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Supplemental Figure 1 Legend: I-SPY COVID trial design. Patients that enter the hospital, have a positive SARS-CoV-2 test by PCR or rapid antigen testing, and are treated with ≥ 6 L/min oxygen or are intubated are screened for trial eligibility. Eligible patients or their designated surrogates are asked if they want to enroll on the therapeutic portion of the trial, in which all enrolled patients receive backbone therapy with or without an additional investigational agent. An observational component, separate from the platform trial, follows ICU patients via medical records under a waiver of consent mechanism. Randomized patients who decline consent to their randomized treatment are automatically enrolled in the observational cohort, for which there is a waiver for consent to collect follow-up data. This approach enables analyses on the ITT population, consisting of all randomized patients. Of note, this figure is published in BMJ Open (reference # 5 in main text—will ask for reprint permissions)

Supplemental Figure 2: Map of participating sites during enrollment for agents presented in this manuscript

Supplemental Figure 3: Timeline of agents

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Supplemental Figure 5: Modeled Time To Death, mITT Population Posterior cumulative mortality functions (solid lines) and 95% quantile credible intervals (shaded area between dotted lines) conditioned on randomisation arm and marginalised over the empirical distribution of screening COVID level (5 vs 6/7) in the mITT population.

Supplemental Figure 6: Time-To-Recovery, ITT Population Aalen-Johansen cumulative incidence curves showing the proportion of recovered patients in the ITT population up to 60 days of follow-up post randomization in the presence of death prior to recovery as a competing risk. Numbers below each panel show patients at risk across time. Shaded areas show 95% pointwise confidence intervals.

Supplemental Figure 7: Mortality, ITT Population Kaplan-Meier curves for overall survival in the ITT population according to treatment arm. The x-axis shows time in days. Numbers below each panel show patients at risk across time. Shaded areas show 95% pointwise confidence intervals.

Supplemental Figure 8: Clinical Events By Arm in mITT Population

Supplemental Table 1. **Modified WHO Ordinal Scale**

Supplemental Table 2. Treatment Regimens

Supplemental Table 3 – Dates of Opening and Closing for First Seven Agents in I-SPY COVID Trial

ONLINE SUPPLEMENT

Supplementary Statistical Methods:

The primary study analyses were conducted in a modified intention-to-treat (mITT) population, consisting of all patients who were randomized and signed informed consent. We also performed pre-specified analyses in the intention-to-treat (ITT) population, defined as all randomized patients irrespective of whether they signed the informed consent and received an investigational agent or not. This analysis was enabled by tracking outcomes in the observational cohort of randomized patients declining consent (Supplemental **Figure 1**). The ITT population retains the original randomization (see consort diagram **Figure 1**) by including the 172 patients declining consent after randomization and is thus unaffected by any potential differences in consent rates across study arms. It should be noted that the ITT and mITT populations are referred to as the Super ITT and ITT populations, respectively, in the protocol and SAP. The background to this nomenclature designation is the difference in the construction of these populations compared to how ITT and mITT populations are constructed in trials employing a randomization procedure where full consent precedes randomization. For simplicity we refer to ITT and mITT in this manuscript.

Bayesian proportional-hazard Weibull regression was used to model the hazard functions for all-cause mortality and for time to mechanical ventilation or death for patients who were not mechanically ventilated at baseline. In these analyses, follow-up times were censored at 60 days if patients were still at risk. Weakly informative priors were used for both models; see SAP Supplement for details. The models were fit using Markov chain Monte Carlo methods with 4,000 draws from the joint posterior distribution; we report medians of the posterior hazard ratio (HR) distributions for investigational agents compared with concurrently randomized controls, and 95% quantile-based credible intervals. Detailed descriptions of the models are found in the SAP Supplement.

Nonparametric Aalen-Johansen cumulative incidence curves were computed to estimate the proportion of recovered patients by treatment arm up to 60 days of follow-up in the presence of death prior to recovery as a competing risk. Kaplan-Meier curves were constructed to estimate survival curves

by treatment arm. In addition, we used Bayesian models to estimate posterior cumulative incidence functions for recovery and survival functions. For recovery, noting that death is a competing event to recovery, this was achieved by fitting an additional Bayesian Weibull model for the cause-specific hazard function for death prior to recovery and, following standard methodology (Putter et al., 2007), the posterior cumulative incidence function for recovery was estimated. The nonparametric and model-based cumulative incidence and survival functions can be used to evaluate the cumulative probability of recovery and survival at specific time points.

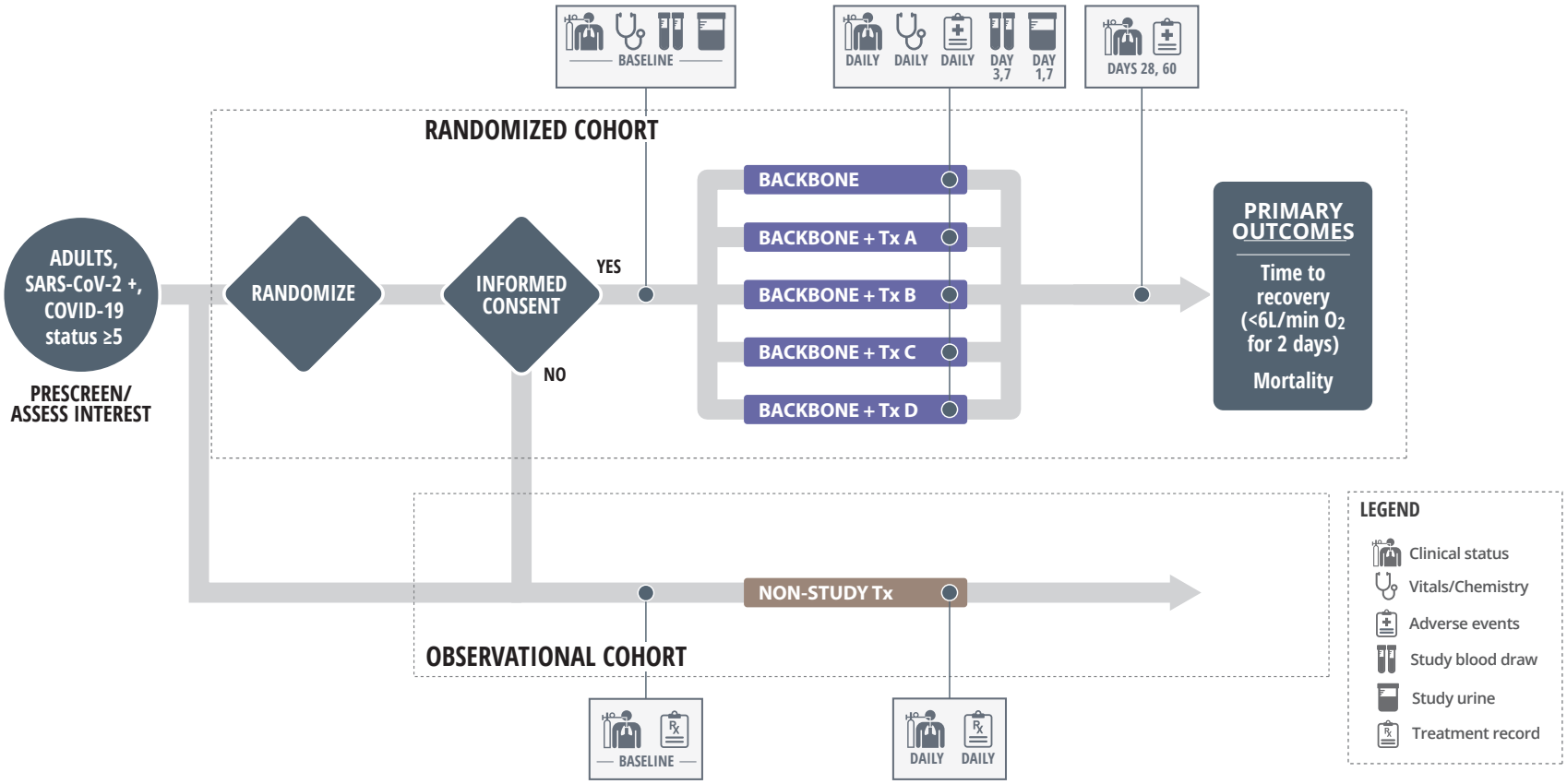
Simulations were used to estimate trial operating characteristics under a variety of possible scenarios ranging from pessimistic, in which the null hypothesis of no benefit holds for every treatment, to optimistic cases in which several of the regimens are truly effective. The power ranged from high (≥85%) for scenarios with big effect sizes on reduction in recovery ($HR_r \geq 1{\cdot}7$) or mortality rates ($HR_m \leq 0{\cdot}5$) to low (around 20%) for small effect sizes ($HR_r = 1{\cdot}2$ and $HR_m = 0{\cdot}8$, respectively). The type 1 error rate varied between 4% and 17%, depending on scenario, which was considered acceptable for a phase 2 signal seeking trial.

Data were analysed biweekly and presented to the independent DMC. The biweekly analyses included evaluations of the pre-specified criteria for graduation, futility, and safety (using concurrently enrolled controls). The analyses were performed by unblinded statisticians and presented to the DMC by the non-voting unblinded DMC statistician.

Efforts to mitigate the potential for operational bias included a clear governance of information dissemination following recommendations from the DMC to close an arm (for graduation or futility). If the DMC recommended arm termination, the DMC Secretary and unblinded non-voting DMC statistician communicated the recommendations to the trial PI, who made the final decision to terminate or not to terminate the arm. After the Study PI had decided to terminate an arm, the Study PI informed the following people: chief data officer, blinded statistician, clinical data management, the unblinded DMC statistician, and the trial Co-PIs. In

turn, the unblinded statistician notified the DMC that the arm is terminated. Site PIs and all other staff remained blinded to information about outcomes until enrollment and 28-day outcomes in each arm were complete. If the study PI decides not to terminate an arm, the study PI informs the DMC secretary and unblinded DMC statistician of the decision to not terminate. The large number of active sites and clinicians involved in the trial as well as the fact that, in general, four trial arms were open in parallel reduces the risk of operational bias during patient enrolment into study arms.

FIGURES



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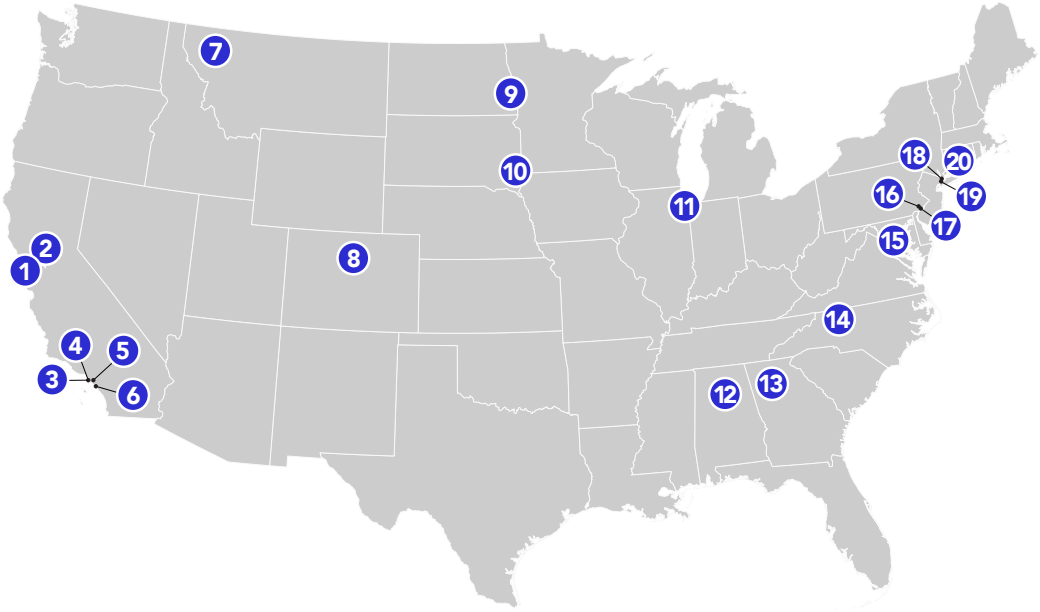
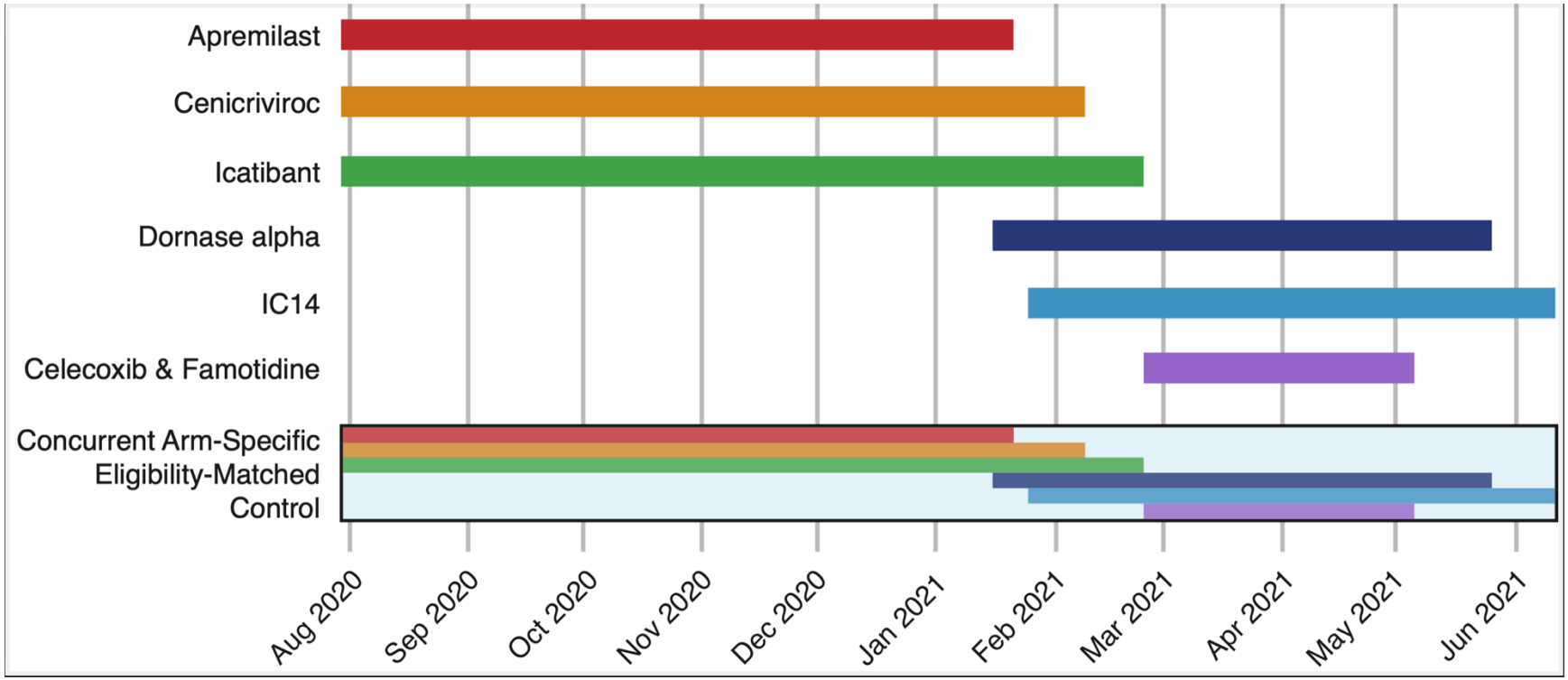


1. University of California San Francisco
2. University of California Davis
3. University of Southern California
4. USC Keck
5. Long Beach Memorial Medical Center
6. Hoag Memorial Hospital Presbyterian
7. Logan Health
8. University of Colorado
9. Sanford Health Fargo
10. Sanford Health Sioux Falls
11. Northwestern University
12. University of Alabama at Birmingham
13. Emory University
14. Wake Forest University
15. Georgetown University
16. Main Line Health Lankenau Medical Center
17. University of Pennsylvania
18. Montefiore Moses
19. Columbia University
20. Yale University

Supplemental Figure 3: Timeline of agents



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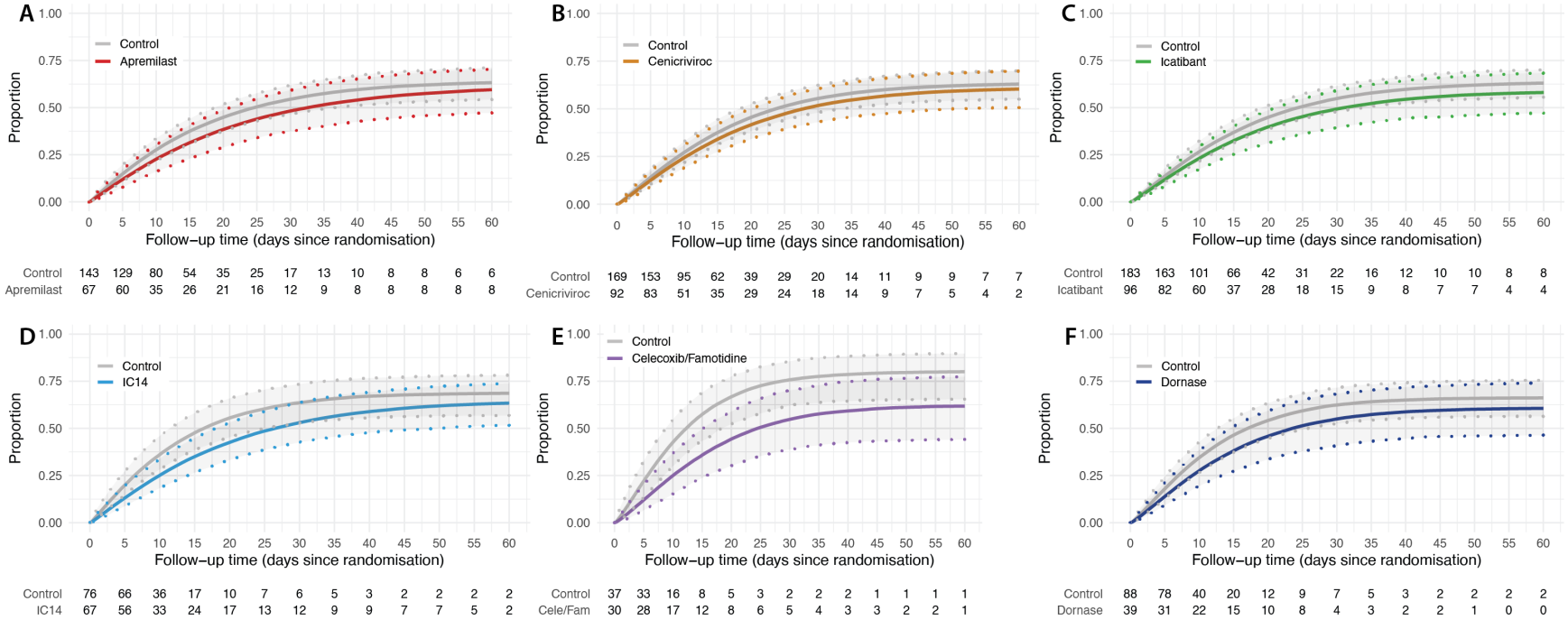

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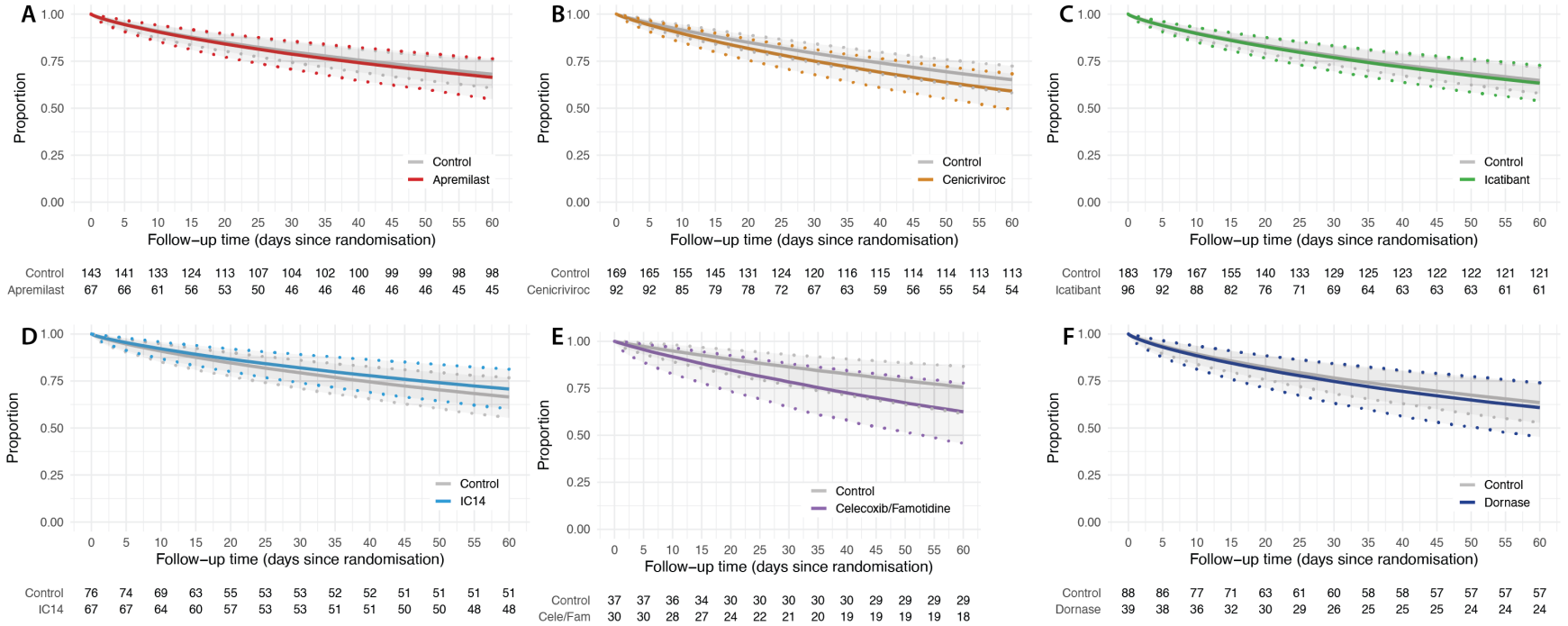

