el}$, where k_{el} is the terminal phase rate constant calculated by a linear regression of the log-linear concentration–time curve
C_{ss}	Concentration at steady state	Lufotrelvir: $AUC_{48–120}/72$ (where $AUC_{48–120}$ is the area under the plasma concentration–time profile from the 48-h time point to the 120-h time point) divided by the 72-h time interval over which C_{ss} was assessed PF-00835231: $AUC_{24–120}/96$ (where $AUC_{24–120}$ is the area under the plasma concentration–time profile from the 24-h time point to the 120-h time point) divided by the 96-h time interval over which C_{ss} was assessed
C_{120}	Concentration at the 120-h time point	Observed directly from data
C_{ss} (dn)	Concentration at steady state normalized to lufotrelvir dose	C_{ss}/dose of lufotrelvir
C_{120} (dn)	Concentration at the 120-h time point normalized to lufotrelvir dose	C_{120}/dose of lufotrelvir

[a]For the 250-mg dose group, only 4 data points were collected. Therefore, based on the data available, only the C_{24} parameter was reported.
[b]Calculated for the active moiety PF-00835231 only.

Supplementary Table 2. Study Populations

Population	Description
Evaluable	All participants who were randomly assigned to study intervention and received ≥1 dose of study intervention for the given part of the study
Safety	All participants who were randomly assigned to study intervention and received ≥1 dose of study intervention
Pharmacokinetic concentration set	All participants who were randomly assigned to study intervention, received ≥1 dose of study intervention, and in whom ≥1 concentration value is reported for the given part of the study
Pharmacokinetic parameter set	All participants who were randomly assigned to study intervention, received ≥1 dose of study intervention, and in whom ≥1 of the pharmacokinetic parameters of interest are reported for the given part of the study
Biomarker analysis set	All participants who were randomly assigned to study intervention, received ≥1 dose of study intervention, and in whom ≥1 of the biomarkers of interest are reported for the given part of the study

Supplementary Table 3. Incidence and Severity of Treatment-Emergent AEs (All Causality)

	Single Ascending Dose															
	Lufotrelvir 500 mg (N=2)				Lufotrelvir 250 mg (N=2)				Placebo 500 mg[a] (N=2)				Placebo 250 mg[b] (N=2)			
	Mild	Mod	Severe	Total	Mild	Mod	Severe	Total	Mild	Mod	Severe	Total	Mild	Mod	Severe	Total
Any AE	0	0	1 (50.0)	1 (50.0)	0	0	1 (50.0)	1 (50.0)	1 (50.0)	0	1 (50.0)	2 (100.0)	1 (50.0)	0	1 (50.0)	2 (100.0)
Blood and lymphatic system disorders	0	0	0	0	0	1 (50.0)	0	1 (50.0)	0	0	0	0	0	0	0	0
Coagulopathy	0	0	0	0	0	1 (50.0)	0	1 (50.0)	0	0	0	0	0	0	0	0
Cardiac disorders	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)	0	0	0	0
Tachycardia	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)	0	0	0	0
Gastrointestinal disorders	1 (50.0)	0	0	1 (50.0)	0	0	0	0	1 (50.0)	0	0	1 (50.0)	1 (50.0)	0	0	1 (50.0)
Abdominal pain	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)	0	0	0	0
Diarrhea	1 (50.0)	0	0	1 (50.0)	0	0	0	0	1 (50.0)	0	0	1 (50.0)	0	0	0	0
Dyspepsia	0	0	0	0	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)
General disorders and administration site conditions	0	0	0	0	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)
Edema	0	0	0	0	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)
Infections and infestations	1 (50.0)	0	0	1 (50.0)	0	0	0	0	1 (50.0)	0	0	1 (50.0)	0	0	1 (50.0)	1 (50.0)
COVID-19 pneumonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (50.0)	1 (50.0)
Folliculitis	1 (50.0)	0	0	1 (50.0)	0	0	0	0	0	0	0	0	0	0	0	0
Tinea cruris	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)	0	0	0	0
Investigations	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)	1 (50.0)	0	0	1 (50.0)
Hematocrit decrease	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)	0	0	0	0
Hemoglobin decrease	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)	0	0	0	0
Liver function test increase	0	0	0	0	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)
Transaminase increase	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)	0	0	0	0
Musculoskeletal and connective tissue disorders	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)	0	0	0	0
Pain in extremity	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)	0	0	0	0
Renal and urinary disorders	0	0	0	0	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)
Hematuria	0	0	0	0	0	0	0	0	0	0	0	0	1 (50.0)	0	0	1 (50.0)
Respiratory, thoracic, and mediastinal disorders	0	0	0	0	0	0	1 (50.0)	1 (50.0)	0	0	0	0	0	0	0	0
Respiratory failure	0	0	0	0	0	0	1 (50.0)	1 (50.0)	0	0	0	0	0	0	0	0
Vascular disorders	0	0	1 (50.0)	1 (50.0)	0	0	0	0	0	0	1 (50.0)	1 (50.0)	0	0	0	0
Subclavian vein thrombosis	0	0	1 (50.0)	1 (50.0)	0	0	0	0	0	0	1 (50.0)	1 (50.0)	0	0	0	0
Total preferred term events, n	2	0	1	3	0	1	1	2	8	0	1	9	4	0	1	5