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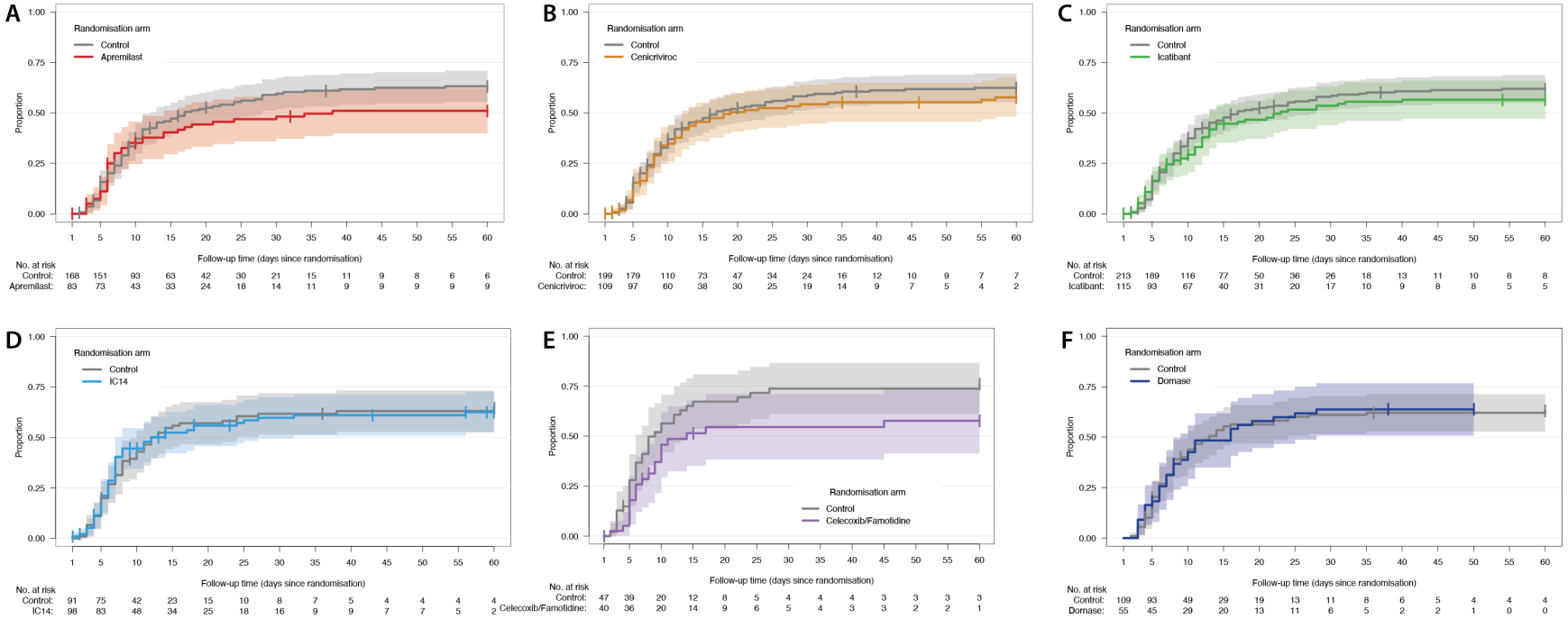

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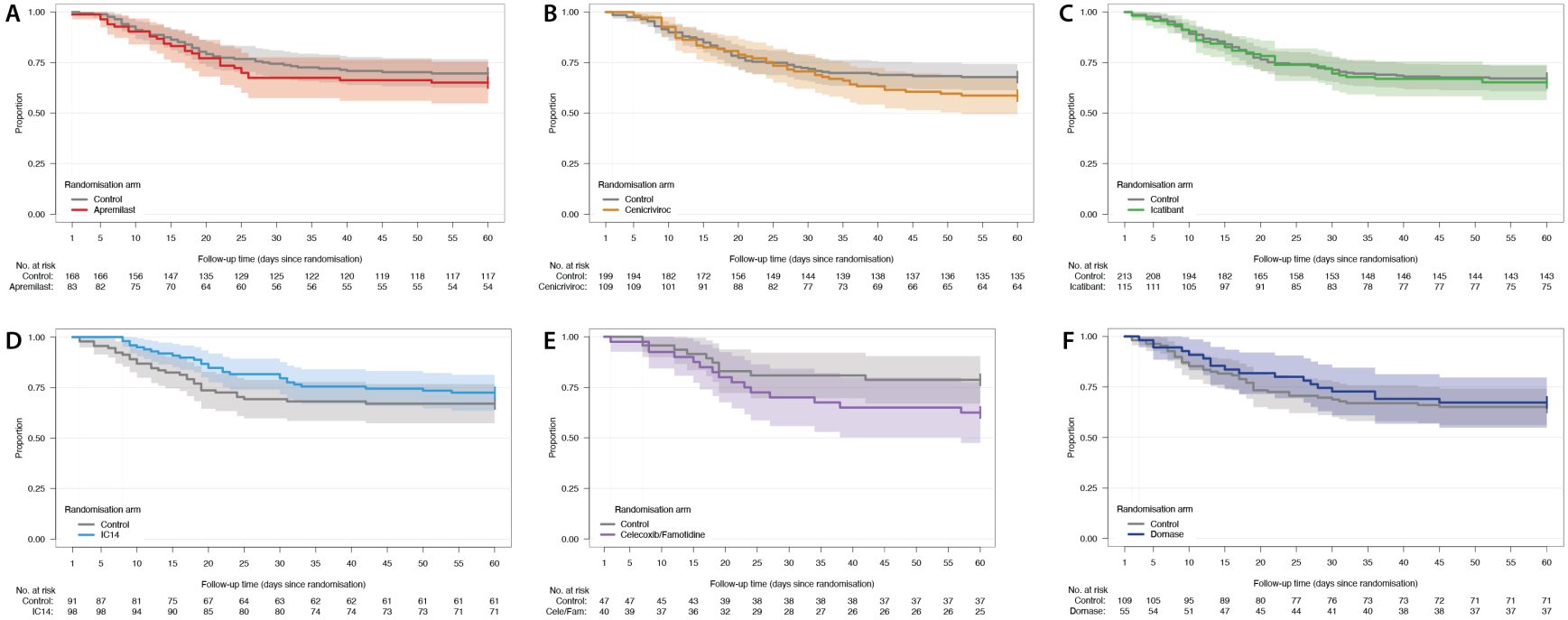

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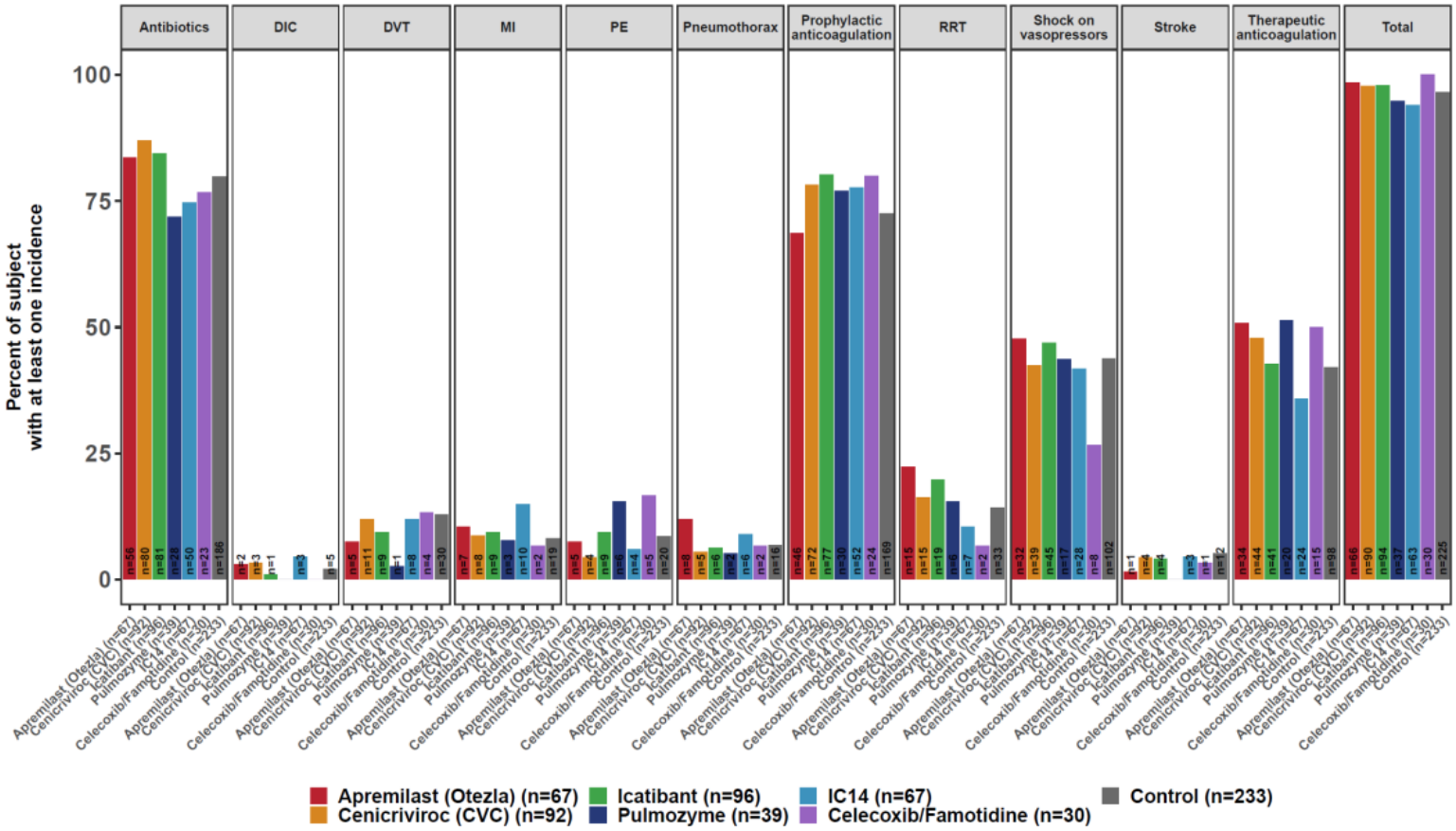

Supplementary Tables

Supplemental Table 1. Modified WHO Ordinal Scale

Score	Description
0	No clinical or virologic evidence of infection
1	Not hospitalized, no limitation on activities
2	Not hospitalized, limitation on activities
3	Hospitalized, not requiring supplemental oxygen
4	Hospitalized, requiring supplemental oxygen (<6 liters/minute by nasal cannula or mask)
5	Hospitalized, on non-invasive ventilation or high-flow oxygen devices (≥6 liters/minute by nasal cannula or mask)
6	Hospitalized, on invasive mechanical ventilation
7	Hospitalized, ventilation plus additional organ support-vasopressors, renal replacement therapy or extracorporeal membrane oxygenation
8	Death

Supplemental Table 2. Treatment Regimens

Arm	Treatment Dose	Route
Control/ Backbone	Remdesivir: 200-mg loading dose on day 1, followed by a daily maintenance dose of 100-mg on days 2 through 10	intravenous
	Dexamethasone: 6 mg intravenous or oral dexamethasone once daily up to 10 days or equivalent for alternate corticosteroid if dexamethasone unavailable	intravenous
Cenicriviroc*	Loading 300 mg qAM followed by 150 mg qPM, 12 hours apart on day 1, then 150 mg BID for total of 14 to 28 days depending on date of hospital discharge	oral
Icatibant*	A safety run-in for the first 10 subjects was conducted using a regimen of 30 mg q8h × 3 days. All subsequent subjects received drug at 30 mg q8h x 6 days.	subcutaneous
Apremilast*	30 mg bid × 14 days	oral
Dornase*	Non-intubated subjects: 2.5 mg BID until hospital discharge, improvement to room air (or baseline oxygen use prior to illness) for 24 hours, or total of 14 days of study drug, whichever comes first. Intubated subjects: 5.0 mg BID in 10 mL normal saline until extubation or 14 days, whichever comes first. If intubated for less than 14 days, extubated subjects received 2.5 mg BID for a total Dornase treatment of 14 days, or until hospital discharge, whichever comes first.	inhaled
celecoxib/ famotidine*	Celecoxib: 400 mg BID for 7 days	oral
	Famotidine: *High dose* 80 mg QID for 7 days followed by 40 mg BID for a course of 14 days	oral
IC14*	4 mg/kg on day 1, followed by 2 mg/kg on days 2, 3, 4	intravenous

*plus backbone

Supplemental Table 3 – Dates of Opening and Closing for First Seven Agents in I-SPY COVID Trial

Arm	Open Date	Close Date
apremilast	July 30, 2020	January 21, 2021
cenicriviroc	July 30, 2020	February 09, 2021
icatibant	July 30, 2020	February 24, 2021
razuprotafib	July 30, 2020	November 30, 2020
celecoxib/famotidine	February 24, 2021	May 06, 2021
dornase	January 16, 2021	May 26, 2021
IC14	January 25, 2021	June 11, 2021

Supplemental Table 4: Additional Detailed Baseline and Day 1 Characteristics By Enrollment Arm

Characteristics	Apremilast (n=67)	Cenicriviroc (n=92)	Icatibant (n=96)	FamCox (n=30)	Dornase (n=39)	IC14 (n=67)	Control (n=233)
Supplemental O2 at Baseline in Liters/min (n (%))							
6 to 10	4 (6%)	8 (9%)	7 (7%)	1 (3%)	2 (5%)	5 (7%)	16 (7%)
11 to 15	4 (6%)	3 (3%)	3 (3%)	1 (3%)	3 (8%)	6 (9%)	19 (8%)
>15	43 (64%)	53 (58%)	52 (54%)	20 (67%)	26 (67%)	49 (73%)	142 (61%)
Unknown	1 (1%)	2 (2%)	3 (3%)	0 (0%)	0 (0%)	1 (1%)	5 (2%)
Not on High-flow O2 (on NIV or MV)	15 (22%)	26 (28%)	31 (32%)	8 (27%)	8 (21%)	6 (9%)	51 (22%)
BMI at Baseline (n (%))							
<18	0 (0%)	0 (0%)	1 (1%)	1 (3%)	0 (0%)	0 (0%)	1 (<1%)
18-25	9 (13%)	13 (14%)	7 (7%)	3 (10%)	5 (13%)	10 (15%)	32 (14%)
>25	55 (82%)	77 (84%)	87 (91%)	23 (77%)	34 (87%)	56 (84%)	195 (84%)
Missing	3 (4%)	2 (2%)	1 (1%)	3 (10%)	0 (0%)	1 (1%)	5 (2%)
Comorbidities at Baseline- In Addition To Those Listed in Table 1 (n (%))							
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)
Unknown	2 (3%)	5 (5%)	2 (2%)	8 (27%)	7 (18%)	18 (27%)	51 (22%)
Any of listed conditions (includes comorbidities in Table 1)	65 (97%)	87 (95%)	94 (98%)	22 (73%)	31 (79%)	49 (73%)	182 (78%)
AIDS	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cerebrovascular Disease	0 (0%)	2 (2%)	10 (10%)	1 (3%)	3 (8%)	1 (1%)	14 (6%)
Hemiplegia/Paraplegia	0 (0%)	0 (0%)	1 (1%)	1 (3%)	0 (0%)	0 (0%)	0 (0%)
Dementia	1 (1%)	2 (2%)	3 (3%)	0 (0%)	2 (5%)	0 (0%)	2 (1%)
Mild Liver Disease	2 (3%)	2 (2%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)	3 (1%)
Moderate/Severe Liver Disease	2 (3%)	1 (1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (2%)
Myocardial Infarction (MI)	1 (1%)	5 (5%)	3 (3%)	1 (3%)	2 (5%)	2 (3%)	5 (2%)
Peptic Ulcer Disease	1 (1%)	2 (2%)	1 (1%)	0 (0%)	0 (0%)	1 (1%)	5 (2%)
Peripheral Vascular Disease	4 (6%)	4 (4%)	4 (4%)	1 (3%)	0 (0%)	1 (1%)	7 (3%)
Rheumatologic Disease	3 (4%)	2 (2%)	6 (6%)	0 (0%)	1 (3%)	6 (9%)	12 (5%)
Receiving Chronic High-Dose Steroids before Admission (n (%))							
Yes	0 (0%)	1 (1%)	0 (0%)	4 (13%)	2 (5%)	2 (3%)	1 (<1%)
No	5 (7%)	23 (25%)	19 (20%)	24 (80%)	30 (77%)	56 (84%)	100 (43%)

Unknown	62 (93%)	68 (74%)	77 (80%)	2 (7%)	7 (18%)	9 (13%)	132 (57%)
Patient Received IL-6 Therapy within 5 half-lives/30 days at Baseline (n (%))							
Yes	0 (0%)	1 (1%)	0 (0%)	8 (27%)	8 (21%)	12 (18%)	11 (5%)
No	5 (7%)	23 (25%)	19 (20%)	20 (67%)	24 (62%)	45 (67%)	90 (39%)
Unknown	62 (93%)	68 (74%)	77 (80%)	2 (7%)	7 (18%)	10 (15%)	132 (57%)
Received COVID-19 Antibody Treatment at Baseline (n (%))							
Yes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3%)	2 (3%)	2 (1%)
No	5 (7%)	20 (22%)	16 (17%)	25 (83%)	27 (69%)	55 (82%)	87 (37%)
Missing	62 (93%)	72 (78%)	80 (83%)	5 (17%)	11 (28%)	10 (15%)	144 (62%)
CRP on Day 1 (mg/L)							
Mean (SD)	95·7 (79·7)	95·2 (83·5)	117·3 (94·4)	103·6 (90·8)	104·1 (95·8)	86·2 (86·5)	109·7 (86·7)
Not available (n (%))	26 (39%)	34 (37%)	41 (43%)	9 (30%)	16 (41%)	22 (33%)	98 (42%)
D-Dimer on Day 1 (ng/mL)							
Mean (SD)	1811·4 (4311·1)	790·8 (985·5)	3326·2 (10805·4)	2136·4 (4940·8)	4524·9 (16500·3)	2565·2 (3307·9)	4039·6 (11741·2)
Not available (n (%))	24 (36%)	36 (39%)	42 (44%)	14 (47%)	16 (41%)	25 (37%)	94 (40%)

Supplemental Table 5: Observed Recovery and All-cause Mortality proportions At 60 Days, By Agent and Concurrent Controls, Intention-To-Treat Population

Agent	Recovered	Died
Apremilast (n=83)	40 (48%)	29 (35%)
Apremilast controls (n=168)	98 (58%)	51 (30%)
Cenicriviroc (n=109)	59 (54%)	45 (41%)
Cenicriviroc controls (n=199)	116 (58%)	64 (32%)
Icatibant (n=115)	60 (52%)	40 (35%)
Icatibant controls (n=213)	123 (58%)	70 (33%)
IC14 (n=98)	57 (58%)	27 (28%)
IC14 controls (n=91)	56 (62%)	30 (33%)
Celecoxib/Famotidine (n=40)	21 (52%)	15 (38%)
Celecoxib/Famotidine controls (n=47)	35 (74%)	10 (21%)
Dornase (n=55)	34 (62%)	18 (33%)
Dornase controls (n=109)	67 (61%)	38 (35%)
Razuprotafib (n=29)	20 (69%)	8 (28%)
Razuprotafib controls (n=76)	50 (66%)	19 (25%)

Note that controls may appear in more than one row if they were concurrent to multiple agents

Supplemental Table 6: Posterior Probabilities for Stopping Criteria By Agent, Action, and Outcome: Intention-To-Treat Population.

Outcome	Action		Arm	Value
Recovery (HR > 1 is better)	**Graduation**	Pr(HRr > 1·0 | data) >= 0·975	Apremilast	0·039
			Cenicriviroc	0·162
			Icatibant	0·184
			IC14	0·055
			Celecoxib/Famotidine	0·067
			Dornase	0·334
	Futility	Pr(HRr < 1·5 | data) >= 0·900	Apremilast	1·000
			Cenicriviroc	1·000
			Icatibant	1·000
			IC14	1·000
			Celecoxib/Famotidine	0·999
			Dornase	0·993
Death (HR < 1 is better)	**Graduation**	Pr(HRm < 1·0 | data) >= 0·900	Apremilast	0·223
			Cenicriviroc	0·070
			Icatibant	0·375
			IC14	0·734
			Celecoxib/Famotidine	0·066
			Dornase	0·580
	Futility	Pr(HRm < 1·0 | data) <= 0·500	Apremilast	0·223
			Cenicriviroc	0·070
			Icatibant	0·375
			IC14	0·734
			Celecoxib/Famotidine	0·066
			Dornase	0·580

Supplemental Table 7: Posterior HR By Agent, Intention To Treat Population

Outcome	Arm	Median posterior hazard ratio (95% CrI)
Recovery	Apremilast	0·74 (0·52, 1·02)
	Cenicriviroc	0·86 (0·63, 1·16)
	Icatibant	0·87 (0·64, 1·19)
	Celecoxib/Famotidine	0·67 (0·41, 1·13)
	Dornase	0·92 (0·62, 1·34)
	IC14	0·75 (0·52, 1·08)
Death	Apremilast	1·19 (0·77, 1·82)
	Cenicriviroc	1·32 (0·92, 1·90)
	Icatibant	1·06 (0·72, 1·54)
	Celecoxib/Famotidine	1·71 (0·85, 3·48)
	Dornase	0·95 (0·57, 1·57)
	IC14	0·85 (0·52, 1·41)

Supplemental Table 8: Median Posterior Hazard Ratio for Recovery, mITT Population, Comparing Original Method For Analysis of Patients Discharged with Only One Day With Oxygen Requirement < 6L NC (Censoring) to Treating as Recovered

Agent	# Patients Censored for Discharge at <6L Oxygen Requirement For < 2 Days in the Agent Arm	Posterior Hazard Ratio, Original	Posterior Hazard Ratio, New Analysis (Treat As Recovered)
apremilast	1	0·78	0·75
cenicriviroc	1	0·88	0·85
icatibant	4	0·85	0·86
IC14	3	0·63	0·64
celecoxib/Famotidine	2	0·50	0·56
dornase alfa	1	0·76	0·78

Supplemental Table 9: Posterior HR For Progression to Mechanical Ventilation or Death in Non-Intubated Patients, mITT Population

Arm	Median posterior hazard ratio (95% CrI)
Apremilast	1·22 (0·80, 1·81)
Cenicriviroc	0·95 (0·65, 1·38)
Icatibant	1·36 (0·96, 1·93)
Celecoxib/Famotidine	1·09 (0·53, 2·22)
Dornase	0·96 (0·53, 1·62)
IC14	0·95 (0·58, 1·58)

Supplemental Table 10: Adverse Events Denoted By Safety Working Group as Probably or Definitely Related to Study Drug

	Apremilast	CVC	Icatibant	Celecoxib/ Famotidine	Dornase	IC14
Nausea	0	1 (1%)	0	0	0	0
Hepatobiliary disorders – other, specify	0	2 (2%)	0	0	0	0
Surgical and Medical procedures – other, specify	0	0	0	0	0	0

I-SPY COVID

STATISTICAL ANALYSIS PLAN

VERSION 1.4

DATE: JULY 1, 2021

Table of content

1 Revisions

Version	Date	Reason
1.0	July 27, 2020	Version 1 developed prior to enrolling first patient in the trial
1.1	January 15, 2021	Updating randomization probabilities and minimum number of patients needed for declaring futility
1.2	February 23, 2021	Updates and clarifications following comment from the FDA
1.3	April 8, 2021	Updates and clarifications following comment from the FDA
1.4	July 1, 2021	Introduction of a family of primary endpoints

2 Abbreviations

AE	Adverse event
AESI	Adverse event of special interest
ARDS	Acute respiratory distress syndrome
AUC	Area under the curve
COVID-19	Coronavirus disease 2019
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
DAPC	Data Access and Publications Committee
DCC	Data Coordinating Center
DLT	Dose-limiting toxicity
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
IND	Investigational New Drug
IRB	Institutional Review Board
	I-SPY TRIAL **I**nvestigation of **S**erial Studies to **P**redict **Y**our **T**herapeutic **R**esponse with **I**maging **A**nd mo**L**ecular Analysis
MDACC	MD Anderson Cancer Center
MEB	Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden
QLHC	QuantumLeap Healthcare Collaborative
SAE	Serious adverse event
UCSF	University of California San Francisco
VFD	Ventilator free days
MICE	Multiple imputation by chained equations

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5 Introduction

The goal of the I-SPY COVID trial is to rapidly screen promising agents, in the setting of an adaptive platform trial, for treatment of critically ill COVID-19 patients. In this phase 2 platform design, we will be able to identify agents with a signal suggesting a big impact on reducing mortality and the need for, as well as duration, of mechanical ventilation.

Approximately 10-15% of those infected with the highly contagious SARS-CoV2 virus develop an acute respiratory illness, with a death rate in the 2-10% range. Of patients who present with respiratory distress, 40-60% require ventilation for a mean of 10-14 days, and up to 50% will not survive.

There are numerous potential effective therapies for ARDS that warrant testing in clinical trials. Although several therapies are under investigation, each trial is a one-off effort, many are not randomized, and there is no coordinated rapid cycle learning. An adaptive platform trial is the ideal design, as it allows the rapid screening of many agents simultaneously. Perhaps most important is that there are several agents with potential to abrogate the respiratory deterioration initiated in the setting of COVID-19 that are approved, available in sufficient supply or capable of rapid scaling to reach hundreds of thousands of patients world-wide. These should be the initial focus of testing as success in the setting of a phase 2 screening/signal seeking I-SPY COVID-19 platform trial can lead to the identification of agents to be evaluated in double blind, placebo-controlled confirmatory trials for regulatory approval and rapid dissemination to those in need of treatment.

The approach that we will take in this trial is to focus on finding agents with a big impact on reducing both mortality and time on ventilators for those critically ill as a result of COVID 19. Both of these endpoints are critical to managing the health crisis created by the global pandemic. Within this trial, there will be an effort to collect sufficient data on biomarkers to evaluate, in a pure research setting, whether the data generated can lead to better and more

targeted interventions, and to better understand the response based on severity of disease as well as prespecified subtypes of ARDS.

6 Trial overview

6.1 Study description

This platform trial will provide access to repurposed and investigational agents for critically ill patients infected with SARS-CoV-2 who have severe or life-threatening COVID-19. The main focus of this study is a randomized platform trial for identifying efficacious agents for the treatment of COVID-19. However, an observational cohort of patients with similar clinical stages of COVID-19 will be followed via medical records. Any critically ill patient with known or presumed COVID-19 will be automatically entered into the screening phase of the trial until SARS-CoV-2 infection is confirmed. Basic data will be assembled for each patient (such as ventilatory status and survival). All patients who start high-flow oxygen (WHO COVID-19 level 5; > 6L oxygen by nasal prongs or mask) will be entered in an observational registry which will only require extraction of medical record data. If interested in the therapeutic portion of the trial, registry participants will be asked to sign a consent form for the backbone treatment and a specific investigational agent arm to which they are assigned. Informed consent will be electronically obtained using a CRF part 11 compliant electronic signature system that will be embedded into the trial informatics platform (Docusign). Patient demographics from the electronic medical record will populate the CRFs. On a daily basis, coordinators will assess status, including ventilatory status, survival, and a small set of additional variables. The primary endpoint will be time to recover to a durable level 4 (or less) on the WHO COVID-19 ordinal scale for clinical improvement. For this trial, a durable level 4 is defined as at least 48 hours at COVID level 4 or less (nasal prongs oxygen) without returning to high flow oxygen or intubation (i.e. the endpoint is reached after reaching level 4 and having stayed there for at least 28 hours). Acute care facility resource utilization will be automatically calculated (total length of stay in a critical care setting, days intubated, and survival). Any change in status, including intubation, extubation, death or discharge, will be recorded and verified by the attending physician.

We will use the platform trial approach developed by the I-SPY consortium to rapidly assess potential COVID-19 treatments. Patients will be evaluated based on their initial status (ventilation at entry vs. high flow oxygen). Exploratory biomarkers will be evaluated over time (ARDS phenotypes and other proposed markers) to facilitate clinical learning. Randomization will be 1:1 with one active arm, 1.2:1:1 with two active arms, 1.33:1:1:1 with three active arms, and 1.4:1:1:1:1 with four active arms. The larger allocation to the control arm than to the active arms is made since the control arm is used for all comparisons. The anticipated accrual will be about 50 patients per week. The maximum number of participants assigned to an arm (without meeting criteria for graduation or dropping for futility) will be 125 patients. Agents can graduate after enrollment of 50 evaluable patients (i.e. patients with correct entry criteria and without missing data, which can enter the statistical analysis). Analogously, 40 evaluable patients are needed for drug to be dropped for futility. As the trial proceeds and a better understanding of the underlying mechanisms of the COVID-19 illness emerges, we can expand biomarker and data collection as needed to further elucidate how agents are or are not working. The study design features comparison of investigational agent efficacy using a Bayesian design, which will allow the detection of strong efficacy signals. Initially the control will be patients given current standard of care (supportive care for ARDS, including lung protective ventilation and remdesivir and dexamethasone as backbone therapy). As other treatments become part of standard

supportive care across sites, these will be added to the backbone therapy. If an agent meets the threshold for graduation or futility the company leadership will be informed as will the FDA. The arm with the graduated agent will cease to enroll, allowing a new arm with a different investigational agent to be added.

The trial assesses performance of agents based on disease severity (intubated or not at presentation). As well, the trial will enable investigation of the performance of agents against pre-specified subtypes based on exploratory biomarkers. This latter analysis will be performed as a secondary objective in a pure research setting. Every trial participant will have blood collected at trial enrollment, day 3, and day 7 for pre-specified biomarker and DNA and RNA analysis. Additional biomarkers can be added as the trial proceeds. Patient outcomes will also be evaluated on the basis of whether patients are ventilated initially or not.

This is a Phase 2, open-label study designed to reduce mortality and morbidity as well as the impact on healthcare utilization for critically ill patients with severe or immediately life threatening COVID-19. Agents that graduate from this trial will be recommended for further double blind, placebo-controlled phase 3 trials so that the signal can be validated and enable registration for the indication of acute and severe illness related to COVID-19.

6.2 Objectives and endpoints

The trial objectives and corresponding endpoints are shown in Table 1.

Objectives	Endpoints
Primary Identify agents that will result in substantial improvements to the clinical condition of participants with COVID-19	**Primary** Time to reach a durable (≥48 hours) COVID-19 level 4 or less and time to death Data will be analyzed for 3 groups: ● All ● COVID-19 level 6/7 (those intubated immediately) ● COVID-19 level 5 (high flow oxygen to start) WHO 9-point ordinal scale: 0. No clinical or virologic evidence of infection 1. Not hospitalized, no limitations on activities; 2. Not hospitalized, limitation on activities; 3. Hospitalized, not requiring supplemental oxygen; 4. Hospitalized, requiring supplemental oxygen (up to 6L by nasal cannula or mask delivery system);

	5. Hospitalized, on non-invasive ventilation or high flow oxygen devices (>6L per minute, mask or intranasal cannula); 6. Hospitalized, on invasive mechanical ventilation; 7. Hospitalized, ventilation plus additional organ support—pressors, RRT, ECMO 8. Death.
Secondary ● Health care utilization ● Safety	**Secondary** ● % of COVID-19 level 5 who never progress to COVID-19 level 6/7 or die ● Ventilator-free Days ● Total grade 3 or higher AEs by arm and total number of patients with grade 3 or higher AEs by arm. ● Total grade 3 or higher AEs of special interest by arm and total number of patients with grade 3 or higher AEs of special interest by arms (based upon lab assessments)
Exploratory ● Differential impact on subsets of patients based on exploratory biomarkers and severity of presenting illness ● Establish biomarker-rich database for rapid learning about mechanism of illness and response to different classes of agents ● Develop tools to rapidly assess pharmacodynamics and dose response ● Vital status ● Quality of Life	**Exploratory** ● Mortality and ventilatory requirements for different ARDS subphenotypes, severity of illness at admission to ICU (ventilated or not) and comorbidities ● Evaluate response to classes of agents on the basis of exploratory biomarkers. ● Relationship between dose/exposure and response, measured as outcome (ventilatory requirements/mortality/days in ICU) or biomarker, will be quantitatively evaluated for each agent and integrated across the arms ● Vital status at 28 days after study enrollment ● ePRO 28, 60, and 120 days after enrollment

Table 1. *Trial objectives and endpoints.*

6.3 Trial design overview

I-SPY COVID is a multi-centre, multi-arm, adaptive, open-label, randomised controlled trial. Patients who do not consent to be randomized will enter an observational cohort (by a waiver of consent mechanism), where disease outcomes and other endpoints will be tracked. The trial design for the randomized part of the trial is shown in Figure 1.

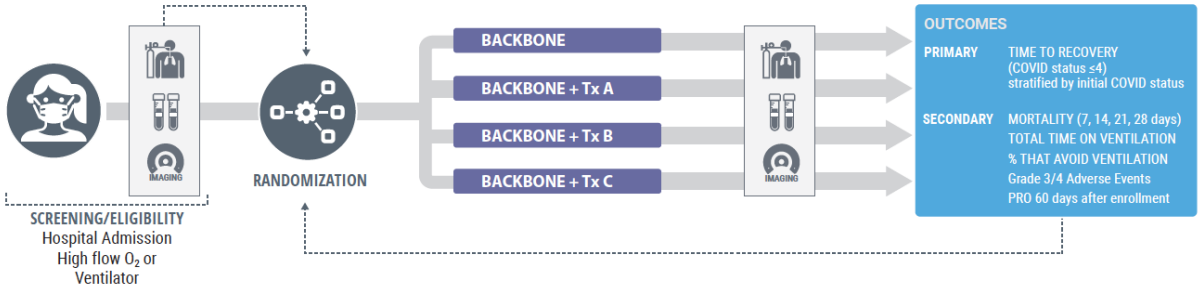


Figure 1. *All patients that enter the hospital and that are placed on high flow oxygen or are intubated will be enrolled in the observational portion of the trial and will be tracked and screened for trial eligibility. Enrolled patients must have had a positive COVID-19 test by PCR. Eligible PCR-positive patients or their designated surrogates will be asked if they want to enroll on the therapeutic portion of the trial. All enrolled patients will receive backbone therapy with or without an additional investigational agent.*

6.4 Eligibility

6.4.1 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for participation in this study:

1. Male or Female, at least 18 years old
2. Admitted to the hospital and placed on high flow oxygen (greater than 6L by nasal cannula or mask delivery system) or intubated for the treatment of (established or presumed) COVID-19.
3. Informed consent provided by the patient or health care proxy.
4. Confirmation of SARS-CoV-2 infection by PCR or Rapid antigen testing for SARS-CoV-2 infection prior to randomization.

6.4.2 Exclusion criteria

1. Pregnant or breastfeeding women (must be documented by a pregnancy test during hospitalization)
2. History of allergic reactions attributed to compounds of similar chemical or biologic composition to study agent based on review of the medical record and patient history.
3. Comfort measures only.
4. Acute or chronic liver disease with a Child-Pugh score greater than 11.
5. Resident for more than six months at a skilled nursing facility.

6. Estimated mortality greater than 50% over the next six months from underlying chronic conditions.
7. Time since requirement for high flow oxygen or ventilation greater than 72 hours.
8. Anticipated transfer to another hospital which is not a study site within 72 hours.
9. Patients with either end-stage kidney disease or acute kidney injury who are on dialysis.

6.5 Randomization

Agents will be assigned to all patients equally as the trial begins. Randomization will be 1:1 with control and one active arm, 1.2:1:1 with control and two active arms, 1.33:1:1:1 with control and three active arms, and 1.4:1:1:1:1 with control and four active arms. We will use concurrent control for the primary analysis and decisions on graduation and dropping for futility, such that any experimental arm is always compared with controls accrued during the same time period. This means that any differences in the control arm over time (due to changing standard of care of COVID-19 patients) will be reflected in the comparison. (Cumulative controls can be used as well in sensitivity analyses and to analyze temporal trends.) Since we will use concurrent controls and since the control arm is used for multiple comparisons, it makes sense to make the control arm bigger than the experimental arm to reduce any stochastic noise within that arm (which affects all comparisons). Randomization will be stratified by site and WHO COVID status at baseline. Randomization will practically be conducted using a centralized randomization list, to facilitate transparency and documentation.

As is outlined in the *Trial design overview* section, randomization happens prior to consent. I.e., a potential trial participant is first randomized and then approached for study participation and consent. The order of the randomization and consent process has proved to work well in the I-SPY2 neoadjuvant breast cancer treatment trial and is used in order to facilitate a more patient centered consent process, in which the patient is approached for study participation with respect to *one* treatment (as opposed to all treatments, which would be the case if consent happened prior to randomization). This process avoids a two-step consent and makes it easier to describe potential treatment side effects (as only the side effects of the relevant treatment need to be described). However, although this randomization and consent process has several operational and patient centered advantages, there is a risk that it may lead to different accrual patterns across the different trial arms since patients consent to each arm individually and not to all arms up front. For example, different side effects of different investigational treatments may result in differential participation rates across trial arms. Since all trial patients get standard of care treatment (including Remdesivir as backbone treatment for all I-SPY COVID trial patients) and since the investigational agents have limited and similar side effects, we believe participation rates will be high in all arms, which will decrease the risk of any systematic differences between the participants across trial arms. Nonetheless, we will closely monitor participation rates across arms, and there are signs of systematic differences, we will reevaluate the randomization and consent process.

6.6 Stopping for graduation and futility

The trial is designed to study over time which agents perform best.

Each agent's probability of reducing time to recovery and of reducing overall mortality will be calculated as the trial proceeds:

- Agents may be graduated at an interim point should one or more of these probabilities reach a sufficient level.

- Agents may be dropped from the trial for futility when probabilities drop sufficiently low.

Participants currently receiving the agent will continue on the regimen until they complete the entire course of treatment.

At each interim we calculate the Bayesian posterior distribution for the hazard ratios comparing each experimental treatment to control with respect to recovery and overall mortality. From these posterior distributions we calculate Bayesian posterior probabilities: (1) $Pr(csHR_{r,d} > 1.0)$, the probability the cause-specific hazard ratio (csHR) for recovery (*r*) for treatment *d* is greater than 1.0, representing an improvement vs. control/backbone therapy; (2) $Pr(csHR_{r,d} < 1.5)$, the probability the cause specific hazard ratio for treatment *d* offers less than a 50% improvement over control; (3) $Pr(HR_{m,d} < 1)$, the probability that the hazard ratio for overall mortality (*m*) for treatment *d* is less than 1; and (4) $Pr(csHR_{m,d} > 1.3)$, the probability the cause-specific hazard ratio for mortality for treatment *d* does increase the mortality rate (without any prior recovery) compared with the control arm by more than 30%. Concurrent controls will be used when computing these probabilities. Moreover, the calculations will be based on comparisons against only those control arm patients who were eligible to receive that drug.

During the course of follow-up, a specific treatment may graduate for superiority if at least 50 evaluable patients have been enrolled to the treatment arm. Analogously, a specific treatment may be dropped for futility after 40 evaluable patients have been enrolled to the arm.

- An agent *d* will be evaluated by the DMC for *graduation* if the posterior probability for its cause-specific hazard ratio for recovery vs. backbone (adjusted for COVID-19 level status at baseline) is greater than one is greater than or equal to 0.975, $Pr(csHR_{r,d} > 1) \geq 0.975$ OR if the posterior probability for its hazard ratio for overall mortality vs. backbone (adjusted for COVID-19 level status at baseline) is smaller than one is greater than or equal to 0.9, $Pr(HR_{m,d} < 1) \geq 0.9$.
- An agent *d* will be evaluated by the DMC for *futility* if the posterior probability for its cause-specific hazard ratio for recovery vs. backbone (adjusted for COVID-19 level status at baseline) is less than 1.5 is greater than or equal to 0.9, $Pr(csHR_{r,d} < 1.5) \geq 0.9$ AND if the posterior probability for its hazard ratio for overall mortality vs. backbone (adjusted for COVID-19 level status at baseline) is greater than one is greater than or equal to 0.5, $Pr(HR_{m,d} > 1) \geq 0.5$.
- In addition, we have a criterion for *safety*: If $Pr(csHR_{m,d} > 1.3) \geq 0.7$, where $csHR_{m,d}$ is the cause specific hazard ratio for mortality for treatment *d*. If this criterion is met, it will trigger a DMC review of data for treatment *d* against controls to decide whether it is regarded as acceptable to continue enrolling patients to this arm (at the next DMC meeting immediately after the safety criterion was met).

If the maximum sample size of participants is reached, assignments to the regimen will end and participants currently receiving the agent will continue on the regimen until they complete the entire course of treatment.

If an investigational agent reaches a threshold for graduation or futility, the DMC will review the findings and make a recommendation to Principal Investigators (PIs) for final approval. In addition to examining $csHR_{r,d}$ and $HR_{m,d}$, the DMC will also review and evaluate the cumulative incidence function for recovery and for mortality. The DMC will, during the review process, also evaluate additional mortality data, specifically the proportion of patients at risk of both study outcomes in the treatment and control arm at day 7, 14, 21, and 28. If all these statistics are favorable, i.e. do not indicate increased mortality, the drug will graduate. The proportion of patients still at risk of both outcomes of the study (i.e. recovery and death) is given by the

survival function, which we will also report to the DMC and in publications. During the review by the DMC and PIs, participants currently receiving that agent will continue on the regimen until they complete the entire course of treatment. No additional patients will be randomized to the agent arm after graduation.

If an agent is found not to reach a specified threshold of improvement in the primary endpoint, it may be dropped for futility; the DMC will review the findings, and if they agree, recommend to the PIs that the agent be dropped from the trial. During review by the DMC and PIs, no participants will be randomized to that regimen or agent. Participants who have not completed the course of the agent will continue to receive the agent until a determination is made. Once an agent is dropped from the trial, the option to continue or drop the agent will be at the discretion of the participant and his or her treating physician. Participants who do not continue on the agent will continue on-study but will revert to the standard/control regimen; their outcomes will remain part of the arm to which they were assigned. Once an agent is dropped from the trial, the option to continue or drop the agent will be at the discretion of the participant and his or her treating physician. Participants who do not continue on the agent will continue on-study but will revert to the standard/control regimen; their outcomes will remain part of the arm to which they were assigned.

If an investigational agent is removed from the trial due to serious side effects from the agent, use of that agent for all participants will be stopped. Participants will continue on-study but will revert to the standard/control regimen and their outcomes will remain part of the arm to which they were randomized.

Up to four investigational agents will be active at any given time. The number of agents considered will be restricted by the study's accrual rate and the ability to "process" the trial agents expeditiously in order to give companies timely information concerning the potential role of the agent in treating COVID-19 ICU patients. Trial data will also be used to test, qualify, and validate biomarkers as predictors of response to specific therapeutic agents. This trial is an opportunity to integrate information from emerging biomarkers and thereby accelerate identification of optimal therapies for patients with severe or life-threatening COVID-19.

6.7 Blinding

I-SPY COVID is an open-label study. However, while the study is in progress, access to tabular results of study outcomes by treatment allocation will not be available to the research team, clinical investigators, clinical teams, or members of the steering committee (unless the DMC advises otherwise). The DMC and trial statisticians will be unblinded.

6.8 Data collection

Study data collection will be performed through the OpenClinica Clinical EDC system, where Clinical Research Coordinators (CRCs) at the respective I-SPY COVID-19 trial sites will have secure login credentials allowing them to register and enter relevant study data information. OpenClinica allows for providers to refer patients through referral electronic Case Report Forms (eCRFs), that can be reviewed by CRC's to assess eligibility and initiate the consent process before formal enrollment in the trial. All eCRFs will be developed using Clinical Data Interchange Standards Consortium standards. For adverse event reporting, the standard safety terminology used is the National Cancer Institute's (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v5.0. eCRFs will be structured to balance ease of data entry with

mapping complexity for the target Clinical Data Interchange Standards Consortium Study Data Tabulation Model datasets. Management and revisions to eCRF data elements will be managed through by the informatics group with guidance from the FDA to insure alignment with other COVID-19 and ARDS studies. The OpenClinica EDC is directly integrated with the randomization engine which houses the adaptive algorithm written in the R statistical programming language. In order to perform the required calculations for randomization and treatment arm assignment, all data relevant to the statistical modeling will be de-identified prior to submission to the engine. For dissemination of analysis datasets to statisticians and the DMC, a target Define.xml will be used in parallel with the eCRFs. Define-XML is required by the United States Food and Drug Administration (FDA) for every study in each electronic submission to inform the regulators which datasets, variables, controlled terms, and other specified metadata were used. Field names will be aligned with Clinical Data Acquisition Standards Harmonization ARDS and COVID-19 domains where possible and codelists matched to the appropriate NCI controlled Terminology.

Data visualizations will be deployed and accessible via the Tableau Server platform, allowing decision support displays for access by the project management office, data managers and the DMC.

6.8.1 Electronic Case Report Forms (eCRFs)

Participant data will be collected using protocol-specific electronic case report forms (eCRFs) utilizing CDISC data standards specific to ARDS and COVID-19. Study staff will enter data into the EDC. Instructions on how to use the EDC are part of the manual which is part of the MOP.

The ability to randomize a participant and to ensure the randomization engine is updated with the most current response data is dependent on timely completion of the CRFs listed in Table 2.

Form	When form is to be completed
Eligibility Checklist / Screen Admission to ICU	Completed for each participant considered for I-SPY COVID-19
Registration Form / Coordinator data at consent	For each participant signing a screening consent form.
Verification Form	Change in COVID-19 status level from 5 to 6/7 (intubation) or from 6 to 5 (extubation), and 5 to 4 (primary endpoint) and from 3 to 2 (at discharge from hospital)
Daily Coordinator Checklist	Daily intake on labs, medications, COVID-19 WHO designation status levels
Randomization Form	Once after participant has been randomized
SAE Form	As needed.
Lost to Follow-up Form and No Longer Lost to Follow-Up Form	As needed.

Off-study Form	As needed.
Protocol Violation Form	As needed.

Table 2. *Case Report Form Submission Schedule*

6.9 Monitoring

A DMC has been formed to assure participant safety in this clinical trial. As outlined in the I-SPY COVID TRIAL DMC Charter, DMC members will also have additional responsibility for assurance that the trial is conducted to a high standard, and they may be involved in conduct and interpretation of data analyses for efficacy in addition to their primary responsibility for participant safety. The responsibilities of this group include reviewing quantitative recruitment and compliance progress for the study, and recommending modifications of the trial protocol and/or administrative structure in the event these goals are not met. The committee will also review toxicity and endpoint data. The committee will submit written recommendations on the progress of the study to the study Principal Investigator and QLHC.

In addition, QLHC (or their designee), the lead clinical site (UCSF), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

6.10 Trial reporting

The trial will be reported according to the principles of the CONSORT statements. The exact composition of the trial publications depends on the continued development of the epidemic, the availability of drugs, and the results from the analyses conducted within the trial.

7 Analysis of the trial

7.1 Analysis populations

The *intention to treat (ITT) population* will consist of all participants randomised and consenting to be part of the study, irrespective of treatment received. Participants will be analysed according to the treatment they are randomised to, regardless of whether they actually received this treatment at all or whether they received it for the entire follow-up period. The ITT population will be used for analysis of effectiveness.

The *per protocol (PP) population* will be defined by the participants who were randomized to a specific treatment and received it according to the prescribed dose. Patients who received a different treatment than the one they were randomised to will be excluded from the analyses. If, during the follow-up, participants switch from their assigned treatment to a different treatment (including treatment discontinuation), their follow-up time will be censored at the time of their switch.

The number and proportion of patients who did not receive the treatment they were allocated to will be reported. If any other trial treatment options were known to be received, instead of or in addition to, the allocated treatment during the follow-up period after the first randomisation,

these will be collected and reported. Details on the number of days (or doses) of treatment received will be reported for all trial treatments received where available.