	Multiple Ascending Dose											
	Lufotrelvir 500 mg (N=6)				Lufotrelvir 250 mg (N=7)				Placebo (N=4)			
	Mild	**Mod**	**Severe**	**Total**	**Mild**	**Mod**	**Severe**	**Total**	**Mild**	**Mod**	**Severe**	**Total**
Any AE	1 (16.7)	1 (16.7)	0	2 (33.3)	1 (14.3)	1 (14.3)	2 (28.6)	4 (57.1)	1 (25.0)	1 (25.0)	1 (25.0)	3 (75.0)
Blood and lymphatic system disorders	0	0	0	0	1 (14.3)	0	0	1 (14.3)	0	0	0	0
Thrombocytopenia	0	0	0	0	1 (14.3)	0	0	1 (14.3)	0	0	0	0
Cardiac disorders	0	0	0	0	1 (14.3)	0	0	1 (14.3)	0	0	0	0
Bradycardia	0	0	0	0	1 (14.3)	0	0	1 (14.3)	0	0	0	0
Ear and labyrinth disorders	0	0	0	0	1 (14.3)	0	0	1 (14.3)	0	0	0	0
Tympanic membrane perforation	0	0	0	0	1 (14.3)	0	0	1 (14.3)	0	0	0	0
Gastrointestinal disorders	0	0	0	0	0	1 (14.3)	0	1 (14.3)	0	0	0	0
Abdominal pain upper	0	0	0	0	0	1 (14.3)	0	1 (14.3)	0	0	0	0
General disorders and administration site conditions	2 (33.3)	0	0	2 (33.3)	0	1 (14.3)	0	1 (14.3)	0	1 (25.0)	0	1 (25.0)
Discomfort	0	0	0	0	0	1 (14.3)	0	1 (14.3)	0	0	0	0
Inflammation	0	0	0	0	0	0	0	0	0	1 (25.0)	0	1 (25.0)
Infusion site extravasation	1 (16.7)	0	0	1 (16.7)	0	0	0	0	0	0	0	0
Noncardiac chest pain	1 (16.7)	0	0	1 (16.7)	0	0	0	0	0	0	0	0
Edema peripheral	1 (16.7)	0	0	1 (16.7)	0	0	0	0	0	0	0	0
Infections and infestations	0	0	0	0	0	0	1 (14.3)	1 (14.3)	1 (25.0)	1 (25.0)	0	2 (50.0)
Bronchitis bacterial	0	0	0	0	0	0	0	0	1 (25.0)	0	0	1 (25.0)
COVID-19 pneumonia	0	0	0	0	0	0	0	0	0	1 (25.0)	0	1 (25.0)
Pneumonia	0	0	0	0	0	0	1 (14.3)	1 (14.3)	0	0	0	0
Stoma site cellulitis	0	0	0	0	0	1 (14.3)	0	1 (14.3)	0	0	0	0
Injury, poisoning, and procedural complications	0	0	0	0	0	0	0	0	1 (25.0)	0	0	1 (25.0)
Fall	0	0	0	0	0	0	0	0	1 (25.0)	0	0	1 (25.0)
Investigations	0	0	0	0	0	0	0	0	1 (25.0)	1 (25.0)	0	2 (50.0)
Fibrin D dimer increase	0	0	0	0	0	0	0	0	0	1 (25.0)	0	1 (25.0)
Liver function test increase	0	0	0	0	0	0	0	0	1 (25.0)	0	0	1 (25.0)
Metabolism and nutrition disorders	0	0	0	0	1 (14.3)	0	0	1 (14.3)	0	0	0	0
Hyperglycemia	0	0	0	0	1 (14.3)	0	0	1 (14.3)	0	0	0	0
Musculoskeletal and connective tissue disorders	0	0	0	0	0	0	0	0	1 (25.0)	0	0	1 (25.0)
Arthralgia	0	0	0	0	0	0	0	0	1 (25.0)	0	0	1 (25.0)
Renal and urinary disorders	0	0	0	0	1 (14.3)	0	0	1 (14.3)	0	0	0	0
Oliguria	0	0	0	0	1 (14.3)	0	0	1 (14.3)	0	0	0	0
Respiratory, thoracic, and mediastinal disorders	0	1 (16.7)	0	1 (16.7)	0	1 (14.3)	2 (28.6)	3 (42.9)	0	0	1 (25.0)	1 (25.0)
Acute respiratory distress syndrome	0	0	0	0	0	0	1 (14.3)	1 (14.3)	0	0	0	0
Dyspnea	0	0	0	0	0	0	1 (14.3)	1 (14.3)	0	0	0	0
Hypoxia	0	0	0	0	0	0	0	0	0	0	1 (25.0)	1 (25.0)