In addition, we define a *super ITT population*, by including participants in the observational cohort. For practical purposes, randomization in I-SPY COVID happens chronologically prior to consent. If a large proportion of patients decline participation after being randomized, this may lead to differences in the ITT population across arms (we therefore monitor the randomization-consent process carefully, see Section 7.8 *Monitoring of the randomization-consent process*). Patients who decline participation after randomization end up in the observational cohort, for which there is a waiver for consent to collect follow-up data. We therefore define the super ITT population as all randomized patients, irrespectively if they ended up consenting to receive the investigational agents or if the ended up in the observational cohort. The super ITT population will thus not be impacted by any potential effect of the randomization-consent process on the patient population in the different trial arms.

The *safety population* will be defined on an as treated basis, i.e. patients are grouped for analysis according to the treatment they actually received (irrespectively of dose).

For all trial population, concurrent controls will be used in the statistical analysis and the calculations will be based on comparisons against only those control arm patients who were eligible to receive that drug.

All trial arms will be analyzed in the same way and therefore no agent specific statistical analysis plans are planned. If agents enter the trial that will require specific analyses, agent specific SAPs will be detailed.

7.2 Primary endpoints

The trial uses a family of two primary endpoints, recovery and overall mortality. The primary analysis of the primary endpoints will be performed on the intention to treat (ITT) population; additional analyses will be performed on the PP population and the super ITT population.

7.2.1 Recovery

We will use Bayesian time-to-event models (with weakly informative priors) to model the cause-specific hazard functions durable COVID level 4 or less (i.e. recovery) and mortality without prior recovery as a function of study arm, adjusting for baseline COVID level. We note that mortality is a competing event to experience the primary recovery endpoint, because patients who die are prevented from reaching COVID-19 level 4, and vice versa.

More specifically, we will specify two separate Bayesian proportional-hazard Weibull models, one for each outcome. For each model, we will let the logarithm of the Weibull distribution's scale parameter u to depend on study arm (x; agent versus control) and on baseline COVID level (c; level 6/7 versus 5), so that $\log(u_i) = \alpha + \beta x_i + \gamma c_i$ (the subscript i indexes the individual). The distribution's shape parameter (v), which is constrained to be positive, will not depend on any covariates.

The following priors will be specified for the models' parameters:

- α (intercept): Normal(0, sd=20) after centering possible continuous covariates included in the model;
- β (log-hazard ratio for the investigational agent): Normal(0, sd=sqrt(0.5)). This corresponds to 95% equal-tailed prior limits for the cause-specific HR of (0.25, 4.00).
- ɣ (log-hazard ratio for baseline COVID level): Normal(0, sd=sqrt(0.5)). This corresponds to 95% equal-tailed prior limits for the cause-specific HR of (0.25, 4.00).
- *v* (shape parameter): Exponential(1). This corresponds to one-tailed 95% prior limits of (0, 3).

If additional baseline covariates are added to the model (e.g. in sensitivity analyses), we will specify Normal priors with mean zero for the corresponding coefficients. The standard deviation will be set such as the 95% prior limits will cover a plausible range of values for the cause-specific Hazard Ratio.

We will contrast each of the investigational agents versus the control arm using separate sets of two Weibull models (one for each outcome) so that only concurrent control patients who would have been eligible to receive that agent (i.e., who meet the eligibility criteria for that agent) will be included in the analysis.

From the posterior cause-specific hazard functions based on the models described above and following standard methodology (see for example Putter et al., 2007), we will calculate two posterior cumulative incidence functions, one for each study outcome. These two functions give the (posterior) cumulative probability of reaching each of the 2 study outcomes, in the presence of the other study outcome as a competing event, as a function of time. Consequently, these curves can be used to evaluate the actual cumulative probability of recovery or death (without prior recovery) at specific time points, such as 7, 14 or 28 days after randomization. We will also report the posterior survival function, which gives the probability of being still in the trial (i.e. at risk of both study outcomes) as a function of time. From the posterior cumulative incidence functions, it is also possible to extract posterior survival percentiles (e.g. median time to reaching COVID level 4) (Lee et al., 2010). We will report posterior cumulative incidence functions and survival functions conditional on study arm but standardized (marginalized) over the other covariates included in the Weibull models.

7.2.2 Mortality

We will model the distribution of time from randomization to death, irrespective of whether the patients recovered between those two timepoints. This means that recovery is not a competing event in this analysis.

We will contrast each of the investigational agents versus the control arm using separate Bayesian proportional-hazard Weibull models, whose parameters and priors will be modelled and specified in an identical fashion to the primary analysis for the recovery endpoint (§7.2.1). Posterior conditional and marginal survival probabilities will be derived from the posterior mortality hazard function following standard methodology (Putter et al., 2007).

From this, we will be able to evaluate the posterior distribution for: the hazard ratio for mortality, the proportion of patients still alive over time (Survival function) and its complement to one, i.e. the cumulative proportion of patients who died over time (Cumulative mortality function). These last two quantities can be evaluated in particular at day 7, 14, 21, and 28 and both conditionally

and marginally with the respect to the distribution of the covariates, other than randomization arm, included in the model (e.g: baseline COVID level).

7.3 Secondary endpoints

7.3.1 Safety

The main analysis for safety will be performed on the safety population; additional analyses may be performed on the ITT population, the PP population, and the super ITT population. The safety endpoints are:

- Total grade 3 or higher AEs by arm and total number of patients with grade 3 or higher AEs by arm.
- Total grade 3 or higher AEs of special interest (AESI) by arm and total number of patients with grade 3 or higher AEs of special interest by arms (based upon lab assessments)

A full listing of AESIs is provided in the protocol appendix of the investigational drug with a full cumulative listing.

AEs, SAEs, and AESIs will be summarized in tables by arm. If justified based on the number of AEs in each arm, we wil also analyse the safety endpoints in two different ways: (1) Any AE or AESI using a logistic regression model with arm allocation as the main contrast, adjusted for age, BMI, baseline WHO COVID status, and sex; (2) Total number of AE or AESI using Poission regression with arm allocation as the main contrast, adjusted for age, BMI, baseline WHO COVID status, sex, and exposure time. Noninformative priors will be used. Statistical uncertainty (95% credibility intervals) will be computed from the regression models.

We will use weakly informative priors in order to regularize and stabilize the computational algorithms. The background for the adopted priors is discussed in the book Regression and Other Stories (Gelman 2020).
For a fitted general linear model, the following priors will be specified:

- α (intercept): Normal(m_y, 2.5 s_y), where m_y is equal to the mean of the outcome when all the covariates are equal to their mean values if the outcome is continuous, or equal to 0 otherwise; s_y is the standard deviation of the outcome if the outcome is continuous, or otherwise 1;
- β: Normal(0, 2.5 s_y/s_x), where s_x is the standard deviation of the corresponding covariate *x*;
- aux: Exponential(1/s_y) for the ancillary parameters of the model such as residual standard deviation for Gaussian.

7.3.2 COVID-19 level 5 patients who do not progress to COVID-19 level 6/7 or die

The main analysis for this endpoint will be performed on the ITT population; additional analyses will be performed on the PP population and the super ITT population.

The analysis will be performed analogously to the mortality endpoint (§7.2.2). More specifically, we will analyze time to COVID level 6/7 or death using a Bayesian Weibull model. From this model, we will report the posterior survival function, which estimates the percentage of patients

who have not yet progressed to COVID level 6/7 or death over time. Only patients who enter the trial on COVID level 5 will be included in this analysis. Therefore, no adjustment for baseline COVID level will be made.

7.3.3 Ventilator-free days

The main analysis for this endpoint will be performed on the ITT population; additional analyses will be performed on the PP population and the super ITT population. Ventilator free days (VFD) are defined as:

- VFD = 0: If the patient dies before 28 days.
- VFD = (28 - x): x is the number of days spent receiving mechanical ventilation.
- VFD = 0: If the patient requires mechanical ventilation for 28 days or more.

So, if a patient receives invasive mechanical ventilation and survives to the first of hospital discharge or Study Day 28, the number of VFDs is calculated as 28 minus the number of calendar days from the first day on which the patient received invasive mechanical ventilation until the last day on which the patient received invasive mechanical ventilation. Days on which the patient does not receive invasive mechanical ventilation that occur between days on which the patient did receive mechanical ventilation do not count towards the number of VFDs. So for a patient who was intubated on day 10, extubated on day 18, and survived to day 29, the patient would have 28 – 9 (total number of days of ventilation) = 19 VFDs. This approach is being used in other ongoing clinical trials of COVID-19 that do not focus exclusively on ventilated patients.

Following Schoenfeld et al. Crit. Care Med. 2002 Vol. 30, No. 8, we will test for differences in median VFD between trial arms. We will use quantile regression with arm allocation as the main contrast, adjusted for age, BMI, baseline WHO COVID status, and sex. Subgroup analyses will be performed with respect to those who are ventilated at baseline and those who are not ventilated (interactions by subgroup will be tested by including an interaction term in the regression model).

7.4 Exploratory endpoints

Differences between treatment arms with respect to *ePRO* (patient reported outcomes) at 28 days, 60 days and 120 days after enrollment will assessed using appropriate models for analyzing PROs (Pe et al. Lancet Oncol 2018; 19: e459–69). Specifically, linear mixed effect models will be used to assess multiple time point. Time to event models (with interval censoring) may also be used, to analyze time to reach a certain value on the PRO scales. We are using the PROMIS survey to measure quality of life in patients (Cella et al. J Clin Epidemiol. 2010 Nov;63(11):1179-94). PROMIS instruments are scored using item-level calibrations; we will use the HealthMeasures Scoring Service (https://www.assessmentcenter.net/ac_scoringservice). This method of scoring uses responses to each item for each participant. Output of the scoring service includes a T-Score for each measure, which is used to rescale the raw sum score into a standardized score with a mean of 50 and a standard deviation (SD) of 10. A higher PROMIS T-score represents more of the concept being measured. Thus, a person who has T- scores of 60 for the Global Physical Health or Global Mental Health scales is one standard deviation better (more healthy) than the general population. For signs and symptoms, patient responses are provided on a 5-point Likert scale, and will use the PRO-CTCAE questionnaire (Kluetz et al. Am Soc Clin Oncol Educ Book. 2016;35:67-73), which was developed to evaluate symptomatic toxicities in adults.

Differences between treatment arms with respect to *vital status* (patients dead or alive) at 28 days will be analyzed from the survival functions using the approach described in Section 7.3.1.

7.5 Descriptive analyses

7.5.1 Trial flowchart

The flow of participants through the trial will be summarised for each separate pairwise comparison using a CONSORT diagram. The flow diagrams will describe the numbers of participants randomly allocated, who received allocation, withdrew consent, and included in the ITT analysis population (as well as the super ITT population).

7.5.2 Baseline characteristics

Baseline characteristics will be described descriptively and will include:

- Requirement for respiratory support at randomisation (i.e. baseline COVID WHO level)
- Age
- Sex
- Ethnicity
- BMI
- Vaccination status

Summary statistics will be used for each variable: median and interquartile ranges for continuous variables, and number and percentages for categorical variables.

7.6 Subgroup analyses

Results may be stratified by the following subgroups (with bolded ones considered as the most important):

- **Requirement for respiratory support at randomisation (Oxygen only; Ventilation or ECMO)**
- On and off vasopressors at time of enrolment
- Time since illness onset (≤7 days; >7 days)
- **Age (<70; 70-79; 80+ years)**
- **Sex (Male; Female)**
- **Ethnicity (White; Black, Asian or Minority Ethnic; Unknown)**
- **BMI**
- ARDS subgroups (see trial protocol for details)
- Exploratory biomarkers

As drugs may be differentially effective in different stages of the disease, we will also test for an interaction effect between treatment and COVID-19 status level at baseline (following the best practices described in (Wang et al. N Engl J Med 2007; 357:2189-2194).

7.7 Missing data

Due to the nature of the disease and the trial, we expect missing data will be minimal. All reasonable efforts will be taken to minimize loss to follow-up.

Missing primary endpoint values will be censored at the last observed time point (this is also the case for all time-to-event endpoints specified above).

Adverse events will not be imputed, since the absence of adverse events implies that no adverse event occurred.

We will use multiple imputation by chained equations (MICE) to impute missing data for non–time-to-event outcomes (ventilator-free days and ePRO scores) and baseline covariates.

For ventilator-free days, the MICE procedure will include the following variables (between parentheses we indicate the model used to impute it): baseline COVID level (no missing values by design), age (Predictive Mean Matching (PMM), with donor pool (k)=5), sex (logistic regression), BMI (PMM, k=5), ethnicity (multinomial regression), randomization arm (no missing values by design), VFD (PMM, k=5). Imputation will be performed separately (stratified) by baseline COVID level (5 vs 6/7).

For ePRO data, the same baseline covariates as above will be included in the MICE procedure together with all ePRO-items. Each individual ePRO-item will be imputed using PMM (k=5). T-score-level imputation will be used to estimate T-scores with missing item scores, also using PMM using the same baseline covariates as specified in the preceding paragraph and k=5.

We will generate 50 imputed datasets, setting the seed for the MICE procedures to 666. The imputed datasets will be analyzed following the approach described by Gelman et al.(A Gelman et al. Bayesian data analysis. CRC press, 2013) and Zhou et al. (Zhou, Xiang, and Jerome P. Reiter. A note on Bayesian inference after multiple imputation. The American Statistician 64.2 (2010): 159-163). Briefly, we will analyze the imputed datasets separately using the appropriate Bayesian regression models specified in the SAP, mix the draws from the posterior distributions from each completed dataset, and finally use the mixed draws to summarize the posterior distribution.

7.8 Sensitivity analyses and supportive analyses

7.8.1 Heterogeneous treatment effects over randomization periods

Since management of COVID-19 patients is quickly evolving and the patient population enrolled in the trial can potentially change over time, it is possible that the estimated treatment effects for the investigational agents vary with time.

We will therefore always use concurrent controls for any comparative analyses of the investigational agents versus the control arm. Moreover, we will, as a sensitivity/supportive analysis, investigate potential heterogeneous treatment effects over randomization periods. This will be done by letting the hazard ratio for the treatment effect for the primary (time to recovery) and the secondary endpoint (mortality) to vary over time, for example by including in the survival models the appropriate interaction terms between the indicator variable for treatment and time, or by estimating separate models for different periods (stratification).

7.9 Monitoring of the randomization-consent process

As described in the *Randomization* section, we will closely monitor the randomization-consent process in order to ensure that it does not show evidence of differential participation rate across trial arms. Specifically, we report and test for differences in the rate of patients approached for trial participation who decline participation, by arm they were randomized to. These results will be included in the DMC report at interim analyses. If there is evidence of differences between arms, this will be discussed with the DMC who will report to the trial steering committee whether any changes to the randomization-consent process should be recommended.

7.10 Comparing active arms against each other

As exploratory analyses, we may compare the effectiveness and efficacy of investigation agents. Since treatments will enter and leave the trial at different time points and since the standard of care (against which all arms will be compared) is expected to evolve quickly, modeling will be required to conduct such comparisons. To do this, we will use methods akin to the platform trial “time machine” constructed by Don Berry et al. and inspired by the concepts described by Scott Berry et al. in their paper “Bridging different eras in sports”, JASA 1999, 661-685. Thus, we estimated the efficacy of each arm relative to the control arm and adjusted for each arm’s time period. This can be viewed as a missing data problem, where results for a specific arm available for the specific time period the arm was active in the trial need to be imputed across the entire trial time period.

7.11 Deviation from pre-specified analyses

The statistical analysis committee will monitor the model behavior, including scientific appropriateness and inappropriate model fit. The proposed Weibull models are flexible and we are confident that they will provide a good model fit to the data. Nonetheless, if the model is deemed to provide an inappropriate fit then the statistical analysis committee will inform the DMC of appropriate adjustments which will be reported to the trial steering committee in a way that does not risk unblinding trial results. Possible adjustments could include:

1. If there are issues within an intervention for limited data the parameter for that intervention can be fixed for model stability (this will likely only be an issue for exploratory analyses, e.g. within subgroups defined by exploratory biomarkers).

Any post-hoc analysis requested by the oversight committees, a journal editor or referees will be labelled explicitly as such. Any further future analyses not specified in the analysis protocol will be documented in the revision history of this document.

7.12 Nonparametric analyses of cumulative incidence functions

We will present nonparametric cumulative incidence functions for the two competing events recovery and death without any prior recovery (using the Aalen-Johansen estimator) and for overall mortality (using one minus the Kaplan-Meier estimator) with 95% pointwise confidence intervals.

8 Simulations of trial operating characteristics

To test the trial's operating characteristics, we simulated the trial under a variety of scenarios, including the true efficacy of the backbone therapy, true efficacy of candidate treatments, and various accrual rates.

Below, we describe some of the simulations performed to investigate the operating characteristics of the trial. Several updates have been done to the trial. Specifically, we have made changes to the randomization probabilities (to decrease the number of patients randomized to the control arm), the number of patients required for dropping an arm for futility (from 50 to 40), and updates to the decision criteria for graduation and dropping for futility (from being based only on time to recovery to include a family of endpoints consisting of time to recovery and time to death). For the descriptions of the simulations, we start by describing simulations for the original specifications of the trial, and then describe the simulations supporting the updates to the trial protocol.

We consider a variety of possible scenarios ranging from pessimistic, in which the null hypothesis of no benefit holds for every treatment, to optimistic cases in which several of the regimens are truly effective. For each scenario, and following the design we built, we enter participants into a virtual trial and simulate outcomes for them. When the "trial" is over we record various summaries of the trial results, including the duration of the period in which participants were randomized to each experimental regimen, whether the treatment graduated, reached futility, or ran to the maximum number of patients. We repeat this trial simulation procedure at least 500 times to find the operating characteristics of the design.

8.1 Base simulations

The data simulations consist of virtually creating multiple instances of how the trial will evolve and end under different assumptions using the following simulation algorithm:

In the virtual trial we repeat daily the following steps (max trial duration 200 days):

1. *Enrollment and simulation of data events of new patients*
   Every day, we enroll 7 new patients and simulate their time-to-event and event type (recovery or death). In particular, we sample both the times-to-recovery and time-to-death from Weibull distributions, using the approach described in Beyersmann, Jan, et al. "Simulating competing risks data in survival analysis." *Statistics in medicine* 28.6 (2009): 956-971. We let the cause-specific hazard functions for the 2 event types to depend both on Randomization Arm (Control or one of 4 Active agents) and baseline COVID level (level 5 vs 6/7).

At the beginning of the trial, we set the randomization ratio to 2:1:1:1:1. We assume that the probability of enrolling a patient with a COVID level 6/7 is 35%.

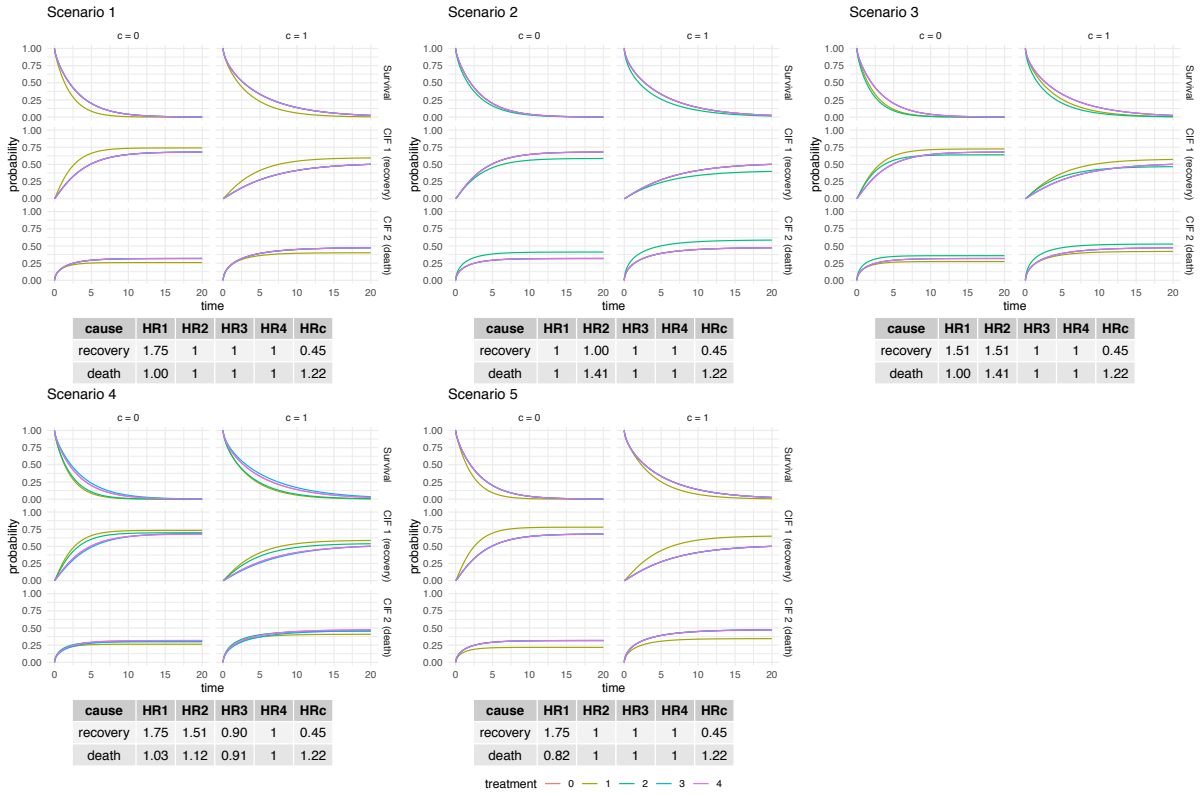
The parameters used to simulate the time-to-event depend on the scenario considered. For example, in the Scenario 1 we sampled the times-to-recovery from a Weibull distribution with shape parameter = 1.15 and a scale parameter (intercept) = -1.725. The cause-specific HR for recovery for the only effective Investigational Agent vs Control was set to 1.75, while it was set to 1 for the other Agents. The cause-specific HR for baseline COVID level (6/7 vs 5) was set to 0.45. The times-to-death were sampled from a Weibull distribution with shape parameter = 0.5 and scale parameter = -1.5. The cause-specific HR for mortality was set to 1 for all Agents, ie we assumed no differences in the cause-specific hazard of dying in the Investigational Agent arms vs Control. The cause-specific mortality HR fir baseline COVID level (6/7 vs 5) was set to 1.22. For the other scenarios, the corresponding cause-specific Hazard Ratios for the 4 Active agents vs Control and for Covid level 6/7 vs 5 are reported at the bottom of Figure 2, which illustrate the Cumulative Incidence Functions for the two event types, conditional on Randomization Arm and baseline COVID level. These curves are largely consistent with Grasselli et al. JAMA. 2020;323(16):1574-1581, Richardson et al. JAMA. 2020;323(20):2052-2059, and the UK Intensive Care National Audit & Research Centre (ICNARC), which repeatedly publishes patient characteristics and outcomes of COVID-19 patients in the UK (https://www.icnarc.org/Our-Audit/Audits/Cmp/Reports).

2. *Update the data events (time and event)*
Every day in the virtual trial, we update the information available at that moment, i.e. how many patients are still alive and under treatment (censored), how many died (competing event), and how many are recovered (main outcome), and the corresponding time-to-event.

3. *Evaluate the accumulating data*
After having observed at least 10 recovered patients, we fit the two described Bayesian Weibull survival models (one per outcome) and update the prespecified prior distributions to obtain the posterior distribution for all models' parameters. The prior distributions for the coefficients of the linear predictor used to model the log-scale parameter of the Weibull distributions are Normal(0, 0.5), while the prior distribution for the shape parameter is Exponential(1).