Pulmonary embolism	0	1 (16.7)	0	1 (16.7)	0	0	0	0	0	0	1 (25.0)	1 (25.0)
Sleep apnea syndrome	0	0	0	0	0	1 (14.3)	0	1 (14.3)	0	0	0	0
Skin and subcutaneous tissue disorders	0	0	0	0	1 (14.3)	0	0	1 (14.3)	0	0	0	0
Rash	0	0	0	0	1 (14.3)	0	0	1 (14.3)	0	0	0	0
Total preferred term events, n	3	1	0	4	6	4	3	13	4	3	2	9

AE=adverse event; COVID-19=coronavirus disease 2019; Mod=moderate; SAE=serious AE.

All data are n (%) unless stated otherwise. Results are for the safety analysis set. If the same participant in a given treatment had more than 1 occurrence in the same preferred term event category, only the most severe occurrence was counted. Participants were counted only once per treatment per event.

[a]Placebo group for the lufotrelvir 500-mg dose.

[b]Placebo group for the lufotrelvir 250-mg dose.

Supplementary Table 4. Overview of Nonclinical Safety Testing Program

Study[a,b]	Study Number[c]	Concentration or Dose	GLP Compliance
Lufotrelvir (Prodrug)[d]			
Single-dose toxicity			
Acute toxicity, 24-h continuous IV infusion with 13-d recovery, rat	100-455 (20GR105)	0, 80, 360, 1000 mg/kg	Yes
Repeat-dose toxicity			
2-wk continuous IV infusion with 2-wk recovery, rat	100-466 (20GR143)	0, 70, 360, 1000 mg/kg	Yes
2-wk continuous IV infusion with 2-wk recovery, monkey	20256263 (20GR144)	0, 70, 360, 1000 mg/kg	Yes
Genotoxicity			
Bacterial reverse mutation assay	AG19PT.503ICH.BTL (20GR106)	1.5–5000 µg/plate	Yes
Induction of micronuclei in cultured human lymphoblastoid TK6 cells	AG19PT.361ICH.BTL (20GR107)	125–500 µg/mL	Yes
Micronucleus in rat peripheral whole blood	100-466 (20GR143)	0, 70, 360, 1000 mg/kg	Yes
Other toxicity studies			
Human hemocompatibility	6000781 (20GR104)	0 (whole blood); Vehicle control[e]: 0, 0.44, 4.4, 44 µg/mL	Yes
PF-00835231 (Active Moiety)			
Single-dose toxicity			
Acute toxicity, 24-h continuous IV infusion with 13-d recovery, rat	100-453 (20GR082)	0[e], 0[f], 0[g], 30[h], 60[h], 120[i] mg/kg	Yes
Genotoxicity			
Bacterial reverse mutation assay	AG16EX.503ICH.BTL (20GR125)	1.5–5000 µg/plate	Yes
Induction of micronuclei in cultured human lymphoblastoid TK6 cells	AG16EX.361ICH.BTL (20GR127)	126–473 µg/mL	Yes
Other toxicity studies			
Human hemocompatibility	6000774 (20GR126)	0 (whole blood); Vehicle control 1[j]: 0, 0.34, 3.4, 34 µg/mL; Vehicle control 2[k]: 0, 0.54, 5.4, 54 µg/mL	Yes