4. *Decision rules*
After having enrolled at least 50 patients to a specific treatment, we decide either to continue or early terminate the randomization to the specific treatment based on threshold rules decided in the simulation study:
Graduation: $P(HR > 1) \geq 0.975$ (DMC will make sure treatment safety in terms of mortality)
Futility: $P(HR > 1.5) \leq 0.1$ or, indicating HR_2 the HR for mortality, $P(HR_2 > 1.3) \geq 0.7$
Stop: if the maximum number of 125 patients have been randomized to a specific treatment

5. *Update randomization probabilities*
Randomization probabilities are updated based on the remaining available treatments, by making sure they sum up to 1. The algorithm is then repeated from 1).



cause	HR1	HR2	HR3	HR4	HRc
recovery	1.75	1	1	1	0.45
death	1.00	1	1	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
recovery	1	1.00	1	1	0.45
death	1	1.41	1	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
recovery	1.51	1.51	1	1	0.45
death	1.00	1.41	1	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
recovery	1.75	1.51	0.90	1	0.45
death	1.03	1.12	0.91	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
recovery	1.75	1	1	1	0.45
death	0.82	1	1	1	1.22

Figure 2. *Survival and cumulative incidence curves for the five scenarios of the base simulations. The cause-specific hazard ratios for the four Active agents vs Control (HR1, …, HR4) and for baseline COVID level 6/7 vs 5 HRc) are reported in the tables below the survival and cumulative incidence curves.*

The results of the base simulations are presented in Table 3, where we report the probabilities of graduation, futility and safety stopping.

scenario	var	trt_1	trt_2	trt_3	trt_4
1	graduation	0.888	0.033	0.03	0.031
1	futility	0.1	0.963	0.968	0.969
1	safety	0.013	0.006	0.007	0.007
1	futility or safety	0.112	0.967	0.97	0.969
2	graduation	0.037	0.038	0.038	0.031
2	futility	0.959	0.908	0.958	0.968
2	safety	0.008	0.163	0.007	0.005
2	futility or safety	0.963	0.962	0.962	0.969
3	graduation	0.639	0.544	0.022	0.034
3	futility	0.356	0.299	0.978	0.965
3	safety	0.008	0.189	0.004	0.006
3	futility or safety	0.361	0.456	0.978	0.966
4	graduation	0.883	0.63	0.008	0.029

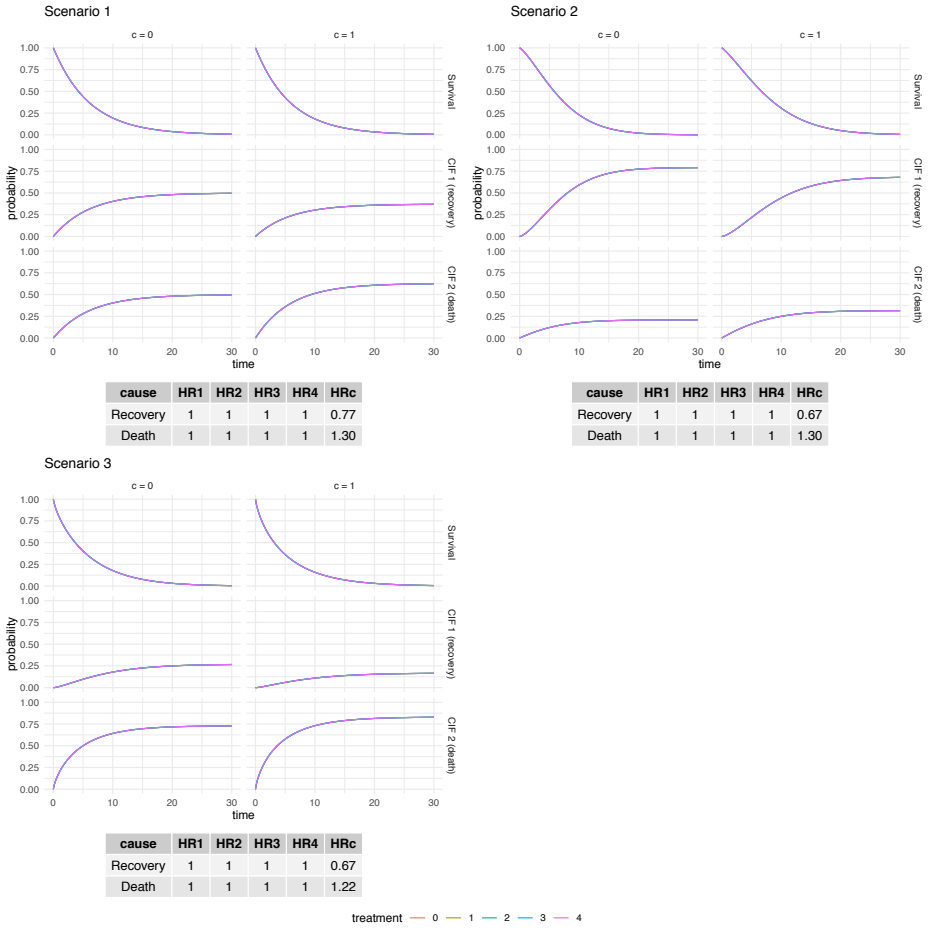
4	futility	0.109	0.338	0.992	0.969
4	safety	0.01	0.035	0.002	0.004
4	futility or safety	0.117	0.37	0.992	0.971
5	graduation	0.903	0.033	0.043	0.032
5	futility	0.095	0.963	0.955	0.962
5	safety	0.002	0.012	0.011	0.015
5	futility or safety	0.097	0.967	0.957	0.968

Table 3. *Probabilities for graduation, or dropping for futility or hitting criterion for DMC safety review for the five scenarios of the base simulations.*

Interactive results from individual simulated trials are available at:
http://alecri.github.io/downloads/example_simulations.html

8.2 Simulations of scenarios covering the null space

To investigate the robustness of simulations results under the null, we simulated scenarios using different shapes for the cumulative incidence and survival functions for the two outcomes (Figure 3). In these simulations, the Active agents have no effect on all the cause-specific Hazard Ratios (they're set equal to 1). The results of these simulations are shown in Table 4. The conclusion of these results is that type 1 error rates are consistent across different specifications of the null.



cause	HR1	HR2	HR3	HR4	HRc
Recovery	1	1	1	1	0.77
Death	1	1	1	1	1.30

cause	HR1	HR2	HR3	HR4	HRc
Recovery	1	1	1	1	0.67
Death	1	1	1	1	1.30

cause	HR1	HR2	HR3	HR4	HRc
Recovery	1	1	1	1	0.67
Death	1	1	1	1	1.22

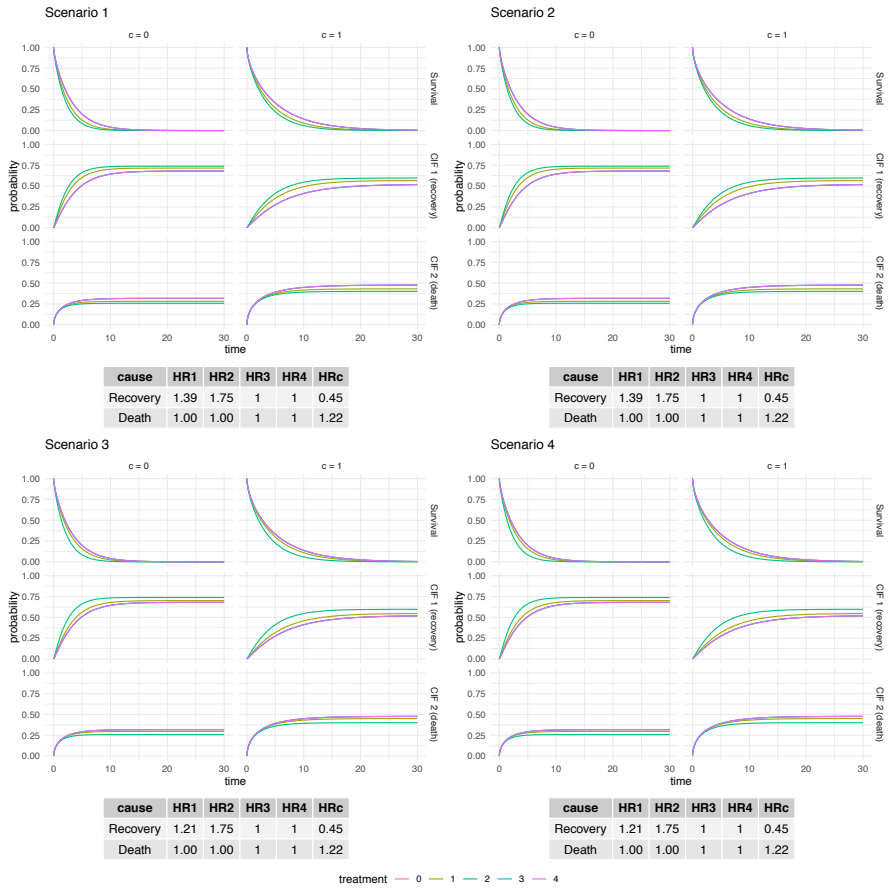
Figure 3. *Survival and cumulative incidence curves for the three scenarios of the simulations used to explore the null space. The cause-specific hazard ratios for the four Active agents vs Control (HR1, …, HR4) and for baseline COVID level 6/7 vs 5 HRc) are reported in the tables below the survival and cumulative incidence curves.*

scenario	var	trt_1	trt_2	trt_3	trt_4
1	graduation	0.052	0.039	0.043	0.046
1	futility	0.933	0.95	0.946	0.945
1	safety	0.025	0.032	0.031	0.028
1	futility or safety	0.947	0.961	0.957	0.954
2	graduation	0.041	0.05	0.058	0.065
2	futility	0.948	0.945	0.931	0.922
2	safety	0.044	0.026	0.039	0.038
2	futility or safety	0.959	0.95	0.942	0.935
3	graduation	0.033	0.035	0.043	0.038
3	futility	0.874	0.858	0.852	0.862
3	safety	0.004	0.013	0.01	0.011
3	futility or safety	0.878	0.868	0.857	0.868

Table 4. *Probabilities for graduation, or dropping for futility or hitting criterion for DMC safety review for the three scenarios of the simulations used to explore the null space.*

8.3 Simulations varying the randomization ratio, the number of subjects before evaluation and the maximum number of enrolled patients

In these simulations, we explored the consequences on early stopping probabilities (for graduation, futility, and safety) of changing the randomization ratio (from 2:1:1:1:1 to 1.4:1:1:1:1) and of decreasing the number of patients needed before an Active agent could be evaluated (from 50 to 40). Furthermore, we explored if enrolling 200 patients instead of 125 would improve the operating characteristics of the trial. The four scenarios considered are illustrated, in terms of Cumulative Incidence and Survival functions, in Figure 4. In scenarios 1 and 3 a maximum of 125 patients can be enrolled in each of the Active agents' arms, while in scenarios 2 and 4, the maximum number of patients is set to 200.



cause	HR1	HR2	HR3	HR4	HRc
Recovery	1.39	1.75	1	1	0.45
Death	1.00	1.00	1	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
Recovery	1.39	1.75	1	1	0.45
Death	1.00	1.00	1	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
Recovery	1.21	1.75	1	1	0.45
Death	1.00	1.00	1	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
Recovery	1.21	1.75	1	1	0.45
Death	1.00	1.00	1	1	1.22

Figure 4. *Survival and cumulative incidence curves for the three scenarios of the simulations used to explore the operating characteristics of the trials varying the randomization ratio, the number of subjects before evaluation and the maximum number of enrolled patients. The cause-specific hazard ratios for the four Active agents vs Control (HR1, …, HR4) and for baseline COVID level 6/7 vs 5 HRc) are reported in the tables below the survival and cumulative incidence curves.*

Results for these simulations are shown in Table 5.

scenario	var	trt_1	trt_2	trt_3	trt_4
1	graduation	0.42	0.85	0.02	0.03
1	futility	0.572	0.142	0.978	0.968
1	safety	0.014	0.01	0.01	0.008
1	futility or safety	0.58	0.15	0.98	0.97
2	graduation	0.448	0.876	0.03	0.018
2	futility	0.534	0.114	0.964	0.98
2	safety	0.02	0.01	0.01	0.01
2	futility or safety	0.552	0.124	0.97	0.982
3	graduation	0.168	0.864	0.022	0.018
3	futility	0.818	0.122	0.972	0.978
3	safety	0.022	0.014	0.008	0.014
3	futility or safety	0.832	0.136	0.978	0.982

4	graduation	0.17	0.852	0.028	0.02
4	futility	0.82	0.14	0.968	0.98
4	safety	0.012	0.008	0.01	0.004
4	futility or safety	0.83	0.148	0.972	0.98

Table 5. *Probabilities for graduation, or dropping for futility or hitting criterion for DMC safety review for the three scenarios of the simulations used to explore the operating characteristics of the trials varying the randomization ratio, the number of subjects before evaluation and the maximum number of enrolled patients.*

8.4 Simulations of family of primary endpoints (time to recovery and time to any cause death)

In these simulations, we explored the consequences of introducing graduation and futility rules based on a family of primary endpoints (recovery and overall mortality). These new stopping rules do not just involve the cause-specific HRs for the "Recovery" endpoint (csHRr) and for the competing event "Death w/o recovery" (csHRd), but also the HR for the "Mortality" endpoint (HRm).

The new stopping rules are the following and supersede those used so far (see "Base simulations"):

- Graduation: Pr(csHRr > 1 | data) ≥ 0.975 or Pr(HRm < 1 | data) ≥ 0.9
- Futility: Pr(csHRr > 1.5 | data) ≤ 0.1 and Pr(HRm < 1 | data) ≤ 0.5
- Safety: Pr(csHRd > 1.3 | data) ≥ 0.7

We used a Bayesian Weibull survival model to model time-to-death, ignoring potential Recovery and censoring subjects still at risk at the time of the analyses. From this model, we obtained the posterior distribution for HR_m. This model was fitted alongside the Weibull models described in "Base simulations". The same priors are specified for this third model's parameters.

The randomization weights (1.4:1:1:1), the maximum number of patients enrolled to each Investigational Agent arm (n=125), and the minimum number of patients needed before an Agent could be evaluated for futility (n=40) and graduation (n=50) have not been changed from the last set of simulations (simulations described above for varying the randomization ratio).

The scenario here considered is similar to Scenario 1 of §8.3 "Simulations varying the randomization ratio…", with the addition of a third effective Investigational Agent (IA 4) on both Recovery (though to a lesser extent than IA 2 and IA 3) and Mortality (see table below).

Endpoint	*IA 1*	*IA 2*	*IA 3*	*IA 4*	*Covid Status*
Recovery (csHRr)	1.00	1.75	1.39	1.13	0.45
Death w/o recovery (csHRd)	1.00	1.00	1.00	0.50	1.22
Mortality (HRm)	1.00	0.80	0.90	0.50	1.60

The results of these simulations are reported in the table below.

	IA_1	IA_2	IA_3	IA_4
graduation	0.042	0.878	0.474	0.866
futility	0.9	0.102	0.466	0.046
safety	0.016	0.016	0.026	0
futility or safety	0.904	0.118	0.488	0.046

Based on these simulations, the operating characteristics of the new decision rules seem reasonable.

To further explore operating characteristics with the change in the primary endpoint definition, we ran additional simulations with different assumed hazard ratios.

The following assumed hazard ratios

	IA 1	IA 2	IA 3	IA 4
Recovery (csHRr)	0.81	1.8	1.5	0.9
Death w/o recovery (csHRd)	0.45	1.2	1.04	1.3
Mortality (HRm)	0.50	1.0	1.03	1.04

yield the following results:

	IA_1	IA_2	IA_3	IA_4
graduation	0.874	0.856	0.554	0.032
futility	0.024	0.078	0.378	0.926
safety	0	0.068	0.078	0.024
futility or safety	0.024	0.144	0.446	0.934

The following assumed hazard ratios

	IA 1	IA 2	IA 3	IA 4
Recovery (csHRr)	1	1.80	1.51	0.81
Death w/o recovery (csHRd)	1	1.73	1.58	1.41
Mortality (HRm)	1	1.40	1.30	1.45

yield the following results:

	IA_1	IA_2	IA_3	IA_4
graduation	0.174	0.642	0.454	0.004
futility	0.552	0.088	0.370	0.99
safety	0	0.278	0.206	0.04
futility or safety	0.552	0.358	0.544	0.992

I-SPY COVID Trial – An Adaptive Platform Trial to Reduce Mortaility and Ventilator Requirements for Critically Ill Patients	
IND #:	150378
Amendment:	7
Location:	Quantum Leap Healthcare Collaborative San Francisco, CA
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Document Approval

Print Name	Signature	Date
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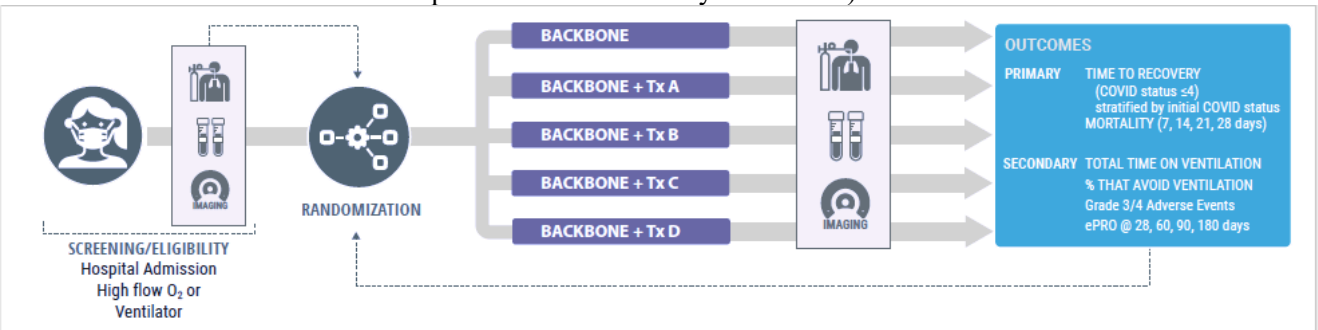
SCHEMA

Figure A: I-SPY COVID-19 TRIAL (An Adaptive Platform Trial to Reduce Mortality and Ventilator Requirements for Critically Ill Patients)



The platform trial design is shown above. Patients that enter the hospital and that are placed on high flow oxygen or are intubated will be screened for trial eligibility. Enrolled patients must have had a positive COVID-19 test by PCR or rapid antigen testing for SARS-CoV-2 infection. Eligible PCR-positive patients or their designated surrogates will be asked if they want to enroll on the therapeutic portion of the trial. All enrolled patients will receive backbone therapy with or without an additional investigational agent.

An observational component, separate from the platform trial, will follow ICU patients via medical records. The ICU patients may or may not be enrolled in the platform trial, must have a positive COVID-19 test by PCR or rapid antigen testing for SARS-CoV-2 infection and must be on high flow oxygen or intubated.

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LIST OF ABBREVIATIONS

Acronym	Description
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
BUN	Blood urea nitrogen
CBC	Complete blood count
CHF	Congestive heart failure
CLIA	Clinical Laboratory Improvement Amendment
COVID-19	Coronavirus disease 2019
CRF	Case report form
CPT	Cell Preparation Tube
CTA	Clinical Trials Agreement
CTCAE	Common Terminology Criteria for Adverse Events
DAPC	Data Access and Publications Committee
DCC	Data Coordinating Center
DLT	Dose-limiting toxicity
DMC	Data Monitoring Committee
ePRO	electronic Patient Reported Outcomes
DMC	Data Monitoring Committee
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GFR	
HIPAA	Health Insurance Portability and Accountability Act
HNSTD	Highest non-severely toxic dose
IASC	Independent Agent Selection Committee
ICH GCP	International Conference on Harmonisation Good Clinical Practice
IND	Investigational New Drug
IRB	Institutional Review Board
I-SPY TRIAL	**I**nvestigation of **S**erial Studies to **P**redict **Y**our **T**herapeutic **R**esponse with **I**maging **A**nd mo**L**ecular Analysis
MDACC	MD Anderson Cancer Center
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NDA	New Drug Application
NIH	National Institutes of Health
NOAEL	No observed adverse effect level
OHRP	Office of Human Research Protections
PBMC	Peripheral blood mononuclear cells
PI	Principal Investigator
PK	Pharmacokinetics

Acronym	Description
QLHC	Quantum Leap Healthcare Collaborative
SAE	Serious adverse event
UCSF	University of California, San Francisco
WBC	White blood cell1.

1. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary Identify agents that will result in substantial improvements to the clinical condition of participants with COVID-19	**Primary** Time to reach a durable COVID-19 level 4 or less and time to death (mortality). Data will be analyzed for 3 groups: ● All ● COVID-19 level 6/7 (those intubated immediately) ● COVID-19 level 5 (high flow oxygen to start) WHO 9-point ordinal scale: 0. No clinical or virologic evidence of infection 1. Not hospitalized, no limitations on activities; 2. Not hospitalized, limitation on activities; 3. Hospitalized, not requiring supplemental oxygen; 4. Hospitalized, requiring supplemental oxygen (< 6L by nasal cannula or mask delivery system); 5. Hospitalized, on non-invasive ventilation or high flow oxygen devices (≥6L per minute, mask or intranasal cannula); 6. Hospitalized, on invasive mechanical ventilation; 7. Hospitalized, ventilation plus additional organ support—pressors, RRT, ECMO 8. Death.
Secondary ● Health care utilization	**Secondary** ● % of COVID-19 level 5 who never progress to COVID-19 level 6/7 ● Ventilator-free Days

• Safety	• Total grade 3 or higher AEs by arm and total number of patients with grade 3 or higher AEs by arm. • Total grade 3 or higher AEs of special interest by arm and total number of patients with grade 3 or higher AEs of special interest by arms (based upon lab assessments)
• Differential impact on subsets of patients based on exploratory biomarkers and severity of presenting illness	• Mortality and ventilatory requirements for different ARDS subphenotypes, severity of illness at admission to ICU (ventilated or not) and comorbidities
• Establish biomarker-rich database for rapid learning about mechanism of illness and response to different classes of agents	• Evaluate response to classes of agents on the basis of exploratory biomarkers.
• Develop tools to rapidly assess pharmacodynamics and dose response	• Relationship between dose/exposure and response, measured as outcome (ventilatory requirements/mortality/days in ICU) or biomarker, will be quantitatively evaluated for each agent and integrated across the arms
• Vital Status	• Vital status at 28 days after study enrollment • ePRO 28 days,60 days, 120 and 180 days after enrollment
• Quality of Life	

2. BACKGROUND

2.1 COVID-19

Introduction: COVID-19 and ARDS

The goal of this project is to rapidly screen promising agents, in the setting of an adaptive platform trial, for treatment of critically ill COVID-19 patients. In this phase 2 platform design, we will be able to identify agents with a signal suggesting a big impact on reducing mortality and the need for, as well as duration, of mechanical ventilation.

Approximately 10-15% of those infected with the highly contagious SARS-CoV2 virus develop an acute respiratory illness, with a death rate in the 2-10% range. Of patients who present with respiratory distress, 40-60% require ventilation for a mean of 10-14 days, and up to 50% will not survive. The unprecedented rate of infection, with greater than 10 million cases world-wide and over 2.5 million of which are in the United States, has already led to more than 500,000 deaths, of which over 125,000 are in the US. [1]

There are numerous potential effective therapies for ARDS that warrant testing in clinical trials. As an example, immunomodulatory agents developed for autoimmune disorders are being considered, as they also target pathways known to be critical for ARDS pathogenesis and blunt the inflammatory cascade in the lungs that is triggered by viral infection. Other important examples include convalescent plasma, COVID-19 specific antibodies, statins, chemokine receptor inhibitors, anti-inflammatory agents and others. Although several therapies are under investigation, each trial is a one-off effort, many are not randomized, and there is no coordinated rapid cycle learning. An adaptive platform trial is the ideal design, as it allows the rapid screening of many agents simultaneously. Perhaps most important is that there are several agents with potential to abrogate the respiratory deterioration initiated in the setting of COVID-19 that are approved, available in sufficient supply or capable of rapid scaling to reach hundreds of thousands of patients world-wide. These should be the initial focus of testing as success in the setting of a phase 2 screening/signal seeking I-SPY COVID-19 platform trial can lead to the identification of agents to be evaluated in double blind, placebo-controlled confirmatory trials for regulatory approval and rapid dissemination to those in need of treatment.

The approach that we will take in this trial is to focus on finding agents with a big impact on reducing both mortality and time on ventilators for those critically ill as a result of COVID 19. Both of these endpoints are critical to managing the health crisis created by the global pandemic. We have chosen time to recovery for our primary endpoint, with a robust measure of recovery (at least 48 hours at COVID level 4 or less – nasal prongs oxygen- without returning to high flow oxygen or intubation). To facilitate rapid accrual, we will focus on mission critical information using a OneSource approach to minimize duplicate data collection. Within this trial, there will be an effort to collect sufficient data on biomarkers to evaluate, in a pure research setting, whether the data generated can lead to better and more targeted interventions, and to better understand the response based on severity of disease as well as prespecified subtypes of ARDS.

2.1.1 **Remdesivir therapy for COVID-19**

Remdesivir is a nucleoside ribonucleic acid (RNA) polymerase inhibitor with activity against SARS CoV-2. Remdesivir will be part of the backbone therapy for the I-SPY COVID-19 trial due to encouraging recent clinical results. The NIAID recently announced a preliminary data analysis of their Remdesivir trial data from 1,063 patients, which found that patients taking the drug recovered 31% faster than those taking the placebo. In remdesivir patients, the median time to recovery was 11 days. In placebo patients, it was 15 days. The mortality rate was lower in the remdesivir group (8%), when compared with those taking the placebo (11%), but this is not yet a statistically significant finding. On the basis of this

[1] As of 01-Nov-20 there are: 46.5 million cases worldwide, 9.3 million cases in the US. There are 1.2 million deaths globally and 231k deaths in the US.

data, Remdesivir has received Emergency Use Authorization and subsequent full approval from the FDA. Given the potential for interference with the activity of remdesivir, hydroxychloroquine will not be used in this trial.

2.2 Relevance of the I-SPY Breast Cancer Trial

The **I**nvestigation of **S**erial Studies to **P**redict **Y**our **T**herapeutic **R**esponse with **I**maging **A**nd mo**L**ecular analysis (I-SPY TRIAL) was designed to integrate clinical, laboratory and bioinformatics investigators in a platform model to evaluate neoadjuvant chemotherapy by bringing together data from multiple molecular biomarker studies with imaging. The intent was to evaluate and develop biomarkers of early response to standard chemotherapy, and to develop a strategy to improve outcomes of women with early, high-risk breast cancer who do not have an optimal response to current standard therapy). All participants received neoadjuvant chemotherapy to test a comprehensive panel of biomarkers, including MR imaging (MRI), for their ability to predict tumor response. Early endpoints being tested as predictors of three-year survival included MR changes (volume and longest diameter) and changes in gene expression. The intermediate endpoint was pCR (absence of invasive tumor in breast or lymph nodes at time of surgery), and the longer term endpoint was three-year RFS.

Quantum Leap Healthcare Collaborative (QLHC) manages the I-SPY 2 clinical trial, a pre-competitive consortium which includes a network of over 20 hospitals, hundreds of clinicians, scientists, computational biologists, and industry collaborators. QLHC, along with the working group chairs of the I-SPY 2 consortium, has demonstrated the ability to operationalize a collaborative but highly efficient process, with standing contracts for sites, standardized contracts with pharma, universal consents, and a streamlined master platform trial process with institutional review boards. To date, 22 phase 2 agents and combinations have been evaluated or are in the testing process in >1500 patients in the high-risk early stage neoadjuvant breast cancer setting over the last decade. The master platform trial structure allows the master protocol and universal consent to be approved initially by the FDA and all participating institutional review boards (RBs). All agents can enter and leave the trial seamlessly. Details about the agents and their associated consent forms are submitted as an amendment, and only those parts pertaining to the specific agent are reviewed. Amendments can be approved and then be in a queue awaiting activation. Accrual never stops as agents enter or exit the trial. The strength of the consortium is the re-engineering of trial and data collection processes as well as the culture of innovation and collaboration. We will repurpose this substantial capability for improving cancer care to the rapid development of new treatments for COVID-19.

The I-SPY COVID-19 response study will utilize the existing reporting structure and establish domain-specific working groups with guidance from the I-SPY 2 investigators and leadership. Some of the working groups will be the same (IRB working group, informatics working group), some will have shared input (Agents, and Biomarkers, Safety, Advocates) while others will be constituted separately with appropriate domain expertise (DMC, Clinical Operations). Our I-SPY 2 working group leaders will work with the COVID-19 teams to help get each group rapidly up to speed. Patients or their legally appointed representative (LAR) can directly consent by a paper signature and or electronic consent, such as Docusign or equivalent 21CFR Part 11 compliant system. Site coordinators for the I-SPY 2 TRIAL can support consent and data collection and can help to cross train new coordinators. The I-SPY COVID-19

consortium will start in approximately 15 active investigational I-SPY sites, and expand as needed or desired. UCSF will serve as the Program Management Office (PMO) and central lab. QLHC will be the non-profit sponsor and provide the operational infrastructure for the trial. Each working group is overseen by a working group chair and consists of trial site investigators (critical care and pulmonary physicians), infectious disease experts, drug development experts across related fields and translational scientists, project management office (PMO) and QLHC representatives. As in I-SPY 2, trial oversight will include key leadership of the FDA.

Each agent being investigated will be assigned two "chaperones" who will be responsible for overseeing the agent/agent combination within the trial, assembling final data and analysis.

Target enrollment is 50 patients a week and thus will require our Data Monitoring Committee (DMC) to meet twice a month and in the interim as needed. We will also have a Pharmacology and Safety Working Group to work with investigators to optimize drug delivery and management. General trial operations, site coordination and management, reporting, coordinator management, drug supply, protocol management is logistically overseen by the Operations Working Group and the I-SPY PMO.

2.3 Rationale: Adaptive Design Approach

Purpose and Study Rationale

Study Description:

This platform trial will provide access to repurposed and investigational agents for critically ill patients infected with SARS-CoV-2 who have severe or life-threatening COVID-19. The main focus of this trial is the platform study described herein for identifying efficacious agents for the treatment of COVID-19. Any critically ill patient with known or presumed COVID-19 will be automatically entered into the screening phase of the trial until SARS-CoV-2 infection is confirmed. Basic data will be assembled for each patient (such as ventilatory status and survival). If interested in the therapeutic portion of the trial, potential participants will be asked to sign a consent form describing the backbone treatment and the two specific investigational agent arms to which they may be randomized. Informed consent will be electronically obtained using a CFR part 11 compliant electronic signature system that will be embedded into the trial informatics platform (Docusign). Patient demographics from the electronic medical record will populate the CRFs. On a daily basis, coordinators will assess status, including ventilatory status, survival, and a small set of additional variables. The primary endpoint will be time to recover to a durable level 4 (or less) on the WHO COVID-19 ordinal scale for clinical improvement. For this trial, a durable level 4 is defined as at least 48 hours at COVID level 4 or less (nasal prongs oxygen) without returning to high flow oxygen or intubation. Acute care facility resource utilization will be automatically calculated (total length of stay in a critical care setting, days intubated, and survival). Any change in status, including intubation, extubation, death or discharge, will be recorded and verified by the attending physician.

We will use the platform trial approach developed by I-SPY to rapidly assess potential COVID-19 treatments. Patients will be evaluated based on their initial status (ventilation at entry vs. high flow oxygen). Exploratory biomarkers will be evaluated over time (ARDS phenotypes and other proposed markers) to facilitate clinical learning. Randomization is 1.2:1:1 for ratio of the control to two

investigational arms. The anticipated accrual will be 50 patients per week. The maximum number of participants assigned to an arm without graduation will be 125 patients. Agents can be dropped for futility after enrollment of 40 patients. We anticipate that 10 investigational agents can be evaluated in the span of 4-6 months, depending on the time course of COVID-19 infections across the US. As the trial proceeds and a better understanding of the underlying mechanisms of the COVID-19 illness emerges, we can expand biomarker and data collection as needed to further elucidate how agents are or are not working. The study design features comparison of investigational agent efficacy using a Bayesian design, which will allow the detection of strong efficacy signals with the fewest possible patients. Initially the control will be patients given current standard of care (supportive care for ARDS, including lung protective ventilation and remdesivir and dexamethasone as backbone therapy). As other treatments (for example, anticoagulation) become part of standard supportive care across sites, these will be added to the backbone therapy. If an agent meets the threshold for graduation the company leadership will be informed as will the FDA. The arm with the graduated agent will cease to enroll, allowing a new arm with a different investigational agent to be added. Prior to any agent entering the trial, we will create a list of senior executives who will be told information about the status of their drug. Information about agent disposition will be as follows:

1) Agents that Graduate: company will be informed at the time the disposition is made (select company leadership group)**;** participants currently receiving that agent will continue on the regimen until they complete the entire course of treatment.
2) Agents that Reach Maximum Accrual: company will be informed at the time the disposition is made (select company leadership group)**;** information will be made available to the investigators 30-45 days after the last patient is treated
3) Agents that Drop for Futility: company and investigators will be informed; the decision to stop treatment of patients on the arm is at investigator's discretion, if the patient is doing well and the investigator wants to continue treatment. If an agent is dropped because of safety it will be discontinued for all patients.

The trial assesses performance of agents based on disease severity (intubated or not at presentation). As well, the trial will enable investigation of the performance of agents against pre-specified subtypes based on exploratory biomarkers. This latter analysis will be performed as a secondary objective in a pure research setting. Every trial participant will have blood collected at trial enrollment, day 3, and day 7 for pre-specified biomarker and DNA and RNA analysis. Additional biomarkers can be added as the trial proceeds. Patient outcomes will also be evaluated on the basis of whether patients are ventilated initially or not.

This is a Phase 2, open-label study designed to reduce mortality and morbidity as well as the impact on healthcare utilization for critically ill patients with severe or immediately life threatening COVID-19. Agents that succeed or "graduate" from this trial will be recommended for further double blind, placebo-controlled phase 3 trials so that the signal can be validated and enable registration for the indication of acute and severe illness related to COVID-19.

Observational Component:

All Covid confirmed patients who start high-flow oxygen (WHO COVID-19 level 5; ≥6L oxygen by nasal prongs or mask) will be entered in an Observational Component which will collect data via extraction of medical records. Patients in the Observation Component also will have the daily COVID status and drug administration form CRFs completed.

2.4 Study Agent Rationale

The I-SPY COVID-19 investigational agents are described in appendices D–x. As investigational agents are added to the trial, they will appear as subsequent appendices.

2.4.1 Agent Selection

The initial process of candidate agent review begins with the I-SPY COVID-19 Agent Selection Working Group. This group consists of trial collaborators with critical care, infectious disease, drug development, and I SPY experience, many of whom contributed to the initial list of possible agents.

This list includes cytokine-directed agents (bradykinin B2 antagonists, pKal inhibitors that target the kallikrein system that contributes to vascular permeability and sustains the inflammatory cascade; agents that inhibit the release of cytokines that lead to acute lung injury, such as PDe4i (Apremilast); CCR2/5 inhibitor (Cenicriviroc); a Tie2 inhibitor such as Razuprotafib and mucolytics such as Pulmozyme. Agents that target IL-1 and IL-6 pathways are already in testing elsewhere, and results of these studies will inform I-SPY for COVID-19-related use of these agents or perhaps less immunosuppressive versions with shorter half-life. As well, the committee will consider agents that directly target the virus including antibodies to the virus, which can be given in the form of IVIG prepared from convalescent plasma (Takeda) or purified antibodies (Vir/GSK).

Through a partnership with the COVID-19 R&D Consortium, several agents are being proposed that have a scientific rationale for efficacy in the setting of pulmonary disease from COVID-19 and which are already approved for other indications. These agents have the capacity to scale globally and to be available for treatment in 2020. The Agents Selection Working Group consisting of experts from ARDS, pharma, and drug development is continuously screening many other proposed agents for the trial and determining the suitability of proposed agents to meet the requirements for scientific rationale, safety, and scalability.

2.5 General Approach in Evaluating Agent(s) and Biomarkers

The I-SPY COVID-19 trial will simultaneously examine the efficacy of multiple investigational agents or agent combinations. Each participant will be given the current standard of care (supportive care for ARDS, including, if needed, lung protective ventilation) and will be randomized to either a backbone of remdesivir or to an experimental arm that includes remdesivir and dexamethasone as a backbone plus an investigational agent. Participants will continue on the study until protocol completion or removal; re-randomization is not planned under the current proposed design. An important objective of the study is to identify agents that are associated with substantial improvements to the clinical condition of these participants. Another goal is to identify agent-associated enhanced survival in these participants. Regimens will be dropped if they are not sufficiently effective.

The Master Investigational New Drug (IND) application will be amended to include additional investigational agents/agent combinations as updated safety and efficacy information becomes available and initial treatment groups are dropped for futility or graduated.

Access to the I-SPY COVID-19 trial data and biospecimen repository is governed by the I-SPY COVID-19 Data Access and Publications Committee (DAPC). Investigators can contribute either biospecimens or processed data. Researchers interested in obtaining access to the I-SPY COVID-19 dataset for analysis should submit a completed concept sheet to the DAPC. Those researchers interested in evaluating a biomarker platform in the I-SPY COVID-19 trial will be designated the Platform Chaperone once their concept sheet is approved by the DAPC. The Platform Chaperone will have continued involvement with other I-SPY COVID-19 researchers interested in utilizing their platform; however, the Platform Chaperone will not own the data obtained by the other I-SPY COVID-19 researchers. Requests for biospecimens are sent to the I-SPY COVID-19 Biomarker Committee who will review and recommend requests for biospecimens to the I-SPY COVID-19 DAPC for final approval.

3. SUMMARY OF STUDY PLAN

3.1 Screening Phase

- Severe disease or life threatening COVID-19
- Critical care status (regardless of location of admission)
- Assessment of co-morbidities, BMI, respiratory rate, intubation status

3.2 Treatment Phase

- Investigational agents will be given as directed for each specific arm being investigated. Up to two agents can be active at any given time
- Study blood draws for research will be collected on days 1, 3 and 7 if blood samples can be safely collected and banked. Biomarker analysis will be performed on one plasma tube for those on the randomized phase of the trial for:
    - DNA and RNA
    - Cytokines
    - Other inflammatory markers (C-reactive protein, others)
    - Others to be determined
- Daily collection of ventilator status, vital and COVID-19 status level and values of specific laboratory tests per the requirements of the protocol
- All adverse events will be collected daily

3.3 Randomization of Patients to Investigational Agents

Agents will be assigned to all patients equally as the trial begins. The trial is designed to study over time which agents perform best.

Each agent's probability of reducing time to recovery and mortality will be calculated as the trial proceeds:

- **Agents will be graduated at an interim point should one or more of these probabilities reach a sufficient level.**
- **Agents will be dropped from the trial for futility when probabilities drop sufficiently low.**

If the maximum sample size of 125 participants assigned to a regimen is reached without reaching a graduating threshold, assignments to the regimen will end. Participants currently receiving the agent will continue on the regimen until they complete the entire course of treatment.

At each interim we calculate the Bayesian posterior distribution for the hazard ratio comparing each experimental treatment to control. From these posterior distributions we calculate Bayesian posterior probabilities: (1) $Pr(HR_d > 1.0)$, the probability the hazard ratio (HR) for treatment *d* is greater than 1.0, representing an improvement of time to recovery vs. control/backbone therapy; (2) $Pr(HR_m < 1 \mid data) \geq 0.9$, the probability the HR for treatment *d* is smaller than 1.0, representing an improvement in survival vs. control/backbone therapy; and (2) $Pr(HR_d < 1.5)$, the probability the hazard ratio for treatment *d* offers less than a 50% improvement over control.

During the course of follow-up, a treatment may graduate for superiority compared to control or stop for futility if

if $Pr(HR_d > 1.0)$ is greater than or equal to 0.975 for at least 50 patients in an arm OR if $Pr(HR_m < 1.0)$ is greater than or equal to 0.9 for at least 50 patients in an arm, the treatment is considered for graduation;

-or-

if $Pr(HR_{r,d} > 1.5 \mid data) \leq 0.1$ AND $Pr(HR_{m,d} < 1 \mid data) \leq 0.5$, the treatment *d* is considered for termination.

If neither is true, then randomization to that treatment may occur up to a maximum of 125 patients.

If either decision threshold is met, the DMC will review the findings while also considering secondary endpoints and safety, and if they agree, will recommend to the PIs that the agent be graduated and publicly announced or terminated from the trial for futility.

Once an agent is dropped from the trial, the option to continue or drop the agent will be at the discretion of the participant and his or her treating physician. Participants who do not continue on the agent will continue on-study but will revert to the standard backbone/control regimen; their outcomes will remain part of the arm to which they were assigned.

If an investigational agent is removed from the trial due to serious side effects from the agent (e.g., increased mortality), use of that agent for all participants will be stopped. Participants will continue on-study but will revert to the standard/control regimen and their outcomes will remain part of the arm to which they were randomized.

The above assignment and stopping rules for randomization apply to all regimens in the trial irrespective of study entry.

Up to 2 investigational agents may be active at any given time. The number of agents considered will be restricted by the ability to "process" the trial agents expeditiously in order to give treating physicians, the FDA, and companies timely information concerning the potential role of the agent in treating COVID-19. Agents can seamlessly be added or dropped from the trial without interruption to the trial. Agents can be approved by IRBs and be in the queue for addition once other agents leave. Trial data will also be used to test, qualify, and validate biomarkers as predictors of response to specific therapeutic agents, but not in real time until such time as it is clear that a particular biomarker is critical to response evaluation.

When a situation arises where one or more sites cannot participate in a specific arm, randomization will be adjusted so that only those sites that have approved the arms will enroll subjects into the arm. If fewer than 80% of sites can participate in a specific arm, then the arm will not be included in the trial.

3.4 Graduation or Futility Stopping

During the course of follow-up, a treatment may graduate for superiority compared to control if at least 50 evaluable patients (i.e. patients with correct entry criteria and without missing data, which can enter the statistical analysis) have been enrolled to the given treatment arm. Analogously, 40 evaluable patients are needed to drop a treatment for futility.:

- An agent may be recommend for graduation if the posterior probability for its hazard ratio for time to recovery vs. backbone (adjusted for COVID-19 level status at baseline) is greater than one is greater than or equal to 0.975, $Pr(HR_{r,d} > 1) \geq 0.975$ OR if the posterior probability

for its hazard ratio for mortality vs. backbone (adjusted for COVID-19 level status at baseline) is smaller than one is greater than or equal to 0.9, $Pr(HR_{m,d} > 1) \geq 0.9$, where $HR_{r,d}$ is the hazard ratio for recovery for treatment *d*, and $HR_{m,d}$ is the hazard ratio for mortality for treatment *d*.

A treatment may stop for futility if at least 40 patients have succeeded in reaching the endpoint:

- An agent may be recommend for futility if $Pr(HR_{r,d} > 1.5 \mid data) \leq 0.1$ AND $Pr(HR_{m,d} < 1 \mid data) \leq 0.5$.

In addition, we have a criterion for safety: If $Pr(HR_{m,d} > 1.3) \geq 0.7$, where $HR_{m,d}$ is the hazard ratio for mortality for treatment *d*. If this criterion is met, it will trigger a DMC review of data for treatment *d* against controls to decide whether it is regarded as acceptable to continue enrolling patients to this arm (at the next DMC meeting immediately after the safety criterion was met).

If the maximum sample size of participants is reached, assignments to the regimen will end. Participants currently receiving the agent will continue on the regimen until they complete the entire course of treatment.

If an investigational agent reaches a threshold for graduation, the DMC will review the findings and make a recommendation to Study Principal Investigators (PIs) for final approval. In addition to examining $HR_{r,d}$ and $HR_{m,d}$ the DMC will also review and evaluate the cumulative incidence function for recovery. The DMC will, during the review process, also evaluate mortality data in addition to $HR_{m,d}$. This will be done by studying key mortality statistics: (1) Proportion of patients alive in the treatment and control arm at day 7, 14, 21, and 28; (2) The cumulative incidence function for mortality. If these statistics are favorable, i.e. do not indicate increased mortality, the drug will graduate. The proportion of patients still at risk of both outcomes of the study (i.e. recovery and death) is given by the survival function, which we will also report to the DMC and in publications. During the review by the DMC and PIs, participants currently receiving that agent will continue on the regimen until they complete the entire course of treatment. No additional patients will be randomized to the agent arm after graduation.

If an agent is found not to reach a specified threshold of improvement in response, it may be dropped for futility; the DMC will review the findings, and if they agree, will recommend to the PIs that the agent be dropped from the trial. During review by the DMC and PIs, no participants will be randomized to that regimen or agent. Participants who have not completed the course of the agent will continue to receive the agent until a determination is made. Once an agent is dropped from the trial, the option to continue or drop the agent will be at the discretion of the participant and his or her treating physician. Participants who do not continue on the agent will continue on-study but will revert to the standard/control regimen; their outcomes will remain part of the arm to which they were assigned. Once an agent is dropped from the trial, the option to continue or drop the agent will be at the discretion of the participant and his or her treating physician. Participants who do not continue on the agent will continue on-study but will revert to the standard/control regimen; their outcomes will remain part of the arm to which they were assigned.

If an investigational agent is removed from the trial due to serious side effects from the agent, use of that agent for all participants will be stopped. Participants will continue on-study but will revert to the standard/control regimen and their outcomes will remain part of the arm to which they were randomized.

The above assignment and stopping rules for randomization apply to all regimens in the trial irrespective of study entry.

Up to two investigational agents will be active at any given time. Trial data will also be used to test, qualify, and validate biomarkers as predictors of response to specific therapeutic agents. This trial is an opportunity to integrate information from emerging biomarkers and thereby accelerate identification of optimal therapies for patients with severe or life-threatening COVID-19.

3.5 Trial Informatics

The randomization will be a ratio of 1.2:1:1 control patients to those assigned to investigational agents in each of two experimental treatment (non-control) arms. Because all comparisons will be an experimental arm vs. control, increasing the randomization weight to the control increases statistical efficiency. Agents will be able to drop for futility or worse outcome, graduate due to superiority vs. control, or reach a maximum accrual without graduation (maximum of 125 patients). The primary endpoint family will be time to reach level 4 on the WHO COVID-19 scale for at least 48 hours (hazard ratio for time to recovery larger than 1) OR time to death (hazard ratio for time to death smaller than 1). Secondary endpoints will include time on the ventilator and adverse events.