GLP=Good Laboratory Practice; IV=intravenous; OECD=Organization for Economic Co-operation and Development; PEG=polyethylene glycol; SBE-β-CD=sulfobutylether-β-cyclodextrin; TK6=thymidine kinase 6.
[a]All GLP studies were conducted in an OECD Mutual Acceptance of Data member state.
[b]All in vivo studies were conducted with male and female animals.
[c]Where applicable, the sponsor reference number is provided in parentheses.
[d]Phosphate ester prodrug of PF-00835231 (active moiety).
[e]Citrate buffered saline, pH 4.5.
[f]Vehicle 1: 2.96 mg/mL ethanol, 12.6 mg/mL PEG 400, 16 mg/mL SBE-β-CD in citrate buffered saline.
[g]Vehicle 2: 14.8 mg/mL ethanol, 63 mg/mL PEG 400, 80 mg/mL SBE-β-CD in citrate buffer.
[h]Formulated with Vehicle 1.
[i]Formulated with Vehicle 2.
[j]2.96 mg/mL ethanol, 12.6 mg/mL PEG 400, 16.2 mg/mL SBE-β-CD, 7.31 mg/mL sodium chloride, 5.08 mM citrate buffer.
[k]14.8 mg/mL ethanol, 63.0 mg/mL PEG 400, 80.0 mg/mL SBE-β-CD, 5.4 mM citrate buffer.

Supplementary Table 5. Summary of Key Findings in Rat In Vivo Safety Studies

Study	Doses Tested	Key Findings
24-h continuous IV infusion with 13-d recovery in rats	PF-07304814: 0, 80, 360, 1000 mg/kg PF-00835231: 0, 30, 60, 120 mg/kg (note: For PF-00835231, 120 mg/kg was the highest feasible dose based on solubility)	Changes typically seen in rodent infusion studies at the infusion sites or in other tissues with similar incidence and severity across all groups including control. Consisted of hemorrhage, intimal proliferation, mixed leukocyte infiltrate, and/or thrombi. Small thrombi were rarely present in small pulmonary vessels in control and treated animals without dose relationship, which is consistent with secondary findings known to occur in infusion studies. The NOAELs were the highest tested doses of 1000 mg/kg/d (lufotrelvir) and 120 mg/kg (PF-00835231).
2-wk continuous IV infusion with 2-wk recovery in rats	PF-07304814: 0, 70, 360, 1000 mg/kg	No lufotrelvir– (or PF-00835231–) related changes beyond those expected in a rodent continuous infusion study, including low-grade inflammation and thrombi at infusion site or in lungs. There were no lufotrelvir– (or PF-00835231–) related effects on respiratory function. The NOAEL was 1000 mg/kg/d, the highest dose tested.