Study data collection will be performed through the OpenClinica Clinical EDC system, where Clinical Research Coordinators (CRCs) at the respective I-SPY COVID-19 trial sites will have secure login credentials allowing them to register and enter relevant study data information. OpenClinica allows for providers to refer patients through referral electronic Case Report Forms (eCRFs), that can be reviewed by CRC's to assess eligibility and initiate the consent process before formal enrollment in the trial. All eCRFs will be developed using Clinical Data Interchange Standards Consortium standards. For adverse event reporting, the standard safety terminology used is the National Cancer Institute's (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v5.0. eCRFs will be structured to balance ease of data entry with mapping complexity for the target Clinical Data Interchange Standards Consortium Study Data Tabulation Model datasets. Management and revisions to eCRF data elements will be managed through by the informatics group with guidance from the FDA to insure alignment with other COVID-19 and ARDS studies. For dissemination of analysis datasets to statisticians and the DMC, a target Define.xml will be used in parallel with the eCRFs. Define-XML is required by the United States Food and Drug Administration (FDA) for every study in each electronic submission to inform the regulators which datasets, variables, controlled terms, and other specified metadata were used. Field names will be aligned with Clinical Data Acquisition Standards Harmonization ARDS and COVID-19 domains where possible and codelists matched to the appropriate NCI controlled Terminology.

Data visualizations will be deployed and accessible via the Tableau Server platform, allowing decision support displays for access by the project management office, data managers and the DMC.

4. PARTICIPANT SELECTION

4.1 Screening process

- The I-SPY COVID-19 TRIAL will screen adult patients that enter the hospital with suspected or confirmed COVID-19. Prior to enrollment in the therapeutic portion of the trial, the diagnosis of COVID-19 must be confirmed by PCR or rapid antigen testing for SARS-CoV-2 infection.

- Eligible adult patients with PCR confirmation of COVID-19 or legally authorized representive will be asked if they want to enroll in the therapeutic portion of the trial. A Legally authorized representative (LAR, also called a designated surrogate) means an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedure(s) involved in the research (45 CFR 46.102(c)).

- Separate from the therapeutic component, the Observational Component will include all COVID-19 confirmed patients who are at least 18 years old and who require high flow oxygen. The Observational Component will be conducted under a waiver of informed consent.

4.2 Inclusion Criteria for Treatment Phase of I-SPY COVID-19 TRIAL

Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for participation in this study:

A. Male or Female, at least 18 years old

B. Admitted to the hospital and placed on high flow oxygen (≥6L by nasal cannula or mask delivery system) or intubated for the treatment of (established or presumed) COVID-19.

C. Informed consent provided by the patient, LAR or health care proxy.

D. Confirmation of SARS-CoV-2 infection by PCR or Rapid antigen testing for SARS-CoV-2 infection prior to randomization.

4.3 Exclusion Criteria

A. Pregnant or breastfeeding women (must be documented by a pregnancy test during hospitalization)

B. History of allergic reactions attributed to compounds of similar chemical or biologic composition to study agent based on review of the medical record and patient history.

C. Comfort measures only.

D. Chronic liver disease with a Child-Pugh score greater than 11.

E. Resident for more than six months at a skilled nursing facility.

F. Estimated mortality greater than 50% over the next six months from underlying chronic conditions.

G. Time since requirement for high flow oxygen or ventilation greater than 5 days.

H. Anticipated transfer to another hospital which is not a study site within 72 hours.

I. Patients with either end-stage kidney disease or acute kidney injury who are on dialysis.

J. Co-enrollment in clinical trials of pharmacologic agents requiring an IND

K. On 3 or more vasopressors

4.4 Inclusion of Women and Minorities

This study will be carried out in men and women.

4.5 Recruitment and Retention Plan

Participant eligibility will be systematically assessed at each of the participating I-SPY COVID trial study sites. A screening log will be kept documenting the review of potentially eligible participants as well as reasons for non-enrollment. Sites will provide detailed information to all relevant treating physicians on the conduct of the trial to optimize physician participation. Monthly conference calls will review recruitment at each site so that sites not meeting recruitment goals can be identified early and interventions to improve recruitment can be instituted.

Patients will be asked if they want to participate in the treatment phase of the trial. If they are interested in participating in the trial, they will be given an informed consent with appendices that describe the currently active agents. After they consent to participation in the treatment phase of the trial, they will be randomized to a study arm.

5. THERAPEUTIC AGENT ADMINISTRATION

5.1 Standard Treatment Plan for Control (Backbone) Arm

Participants randomized to the backbone control will be given standard of care (supportive care for ARDS, including remdesivir and, if needed, lung protective ventilation). Because dexamethasone was shown to have benefit in at least one large randomized clinical trial (RECOVERY, NEJM 2020), patients in the backbone control arm should receive dexamethasone 6 mg IV or PO for a total of 10 days during the hospitalization or until or hospital discharge. Higher doses of steroids are allowed if the medical team plans to use higher dose steroids for a clinical indication. If dexamethasone is not available, options include methylprednisolone 32 mg IV daily OR prednisone 40 mg daily (these are dexamethasone equivalent doses). If the patient comes into the study on another steroid, that patient will be switched to dexamethasone. As other treatments (for example anticoagulation) become part of supportive care across sites, these will be added to the backbone therapy.

5.2 Investigational Agent Treatment Plan

Participants randomized will receive the standard of care backbone plus an investigational agent as described in each appendix.

5.3 Concomitant Medication

All concomitant medications will be recorded on a daily basis and cumulative dose will be assessed. We discourage the use of agents not shown to have benefit in a randomized controlled trial.

5.4 Co-enrollment in other non therapeutic trials

Co-enrollment in clinical trials of pharmacologic agents requiring an IND will not be allowed. Co-enrollment in clinical trials of supportive care measures (e.g., proning, helmet ventilation) and of pharmacologic measures within the standard of care will only be allowed if all arms will be eligible so as to avoid bias. The DMC will review any such trial and allow it only if it is determined that it will not bias the trial results.

5.5 Toxicity Management and Dose Modifications

5.5.1 Toxicity Management for Standard Therapy

Toxicity management and dose modifications for standard therapy are outlined in each investigational arm appendix.

5.5.2 Toxicity Management for Investigational Agents

Toxicity management and dose modifications for investigational agents are outlined in each specific agent appendix (Appendices D–x).

5.5.3 Liver Toxicity Management

Liver toxicity will be closely scrutinized for those investigational agents for which there are known concerns over liver toxicity, as described in the relevant appendix. Close monitoring of patients receiving these investigational agents should be initiated for the following participants:

- Participants with abnormal LFTs (ie, AST, ALT, alkaline phosphatase and/or total bilirubin) which are > ULN at the time of discharge and whose discharge LFTs > baseline.

The participant should be evaluated for liver biochemistry elevation potentially meeting Hy's law criteria as soon as possible, preferably within 24 to 48 hours from the time the investigator becomes aware of the abnormal results. Evaluation should typically include repeat testing of all 4 of the usual serum biochemical measures (ALT, AST, ALP, and TBL) to confirm the abnormalities and to determine if they are increasing or decreasing. Testing should be repeated until the levels decrease or stabilize.

For participants discharged from the hospital, but still receiving the investigational agent, the participant should promptly be retested (locally or at the treating hospital). If locally, normal laboratory ranges should be recorded, results should be made available to the sponsor's study physician and the investigator immediately, and the data should be included in the eCRF. If repeat testing within this time frame is not possible, the study intervention should be discontinued.

It is critical to initiate close monitoring immediately upon detection and confirmation of signals of liver biochemistry elevation potentially meeting Hy's law criteria as early as possible and not to wait until the next scheduled visit or monitoring interval. Close monitoring of the participant should be initiated in conjunction with the sponsor and consideration given to the following, as applicable and relevant:

- Obtain a more detailed history of symptoms and prior or concurrent diseases.
- Obtain a history of concomitant drug use, including nonprescription medications, herbal products and dietary supplements, alcohol and recreational drug use, and special diets.
- Obtain a history of exposure to environmental chemical agents.
- Consideration of evaluations including applicable laboratory tests (eg, direct bilirubin, INR), physical assessments, and other assessments (eg, imaging)
    - As clinically appropriate, rule out other potential causes of biochemical abnormalities, eg, acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; NASH; hypoxic/ischemic hepatopathy; and biliary tract disease.
- Consider gastroenterology or hepatology consultations.

If any of the following criteria are met, discontinuation of study intervention should be considered (if indicated, prior to receipt of confirming retest biochemistry laboratory test results) and the sponsor notified of the discontinuation:

- ALT or AST ≥ 3 × ULN and the participant is symptomatic with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia (> 5%)
- ALT or AST ≥ 3 ✕ ULN and total bilirubin > 2 ✕ ULN or INR > 1.5
- ALT or AST ≥ 5 × ULN for more than 2 weeks
- ALT or AST ≥ 8 × ULN

All participants showing liver biochemistry elevation meeting potential Hy's law criteria should be reported and followed until all abnormalities return to normal or to the baseline state.

6. INVESTIGATIONAL AGENT INFORMATION (See Appendices B and D–x)

The I-SPY COVID-19 TRIAL protocol and IND are structured to enable the seamless addition and release of investigational agents over the course of the trial. When an investigational agent is added or released from use in this trial, only relevant appendices require updating, specifically Appendix B and the corresponding investigational agent's appendix.

Appendix B: Overview of all investigational agents in the study
Appendix D–x: All investigational agent-specific information (one investigational agent per appendix). As investigational agents are added to the trial, they will appear as subsequent appendices.

Each investigational agent falls into one of three categories as described in Appendix B: 1) Agents approved, pending activation for randomization, 2) Agents approved, activated for randomization, and 3) Agents graduated or dropped, no longer activated for randomization.

Adding an Investigational Agent to the Trial
To add a new investigational agent or (new dose or combination of agents), the trial team prepares the protocol amendment containing: 1) the new investigational agent's Appendix, 2) an updated universal informed consent form, and 3) an updated Appendix B showing the new agent in Table 1.1 (Investigational Agents Approved, Pending Activation for Randomization).

The protocol amendment will be considered a major modification to the protocol and will require a full IRB committee review; however, it will not require stopping accrual to the trial because there will be no change to Table 1.2 (Investigational Agents Approved, Activated for Randomization). New investigational agents will remain in this category until all trial sites have received IRB approval and there is space in the randomization engine for the new agent.

When the randomization engine has room for a new agent to be added, a protocol amendment will be generated updating Appendix B by moving the new investigational agent from Table 1.1 (Investigational Agents Approved, Pending Activation for Randomization) to Table 1.2 (Investigational Agents Approved, Activated for Randomization).

The protocol amendment will be considered a minor modification to the protocol and will require an expedited review by the IRB, which should take about 1 to 2 weeks. The trial will not have to stop accruing during this period, because participants will not be randomized to the new investigational agent until all the sites have IRB approval for the agent.

In summary, to add and use an investigational agent to the trial a protocol amendment to ***add and activate*** the new investigational agent must be submitted and approved by the full IRB committee at each site

Releasing an Agent from the Trial
When an investigational agent is graduated or dropped from the trial, a protocol amendment will be generated. Appendix B will be updated moving the agent from Table 1.2 (Investigational Agents Approved, Activated for Randomization) to Table 1.3 (Investigational Agents Graduated or Dropped, No Longer Active for Randomization).

The protocol amendment will be considered a minor modification to the protocol. The protocol will be submitted concurrently to all the study site's IRBs and will only require expedited IRB review and approval, which should take 1 to 2 weeks. The trial will not have to stop accruing to the other treatment arms during this period.

7. Collection and Evaluation of Biomarkers

7.1 Clinical Biomarkers

We will collect information on biomarkers measured at participating sites for clinical purposes such as IL-6.

7.2 Collection of Exploratory Biomarkers

Substantial heterogeneity within the general ARDS population has likely contributed to the failure of experimental therapies for ARDS in recent large clinical trials, despite promising preclinical data. Identifying subphenotypes of ARDS is one approach to untangling the clinical and biological complexity that many believe is a barrier to discovery of successful new treatments, and our research group has led the field in this area (Calfee et al, Lancet Resp Med 2014; Sinha et al, Lancet Resp Med 2020). The ARDS subtypes we have identified are defined by a combination of physiologic, clinical and biological factors, all of which could be considered as biomarkers for stratification in a platform trial. By identifying meaningful but currently unrecognized subgroups encompassed by the broad consensus definition of ARDS, interventions can potentially be tested more efficiently in targeted cohorts.

Collected blood specimens will be used to enable molecular assays for discovery and assessment of exploratory biomarkers. These biomarkers for response prediction and patient stratification will be evaluated in a pure research setting. Plasma will be collected to enable analyses of the inflammatory biomarkers that define ARDS subtypes (e.g. IL-8, Protein C, TNFr1, and others). Collected cells will be used for DNA and RNA analysis. Likely assays include DNA and RNA sequencing. New technologies for analyzing these samples will be explored once they become available and are judged valuable.

7.3 Repository for Storing, Analyzing, and Comparing Assay Results

All specimens will be recorded and tracked using a laboratory information system. Specimen tracking numbers will be used to track and store assay results. Assay results will be stored in a data repository built for cross-platform analysis. We will use the same platforms for clinical database as well as biomarker database. All tools are cloud based.

8. STUDY ASSESSMENTS AND PROCEDURES

Whenever feasible, assessments may be collected in conjunction with those already scheduled for clinical care reasons. For example, in conjunction with blood draws already being performed for

clinical care reasons. Scheduling may occur so as to minimize additional exposure of health-care workers. Post-discharge visits will take place by telephone or telemedicine.

8.1 Schedule of Assessments

8.1.1 Admission and ICU

Table 8.1 Study Calendar

Study Phase	Screening (D-1 or D1)	Baseline (D1)	Study Intervention Period	Post Hospitalization Period
Screening/Eligibility Assessment[1]	x			
Informed Consent		x		
Enrollment/ Randomization		x		
Clinical Status Assessment[2]	x	x	Daily	D28 D60 D120 D180
Chemistry/Hematology			Daily (until study Day 10)[8]	
Coagulation, Inflammation & Myocarditis			Every other day (QOD) (until study Day 7)[19]	
Physical Exam[3]		x	As clinically indicated	
Vital Signs[4]		x	Daily	
ECG[4]		x	As clinically indicated	
Study Blood Draw[5]		x	(D3W1 & D7W1)	
Remdesivir Administration			Daily per FDA Emergency Use Authorization x 5 days (COVID-19 Level 5) or x 10 days (COVID-19 level 6, 7)	
Investigational Agent Administration			TBD	
Concomitant Meds[6]	x		Daily	
Adverse Events[7]		x	Daily	x[10]

1. If not performed in routine clinical care, a pregnancy test for women of childbearing age will be conducted.
2. While the participant is in the hospital, COVID-19 level status will be assessed daily. Patients released prior to 28 days will get a follow up phone call at 28+/-2 days for additional AE assessment. All patients will be given an ePRO questionnaire at 28+/-2 60+/-5 days, 120+/-5, 180+/-5 days and be notified via both email notification and mobile phone text. The ePRO form can be filled out (1) the participant online or (2) the participant with help via telephone from a central coordinator. Automatic reminders will be sent via the participant's preferred communication method. If forms for ePRO at each time point are not completed after the 2 automatic reminders a central coordinator will call to assist completion. The window for

completing ePRO follow up surveys is 7-days. If the subject cannot be reached on this third attempt and/or cannot complete the ePRO form within 7 days, the form will be 'closed' by the staff for that patient at that time point. The ePRO will be available in English or Spanish language.

3. Physical Exam will be targeted and symptom-directed to evaluate for any possible adverse event.
4. These assessments may be collected in conjunction with those already scheduled for clinical care reasons (eg, in conjunction with blood draws, ECGs, vital sign checks already being performed). Scheduling may occur so as to minimize additional patient contact if contact precautions are in place.
5. Collect blood for plasma, PK and PBMC at time of enrollment, at day 3, and at day 7. Plasma collected on both days will be assessed for pharmacodynamic markers and exploratory biomarkers. PK blood draw should be between 15-120 minutes post dose if possible.
6. Concomitant meds will be collected daily, see section 5.3.
7. Refer to section 11.1.1. Using the CTCAE standard definitions, Grade 3 and 4 toxicities for laboratory assessments will be determined daily based on normalized values for each institution (laboratory value over the reference range upper limit of normal). As well, we will record adverse events that occurred over the past 24 hours as described in 11.1.1.
8. CBC with differential and chem 10 panel. These will be collected while patients are in the ICU. Daily until Study Day 10, after which only needed as clinically indicated, but at least weekly and as per the specific Appendix for which the is enrolled.
9. Coagulation panel, D-dimer, CRP, BNP, and troponins. These will be collected while patients are in the ICU QOD up to 7 days.
10. For patients discharged prior to 28 days, additional AE data will be collected by follow up phone call 28+/-2 days (also administer ePRO 28 +/-2 days), at 60 +/-5 days, 120 +/-5 days and 180 +/-5 days via the ePRO questionnaire (Refer to section 11.1.1).

8.1.2 **Discharged from the ICU, but still hospitalized**

Patients who are hospitalized but discharged from the ICU will have the same schedule of assessments as above, with daily data collected (via Daily eCRF), drug administration form and relevant forms if on study regardless of study arms. Labs will be collected throughout the administration of study drug or up to Day 10 whichever is longer. Additional labs will be collected as clinically indicated, and as described in the Appendices for agent-specific lab draws.

8.1.3 **In ICU past 28 days**

Patients who are hospitalized in the ICU longer than 28 days will have collection of labs per clinical care.

8.2 Baseline Testing/Pretreatment Evaluation

The following data will be collected at the time of consent:

- PCR or rapid antigen testing for SARS-CoV-2 infection confirmation of SARS CoV-2 infection
- BMI
- Age
- Charlson comorbidity assessment (index will be calculated) including the following:
    - COPD
    - Smoker

  - Asthma
  - Diabetes
  - History of MI
  - CHF
  - Kidney Failure
- Liver failure (ChildPugh Score >11)
- Concomitant medications
- COVID-19 level status

8.3 Evaluations During Investigational Agent Treatment

8.3.1 Measures of clinical support

At each study day while hospitalized, the following measure of clinical support should be assessed:

- COVID-19 level status (modified to indicate that 5 is high flow oxygen and 4 is < 6L O_2 via nasal prongs or face mask).
- Renal replacement therapy requirement
- Vasopressor requirement

8.3.2 Ordinate Scale (9-point)

The 9-point ordinal COVID-19 status scale is an assessment of the clinical status at the first assessment of a given study day. Each day, the worst score observed for the previous day will be recorded. ie, on Day 3, Day 2 score is obtained and recorded as Day 2. The scale is as follows:

0. No clinical or virologic sign of infection

1. Not hospitalized, no limitations on activities;
2. Not hospitalized, limitation on activities;
3. Hospitalized, not requiring supplemental oxygen;
4. Hospitalized, requiring supplemental oxygen (< 6L by nasal cannula or mask delivery system);
5. Hospitalized, on non-invasive ventilation or high flow oxygen devices (≥6L per minute, mask or intranasal cannula);
6. Hospitalized, on invasive mechanical ventilation;
7. Hospitalized, ventilation plus additional organ support—pressors, RRT, ECMO
8. Death.

8.3.3 Evaluations/Safety Assessments

Patients on this protocol will be critically ill. Clinicians are capturing data on organ systems continually. This is done both for monitoring or for following a suspected problem. Planned time points for all safety assessments are provided in the Schedule of Assessments (section 8.1). Whenever feasible, assessment may be collected in conjunction with those already scheduled for clinical care reasons.

We will collect data daily on the following clinical assessments.

- Physical Exam: As clinically indicated, a targeted and symptom-directed physical exam to evaluate for any possible adverse events.
- Vital Signs: Temperature, pulse rate, respiratory rate and blood pressure will be assessed and recorded.
- ECG: As clinically indicated to evaluate for any possible adverse events.
- Adverse events will be collected on a daily basis reflecting the previous 24 hours, and will be assessed for all patients.

We will collect data on the following laboratory studies.

Daily Assessments (QD):

- Renal Function: Creatinine
- Liver function: AST/ALT and Bilirubin
- Hematology/infection: CBC and differential

Assessments every other day (QOD):

- Coagulation status: PT, PTT, and D-Dimer
- Inflammatory status: CRP
- Cardiac status: BNP, troponin

Laboratory Safety Assessment (QD):

- Using the CTCAE standard definitions, Grade 3 and 4 toxicities will be determined daily based on normalized values for each institution (laboratory value over the reference range upper limit of normal).

8.4 Evaluations at Completion of Investigational Agent Treatment

The following procedures will be done following discharge from the hospital:

- If study treatment continues beyond hospital discharge, specific instructions about tracking compliance will be in the investigational drug appendix.
- Phone call at day 28 after enrollment to assess patient status and additional AE's if discharged from the hospital prior to 28 days.
- ePRO questionnaire at 28 days 60 days, 120 and 180 days post enrollment that will include collection of additional AEs occurring post hospital discharge.

8.5 Evaluations for Premature Discontinuation of Study Treatment

Dose delays and reductions may be implemented as necessary. Subjects will be withdrawn from the study if persistent or unacceptable treatment-related toxicity is observed.

Since this is an intent-to-treat trial, participants who discontinue their randomized treatment assignment prematurely for any reason will remain "on study" to complete the remaining study procedures and follow-up.

For patients that discontinue treatment early we will continue to collect AEs and outcome data as described in Table 8.1. If a participant withdraws from the investigational arm prematurely we will collect new AEs, safety labs and assessment tests up to study day 28; see specific investigational agent appendices D-*x*.

8.6 Disease Progression

If progression of ARDS requires discontinuation from study medication, then local practice should be followed. For participants who progress on study treatment, clinical assessments will be followed including COVID-19 status level, need for pressors and or dialysis, and laboratory studies collected for clinical care as outlined above. All of these will be collected daily until discharge from the hospital.

8.7 Surveillance

There will be follow-up surveillance after discharge from the hospital in the form of an ePRO questionnaire 28 days, 60 days, 120 days and 180 days after enrollment. (PROMIS quality of life assessment- QOL, addended with select questions from the PRO-CTCAE as indicated in Appendix C. The surveillance data will be collected with an iPAD or computer by the patient. For patients who do not have these resources, the survey can be conducted by phone by a coordinator. If the patient does not fill out the questionnaire themselves, then it should be completed by phone interview with the patient using a study coordinator and noted on the questionnaire. For patients that require a translator, one will be provided. If a patient is hospitalized and ventilated or otherwise incapacitated, the survey will not be administered for that time point. If the patient is hospitalized and convalescing, they will be asked to complete the survey

9. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

9.1 Primary Endpoint

The primary endpoint is time to achieve a durable COVID-19 status level 4 or lower. For this trial, a durable level is defined as at least 48 hours at COVID level 4 or less (nasal prongs oxygen) without returning to high flow oxygen or intubation.

9.2 Secondary Endpoints

- Mortality (cumulative)
    - Proportion of patients alive in the treatment and control arm at day 7, 14, 21, and 28 (The proportion of patients alive, i.e. alive and still at risk of both reaching level 4 and death is given by the survival function).
    - The cumulative incidence function for mortality
    - Hazard ratio for mortality
- % of COVID-19 level 5 who never progress to COVID-19 level 6/7
- Ventilator-free days at Day 28
    - Patients who die will be assigned 0 ventilator-free days
    - Ventilator-free days will be calculated from the WHO Covid Ordinal Scale
- Total grade 3 or higher laboratory AEs, as well serious adverse events (as defined in Section 11) by arm
- Total number of patients with grade 3 or higher laboratory AEs by arm as well as serious adverse events.
- Total grade 3 or higher AEs of special interest by arm and total number of patients with grade 3 or higher AEs of special interest by arms (based upon lab assessments).
- Relationship between dose/exposure and response, measured as outcome (ventilatory requirements/mortality/days in ICU) or biomarker, will be quantitatively evaluated for each agent and integrated across the arms.
- We will analyze patient outcomes and differential treatment responses by predefined ARDS subphenotypes (Sinha et al Lancet Resp 2020) as exploratory endpoints.