IV=intravenous; NOAEL=no-observed-adverse-effect level.

Supplementary Table 6. Summary of Key Findings in Monkey In Vivo Safety Studies

Study	Doses Tested	Key Findings
2-wk continuous IV infusion with 2-wk recovery in monkeys	Lufotrelvir: 0, 70, 360, 1000 mg/kg (note: recovery animals included in control and 360-mg/kg groups only)	All animals at 0, 70, or 360 mg/kg/d survived until end of study. All animals at 1000 mg/kg/d underwent necropsy on Day 8 due to dose-limiting clinical observations, including hunched posture, pale mucous membranes, dehydration, rapid shallow breathing, and high body temperature. No primary target organs of toxicity were identified. However, infusion procedure–related anatomic pathology findings indicating inflammatory effects were seen in all dose groups, including controls. Findings at 70 mg/kg/d generally overlapped in incidence and severity with findings in the control group and were considered not drug related. At 360 mg/kg/d, there was a greater incidence and/or severity of these effects, indicating test drug–related exacerbation of findings that are commonly associated with the continuous infusion procedure. Any microscopic changes reversed or were in the process of recovery after 14 d of drug-free period. The intermediate dose of 360 mg/kg/d was the highest tolerated dose, and the low dose of 70 mg/kg/d was considered to be the NOAEL.

IV=intravenous; NOAEL=no-observed-adverse-effect level.

Supplementary Table 7. Incidence of Main Infusion Site Findings and Pulmonary Thrombus From Dosing-Phase Rats (14-d IV infusion/14-d recovery, Lufotrelvir; n=10/Sex/Dose)

	Males				Females			
Dose, mg/kg/d	**0**	**70**	**360**	**1000**	**0**	**70**	**360**	**1000**
Lung, thrombus	2	0	2	2	0	0	1	1
Infusion site, thrombus	9	9	9	8	8	6	8	4

Supplementary Table 8. Incidence of Main Infusion Site Findings From Recovery-Phase Rats (14-d IV infusion/14-d recovery, Lufotrelvir; n=5/Sex/Dose)

	Males				Females			
Dose, mg/kg/d	**0**	**70**	**360**	**1000**	**0**	**70**	**360**	**1000**
Lung, thrombus	0	0	0	0	0	0	0	0
Infusion site, thrombus	5	4	5	5	5	3	4	3

Supplementary Table 9. Incidence of Procedure- and Lufotrelvir–Related Inflammatory Findings and Thrombus in Dosing-Phase Monkeys (n=3/Sex/Dose)

	Males				Females			
Dose, mg/kg/d	**0**	**70**	**360**	**1000**	**0**	**70**	**360**	**1000**
Brain								
Infiltration, mixed cell, choroid plexus	0	0	0	2	0	0	0	3
Inflammation, mixed cell	0	0	0	0	0	0	0	1
Grand, prostate								
Infiltration, mixed cell	0	0	0	2	-	-	-	-
Heart								
Inflammation, mixed cell	0	0	0	2	1	0	1	1
Infiltration, mixed cell	0	1	0	0	0	1	0	0
Joint, femorotibial								
Inflammation, mixed cell	0	0	0	2	0	0	0	0
Kidney								
Inflammation, mixed cell	0	0	0	2	0	0	0	3
Liver								
Infiltration, mixed cell, portal	1	1	1	3	0	1	2	2
Lung								
Inflammation, mixed cell, interstitial	1	0	1	2	1	0	2	2
Thrombus	0	0	0	2	0	0	0	2
Nerve, sciatic								
Infiltration, mixed cell	0	0	0	1	0	1	0	1
Site, administration, catheter tip								
Thrombus	1	0	1	1	1	1	0	1
Ureter								
Infiltration, mixed cell, perivascular	0	0	0	1	0	0	0	1
Urinary bladder								
Infiltration, mixed cell	0	0	0	0	0	0	0	2

Supplementary Table 10. Incidence of Procedure- and Lufotrelvir–Related Inflammatory Findings and Thrombus in Recovery-Phase Monkeys (n=2/Sex/Dose)

	Males		Females	
Dose, mg/kg/d	**0**	**360**	**0**	**360**
Heart				
Inflammation, mixed cell	0	1	0	0
Kidney				
Inflammation, mixed cell	0	1	0	0
Liver				
Infiltration, mixed cell, portal	0	1	0	0
Lung				
Inflammation, mixed cell, interstitial	0	1	0	0
Thrombus	0	1	0	0
Site, administration, catheter entrance				
Inflammation, mixed cell	0	2	0	0

Supplementary Table 11. Exposures of Lufotrelvir or PF-00835231 in Nonclinical Safety Studies

Study	Dose, mg/kg/d	Unbound C_{max},[a] µg/mL	Unbound AUC_{last},[a] µg·h/mL
Single-Dose Toxicity Studies			
24-h continuous IV infusion toxicity study of lufotrelvir[b] in rats (n=15/sex/group; study 100-455)			
Lufotrelvir exposure	80	0.68	12.2
	360	2.3	34.4
	1000	8.8	142
PF-00835231 exposure	80	0.75	15.1
	360	2.1	44.1
	1000	5.9	122
24-h continuous IV infusion toxicity study of PF-00835231 in rats (n=15/sex/group; study 100-453)			
PF-00835231 exposure	30[c]	0.71	10.5
	60[c]	0.77	14.0
	120[d]	2.5	34.7
Repeat-Dose Toxicity Studies			
2-wk continuous IV infusion toxicity study of lufotrelvir in rats (n=15/sex/group; study 100-466)			
Lufotrelvir exposure	70	0.40	6.52
	360	2.15	32.7
	1000	4.09	83.8
PF-00835231 exposure	70	0.49	10.4
	360	1.82	35.4
	1000	3.93	82.1
2-wk continuous IV infusion toxicity study of lufotrelvir in monkeys (n=3–5/sex/group; study 20256263)			
Lufotrelvir exposure	70	0.30	5.45
	360	1.79	30.4
	1000	14.0[g]	227
PF-00835231 exposure	70	0.67	12.4
	360	3.92	71.9
	1000	24.8[e]	459

AUC=area under the concentration–time curve; IV=intravenous; PEG=polyethylene glycol; SBE-β-CD=sulfobutylether-β-cyclodextrin.

[a]In these studies, C_{max} and AUC values indicate mean plasma concentrations. Reported values were obtained near termination or as specified. Unbound C_{max} and AUC based on protein binding for each species (lufotrelvir fraction unbound=0.379, 0.361, and 0.184 in Sprague-Dawley rats, cynomolgus monkeys, and humans, respectively; PF-00835231 fraction unbound=0.255, 0.441, and 0.449 in Sprague-Dawley rats, cynomolgus monkeys, and humans, respectively).

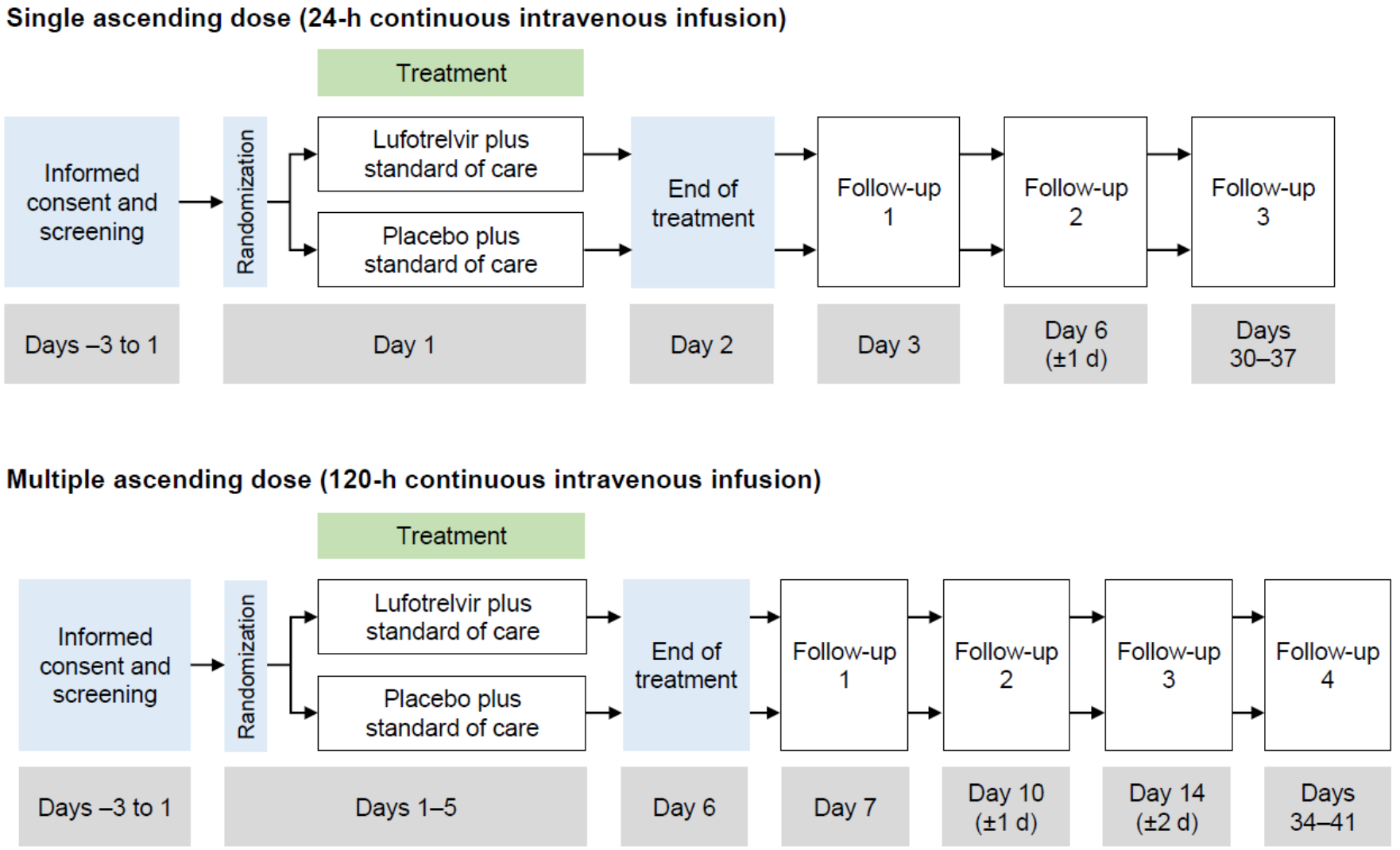
[b]Phosphate ester prodrug of PF-00835231 (active moiety).

[c]Formulated in Vehicle 1 (2.96 mg/mL ethanol, 12.6 mg/mL PEG 400, 16 mg/mL SBE-β-CD in citrate buffered saline).

[d]Formulated in Vehicle 2 (14.8 mg/mL ethanol, 63 mg/mL PEG 400, 80 mg/mL SBE-β-CD in citrate buffer).

[e]Day 1 C_{max} values or C_{max} exposure margins were reported for 1000 mg/kg/d monkeys.

Supplementary Figure 1. Study design for the single ascending dose group and multiple ascending dose group.



Supplementary Figure 2. Biomarker analyses for the (A) SAD and (B) MAD groups. Data are mean (SD). Horizontal grey lines represent normal range, except for D-dimer and hs-CRP, where they represent the upper limit of normal. aPTT=activated partial thromboplastin time; hs-CRP=high-sensitivity C-reactive protein; MAD=multiple ascending dose; PT=prothrombin time; SAD=single ascending dose; SD=standard deviation.