9.3 Off-Investigational Agent Criteria

Off-investigational agent criteria are specified in the agent-specific appendix. Participants may stop taking a study agent for the following reasons: toxicity; or participant or physician preference. This is an intent-to-treat trial, so participants will continue to be followed in order to continue to collect study data

according to the schedule of assessments. See MOP for instructions on how to document participant's preference on discontinuing the investigational agent early (see section 8.6).

If an investigational agent reaches a threshold for graduation or is dropped for futility, no additional participants will be randomized to that agent. Participants currently receiving that agent will continue on the regimen until they complete the entire course of treatment (for graduating agents), or the option to continue or drop the agent will be at the discretion of the participant and their treating physician (for agents dropped for futility). In the later instance, participants will continue on-study but will revert to the standard/control regimen and will remain part of the arm to which they were assigned. See §3.4 for additional details regarding participant treatment options when an agent leaves the trial.

9.4 Off-study Criteria

Participants may go off-study either because the protocol intervention and any protocol-required follow-up period is completed or because the participant withdraws consent. See the individual agent appendices for further instruction. See MOP for further instructions for participants withdrawing consent.

9.5 Study Termination

QuantumLeap Healthcare Collaborative (QLHC) as the study Sponsor has the right to discontinue the study at any time.

10. SPECIMEN MANAGEMENT

10.1 Central Laboratories

All study samples will be sent to the I-SPY COVID-19 Central Laboratory (PIs: Drs. Carolyn Calfee and Michael Matthay) at UCSF, for processing of the samples in a timely manner.

Blood samples will be aliquoted for distribution as listed in §10.2.

All quality and quantity specimen data will be stored in the laboratory information system, as well as shipment tracking information. Each laboratory involved in the analysis of samples will be responsible for returning appropriate assay data to the trial data repository.

10.2 Specimen Collection, Handling, and Shipping Procedures

Table 10.1 lists the study blood specimen collections. Details regarding specimen collection, processing, tracking and shipping can be found in the I-SPY MOP.

Table 10.1 Blood sampling

Sample Type	Amount	Time point(s)	Notes (TBD)
EDTA Tube	One (1) 6.5 ml lavender-top vacutainer	Time point 1,3,7 days	For preparation of plasma and buffy coat cells
Tube TBD	One (1) up to 6.5 mL tube for PK analysis	Time point 1,3,7 days	For PK or other TBD biomarker or metabolite analysis
HeparinTube	One (1) 6 ml tube	Time point 1,7	For PBMC
Paxgene	2.5 mL (RNA) 2.5 mL (DNA)	RNA: Time point 1,3,7 days DNA: Time point day 1 only	For isolation of DNA and RNA

10.3 Research Specimen Processing and Laboratory Procedures

The manual of operations will detail the exact methods by which the specimens will be collected, processed, shipped and delivered to the appropriate labs of the study collaborators. Specimen and participant tracking procedures are outlined in the MOP.

10.3.1 **Whole Blood – Plasma and Buffy Coat**

Participants will have blood collected at the time of study enrollment and on study days 3 and 7, if blood can safely be obtained and stored by research personnel. The details of blood collection are shown in the Schedule of Events. Notably, no study participant will have more than 40 ml of blood drawn at any given specimen collection time point. Blood samples may be collected from every consenting participant enrolled in this study. The blood specimens will be collected for research purposes. In addition to nucleic acid collection, buffy coat and plasma will be extracted from blood specimens. Study participants have the option to consent to allow the researchers to retain leftover blood, buffy coat and plasma samples for future research. Specimens will remain at sites until central study storage has been implemented. Central study specimen storage will be housed at UCSF.

10.3.2 **Banked Specimens**

Additional banking procedures and changes in preparation and storage procedures may occur as new technologies and techniques evolve. For this reason, data and specimens obtained from study participants will be kept and stored indefinitely.

10.3.3 **10.3.3 Additional Samples**

10.3.4

Additional samples will be approved for other and exploratory assays. The I-SPY COVID-19 Biomarker Committee will receive, review, and recommend requests for samples to the I-SPY COVID-19 Data Access and Publication Committee for final approval.

Biomarker data will be made available through the I-SPY COVID Data Portal, as indicated by the I-SPY Data Access and Publication Guidelines.

11. RECORDING AND REPORTING OF ADVERSE EVENTS

11.1 Adverse Events

An adverse event is any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). A list of AEs that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in the specific agent appendix as noted under §6.0, Investigational Agent Information, as well as the Investigator's Brochure or package insert.

11.1.1 **Recording AEs**

Patients enrolled into this I-SPY COVID-19 trial are critically ill. We will use the recording framework proposed for critical care trials and adopted elsewhere for I-SPY COVID. Patients with severe COVID-19 and with ARDS will typically experience multiple events that would meet the conventional definition of a serious AE. The key principles are to safeguard the welfare of participants and to ensure systematic capture of organ system abnormalities that may be considered AEs or recognized as important because they are trial endpoints.

To standardize ascertainment of potential AEs occurring in the course of COVID illness , we will standardize laboratory collection with CBC with differential and Chem 10 (LFTs, Bili, Cr) daily and BNP, D-Dimer, Troponins, CRP and coagulation panel QOD as in the **Schedule of Assessments, Section 8.1**. The following AEs will be captured systematically through laboratory monitoring and daily review of patient status while critically ill. For abnormal laboratory tests, we will consider CTCAE v5.0 Grade 3 or higher as clinically significant. Events that will be collected include SAEs, AEs leading to dose modification, AEs leading to treatment or study discontinuation and AEs with CTCAE Grade 3 or higher. We will also evaluate the overall distribution of all collected AEs regardless of grade by treatment arm.

Laboratory assessments will be collected as defined and graded as defined in Table 11.1.

Clinical AEs of concern are defined as:

- Any clinically important untoward medical occurrence in a patient receiving treatment which is different from what is anticipated in the clinical course of a patient with ARDS, or
- Any clinically important, untoward medical occurrence that is thought to be associated with the study treatment, regardless of the "expectedness" of the event for a patient with ARDS

For each agent, we will also identify agent-specific adverse events of interest and systematically record these as well (see Section 11.3).

We will obtain and record laboratory tests and AEs as described in Section 8 until the patient is discharged from the hospital. Data will be collected as described in the Schedule of Assessments (Table 8.1). If the investigational agent is still being administered after hospital discharge, we will continue to monitor for AEs as per the agent-specific appendix.

11.1.2 **Reporting AEs**

We will report all recorded adverse events, which will include all adverse events meeting the respective category definition, regardless of whether they also meet the definition for specific standardized clinical events and regardless of perceived relatedness to investigational agent or COVID. Reports will specifically include SAEs, AESIs, AEs leading to dose modification, AEs leading to treatment or study discontinuation, all AEs with CTCAE Grade 3 or higher. We will report situations where criteria for Hy's Law Drug Induced Liver Injury are met.

Data elements to be reported for these events will include:

- AE reported date
- AE verbatim term
- NCI CTCAE v5.0 AE term (MedDRA lowest level term)
- CTCAE (MedDRA v23) System Organ Class (SOC)
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as an SAE
- Action taken with the study agent
- Outcome of the event
- Whether or not the participant dropped from the study due to AE
- Whether the event is expected (Y/N)
- Whether the event is likely due to COVID progression (Y/N)
- Comments

Aggregate analysis: Distribution of laboratory abnormalities, and systematically collected AEs as described in 11.1.1. If there is a difference in the distribution or severity of AEs, we will evaluate the attribution for the purposes of IND safety reporting.

11.1.3 **Severity of AEs**

All AEs will be identified and assessed for severity using the NCI CTC5.0. CTCAE v5.0 provides a grading scale for each AE listed. A copy of the CTCAE v5.0 can be found at http://ctep.cancer.gov.

All AEs will be coded using MedDRA version 23.0 for reporting to the FDA, DMC, and Institutional Review Boards (IRBs), as required.

11.1.4 **Assessment of Relationship of Reported AEs to Treatment**

The possibility that a reported AE is related to study agent will be classified as one of the following: unrelated, unlikely, possible, probable, and definite as described below:

- Unrelated (There is no evidence of causal relationship).
- Unlikely (There is little evidence to suggest there is a causal relationship (e.g., the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g., the participant's clinical condition, other concomitant treatments).
- Possible (There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events).
- Probable (There is evidence to suggest a causal relationship, and the influence of other factors is unlikely
- Definite (There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out).

11.1.5 **Exploratory Systematic Recording of Specific Clinical Events for AE Analysis**

Clinical assessments will be systematically recorded daily, and are reflected in the case report forms:

- Respiratory Failure: based on the need for mechanical ventilation, assessed daily
- Disseminated Intravascular Coagulation: based on clinical assessment, confirmed by standardized laboratory collection
- Secondary infection: will be assessed daily based on antibiotic use. In addition, we will evaluate all culture data and record the organisms for positive cultures.
- Hypotension: will be assessed daily based on number and dose of vasopressors
- Renal Failure: assessed daily based on standardized laboratory collection as well as renal replacement therapy (dialysis)
- Hepatic Failure: assessed daily based on standardized laboratory collection
- Myocardial infarction: assessed on an ongoing basis with standardized laboratory collection
- Deep venous thrombosis, pulmonary embolism: based on both laboratory data and the use of anticoagulation and clinical assessment
- Pneumothorax
- Stroke
- Any other clinical event of concern: the intent is to collect any clinically important event identified during the daily review of patient status

As an additional analysis, in addition to the adverse event recording described in **Section 11.1.1**, we will analyze excess toxicity related to the investigational agents using laboratory AEs and clinical assessments collected daily and evaluating the distribution of these AEs in the treatment arms relative to the control. Using laboratory assessments of renal function, liver function, coagulation, infection, cardiac function, and hematological parameters, we will determine the number and type of grade 3 and 4 AEs. For each patient, we will assess the total number of grade 3/4 adverse events. For each arm, the number of patients who have at least 1, 2, or 3+ AEs in this category will be determined. The total AEs daily for each arm will also be calculated. The DMC will be able to evaluate data by system (renal, liver, etc) for each arm to determine investigational agent-related safety issues. The system is set up to be able to investigate toxicity and AEs in any organ type on any arm of the trial, and across all arms of the trial.

We will record, at the time of death, the presence of the following: viral pneumonia, bacterial pneumonia, myocarditis, renal failure, liver failure, pulmonary emboli, intracranial hemorrhage, myocardial infarction, and stroke. The distribution and frequency of these associated conditions will be collected for all patients who expire during the course of the study, and will be compared by arm. We will look for any imbalance among the study arms to ascertain whether there is an association with the investigational agent. The DMC and the FDA will have full access to this information.

Where an aggregate analysis of specific events indicates that those events occur more frequently in the drug treatment group than the control group, an IND safety report will be submitted to FDA as described in Section 11.2.3.

Please see Table 11.1 for the CTCAE-based laboratory values and how AE grading will be calculated.

TABLE 11.1

Laboratory Parameter	**Units**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Liver					
AST	units/L	3x ULN	3-5x ULN	>5-20x ULN	>20x ULN
ALT	units/L	4x ULN	3-5x ULN	>5-20x ULN	>20x ULN
Bilirubin	mg/dL	1.5x ULN	1.5-3.0x ULN	3.0-10.0x ULN	>10.0x ULN
Renal					
Creatinine	mg/dL	1.5x ULN	>1.5-3.0x ULN	>.03-6.0x ULN	>6.0x ULN
Cardiac					
Troponin I	ng/mL	1-2X ULN	>2-5 x ULN	>5-20x ULN	>20x ULN
Coagulation					
Fibrinogen	mg/dL	<1-0.75 LLN	>0.75-0.5x LLN	>0.5-0.25x LLN	>0.25x LLN

INR	seconds	>1.2-1.5	>1.5-2.5	>2.5	N.A.
PTT		>ULN-1.5x ULN	>1.5-2.5x ULN	>2.5x ULN; bleeding	N.A.
Neutropenia					
Neutrophils	(x 10^9/L)	<LLN-3.0	<3.0-2.0	<2.0-1.0	<1.0
Other					
Hgb	gm/dL	<LLN-10	<10-8	<8	life-threatening
Platelets	(x 109/L)	<LLN-75	<75-50	<50-25	<25
White Blood Cell Count	(x 109/L)	<LLN-3.0	<3.0-2.0	<2.0-1.0	<1.0
Lymphocyte Count	(x 109/L)	<LLN-0.8	<0.8-0.5	<0.5-0.2	<0.2

11.2 Serious Adverse Events

11.2.1 **SAE Definition**

Serious Adverse Drug Experience is defined in 21 CFR 312.32 as any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Results in death. As described in Section 9.2, mortality is an efficacy endpoint of the study, so all deaths will be recorded in the clinical database. For the purpose of safety reporting, only the subset of events with death as an outcome that are considered by the investigator to have a causal relationship with a study drug (possible, probable or definite) will be reported to the sponsor as individual SAEs as described below. These events will then be assessed by the Medical Monitor and regulatory/safety CRO for expedited reporting to FDA as IND safety reports. Section 13.3 describes the review of aggregate mortality data for all subjects that die by the DMC by treatment arm.

- Is life threatening (Note: the term life-threatening refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).

- Requires inpatient hospitalization or prolongation of existing hospitalization (Note: All study subjects will be hospitalized at the time of study enrollment, by virtue of the disease being studied. Any AE that results in prolonged hospitalization should be documented and reported as an SAE.

- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

- Is a congenital abnormality/birth defect

- Important medical events that may not result in death, be life-threatening, or require hospitalization or prolongation of hospitalization may be considered serious when, based upon the appropriate medical judgment, they may jeopardize the participant or subject and may require medical or surgical intervention to prevent one of the outcomes listed.

All such SAEs will be reported using Adverse Event forms in EDC (i.e. Open Clinica). The safety clinical research organization (CRO) must be notified by phone within 24 hours of knowledge of the event. The organization must submit the SAE Report Form within 48 hours of knowledge of the event. Please refer to the I-SPY Manual of Operations for completion and submission guidelines.

Include the following information when calling the safety CRO:
ISPY COVID Safety Team
CCSA
Tel: 650.691.4400, x118
Email: ispycovidsafety@ccsainc.com:

- Date and time of the SAE
- Date and time of the SAE report
- Subject ID
- Name of reporter
- Call-back phone number
- Affiliation/Institution conducting the study
- Protocol number, title of protocol
- Description of the SAE, including reason serious and attribution to drug(s)

The Safety CRO will triage the reported information and inform the Safety Working Group (SWG) by contacting:

Melissa Coleman, MD
Clinical Instructor
Division of Adult Cardiothoracic Surgery
University of California, San Francisco
500 Parnassus Ave., MUW 405, Box 0188

San Francisco, CA 94143

Joseph Levitt, MD
Stanford Health Care
Lane Building, 300 Pasteur Dr a175,
Stanford, CA 94305
(650)725-7061

11.2.2 **Follow up of SAEs**

SAEs will be followed to resolution/baseline, stability or up to 28 days after completion of study agent, whichever occurs first.

11.2.3 **IND Safety Reports**

As described in Section 11.1.2 individual case reporting for SAEs and other clinical events of concern will be reported for individual cases as well as aggregate analyses. The SWG and safety/regulatory staff will determine which SAEs require expedited FDA submission as IND safety reports.

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of AEs are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and the associated type of event:

Within 7 calendar days, with a follow up full written report within 15 days for:

Any study event that is:
- associated with the use of the study drug, and
- unexpected, and
- fatal or life-threatening

Within 15 calendar days:

Any study event that is:
- associated with the use of the study drug, and
- unexpected, and
- serious, but not fatal or life-threatening

-or-

- a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable)

Any finding from tests in laboratory animals that:
- suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional IND Reporting Requirements:

Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

SAEs and AEs will be communicated to the relevant agent manufacturer per their individual safety reporting requirements regarding timing, frequency, and format.

All investigational sites will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

Over the course of the first month of the trial, we will constitute a safety committee that will review the distribution of grade 3 and 4 adverse events and cause of death (see section 12.5). This will replace the study monitor function.

Follow-up of SAE: Site staff should send follow-up reports as soon as additional information is available or requested by the Safety CRO. Additional information should be entered on the study-specific SAE Report Form in the appropriate format. Follow-up information should be sent to the Safety CRO as soon as available. These events will be collected in the study database.

11.3 Adverse Events of Special Interest (AESIs)

Investigational drugs may have AESIs that need to be reported to the study sponsor. A full listing of AESIs is provided in the appendix of the investigational drug with a full cumulative listing in Appendix B.

- If an AESI is identified, an AESI form should be filled in and sent to the safety CRO within 7 days of event occurrence. Copy of the form is available in the MOP.
- If an AESI is also an SAE, only an SAE form needs to be submitted. The SAE timeline of 24 hour reporting should be followed and the form should be checked to indicate that an AESI has also occurred.

12. STUDY OVERSIGHT AND MONITORING

This study is sponsored by QLHC, a 501(c)3 dedicated to the delivery of innovative healthcare solutions. Other partners involved in the trial's design and development include the FDA, University of California, San Francisco (UCSF), other collaborating regulatory agencies and investigators. I-SPY 2 Project Oversight Team ensures the effective, efficient and ethical conduct of the trial on behalf of QLHC and all participating agencies and sectors. The I-SPY COVID-19 Executive Operations Group is responsible for the scripting and execution of the study protocol, as well as oversight of the trial operations. The I-SPY Committees (Agents, Biomarkers, Informatics, Pathology, Publications, Regulatory and Site Operations) in conjunction with the I-SPY COVID-19 Project Management team will guide the trial's conduct in the United States. Strict monitoring guidelines for phase 2 trials will apply and the utmost effort will be paid to the collection of data that is in compliance with FDA and other participating regulatory agency guidelines.

12.1 Data Management

This study, based on the established I-SPY 2 Data Access and Publication Guidelines, will collect and report clinical trial data using the OpenClinica Electronic Data Capture (EDC) system managed by QLHC. The EDC, which is a 21 CFR Part 11 compliant system, will be the authoritative database for the clinical trial participant data for the sponsor and FDA to audit. The OpenClinica EDC is directly integrated with the randomization engine which houses the adaptive algorithm written in the R statistical programming language. In order to perform the required calculations for randomization and treatment arm assignment, all data relevant to the statistical modeling will be de-identified prior to submission to the engine. All participant case report forms will be submitted according to Table 12.1 in §12.2 and the Data Coordinating Center (DCC) will review data in real time. All application users will be trained to use the system and will comply with the instructions in the protocol-specific "User Manual."

12.2 Electronic Case Report Forms (eCRFs)

Participant data will be collected using protocol-specific electronic case report forms (eCRFs) utilizing CDISC data standards specific to ARDS and COVID-19. Study staff will enter data into the EDC; see submission schedule in Table 12.2. Instructions on how to use the EDC are part of the manual which is part of the MOP.

The ability to randomize a participant and to ensure the randomization engine is updated with the most current response data is dependent on timely completion of the CRFs listed in Table 12.2.

Table 12.2 Case Report Form Submission Schedule

Form	When form is to be completed
Eligibility Checklist / Screen Admission to ICU	Completed for each participant considered for I-SPY COVID-19
Registration Form / Coordinator data at consent	For each participant signing a screening consent form.
Verification Form	Change in COVID-19 status level from 5 to 6/7 (intubation) or from 6 to 5 (extubation), and 5 to 4 (primary endpoint) and from 3 to 2 (At discharge from Hospital)
Daily Coordinator Checklist	Daily intake on labs, medications, COVID-19 WHO designation status levels
Randomization Form	Once after participant has been randomized
SAE Form	As needed.
Lost to Follow-up Form and No Longer Lost to Follow-Up Form	As needed.
Death Form	As needed
Early Termination Form	As needed
Off-study Form	As needed.

Protocol Violation Form	As needed.

12.3 Source Documents

All source documents will be maintained at the investigational sites in the study EDC, as specified in the user manual. Participants' research charts or electronic medical records containing the source documents, including laboratory records for verification of eligibility, as well as other data which will be entered into the eCRFs. As instructed, source documents will be de-identified to maintain participant confidentiality, digitized, and electronically stored in the OpenClinica system. The source documents will be used for off-site quality control and verification by the DCC.

12.4 Data and Safety Monitoring Plan

A DMC has been formed to assure participant safety in this clinical trial. As outlined in the I-SPY COVID TRIAL DMC Charter, DMC members will also have additional responsibility for assurance that the trial is conducted to a high standard, and they may be involved in conduct and interpretation of data analyses for efficacy in addition to their primary responsibility for participant safety. The responsibilities of this group include reviewing quantitative recruitment and compliance progress for the study, and recommending modifications of the trial protocol and/or administrative structure in the event these goals are not met. The committee will also review tabulated aggregate toxicity and endpoint data. Data summaries will be made visually available through Tableau which will be integrated to the study database. The committee will submit written recommendations on the progress of the study to the study Principal Investigator and QLHC.

The DMC includes a panel of experts recruited from outside of the institutions involved in this study. The DMC will meet biweekly during the study. Regular meetings will be held electronically. There are a total of nine members. Two are statisticians, two are advocates, three are critical care physicians not engaged in the trial, including an experienced ex-industry critical care physician and an ethicist. There are also two members with experience in infectious disease (some members fit more than one category). The Chair is an extremely experienced trialist and specialist in infectious disease and oncology. The committee will meet frequently. For a drug to graduate or be dropped for futility, at least two critical care physicians and a statistician must be present as part of a six member minimum quorum.

12.5 Safety Working Group

The I-SPY COVID-19 Safety Working Group aims to report in real time concerns around toxicity as well as provide guidance to the DMC on optimal management of toxicity and care for patients. Membership will consist of two Safety Working Group chairs, chaperones from active drug arms, operations working group chairs, PMO representative, and a Safety CRO representative. Both Safety Working Group chairs, operations working group chairs and the majority of the chaperones are critical care physicians with experience with clinical trials and COVID-19. The Safety Working Group will perform bi-weekly review of a listing of AE/SAEs for each arm in the trial and review all deaths in the trial.

The group will assist in creating amendment and consent language around drug safety, dose modifications, and data collection plan for AEs of special interest. The Safety Working Group will be responsible for providing memorandums outlining safety procedures for immediate implementation and agent amendments to reflect optimal methods to manage toxicity, as well offering best practice suggestions for ongoing training with participating sites on AE/SAE procedures. The chaperones for each agent will have primary responsibility for the requirements needed for the specific agent, which will then be reviewed by the Safety Working Group.

If an arm requires a safety cohort, it will be indicated in the appendix for that agent. Details of the cohort in the appendix include the dosing regimen, the number of patients, any AESI and others. The Safety Working Group and DMC participate in the management and adjudication of safety cohorts, if indicated for an agent arm.

This section describes the process by which safety cohorts are reported and evaluated.

1. Randomization is paused after the appropriate number of evaluable patients has been enrolled.
2. The Safety Working Group assesses the report and provides a recommendation. The possible recommendations can be terminating the arm, continuing enrollment at the standard dosing, modifying the dosing regimen, or extending enrollment to the safety cohort.
3. The DMC will review the report and the Safety Working Group recommendations, in addition to blinded efficacy data, and make a recommendation.
4. Any major or minor changes based on the recommendations will be reviewed and approved by the IRB.
5. Minor changes will be submitted to the FDA in the form of a memo. Major changes will be submitted to the FDA in the form of an amendment, and enrollment will remain paused until approval is obtained from the FDA and IRB.

12.6 QLHC or FDA Monitoring

QLHC (or their designee), the lead clinical site (UCSF), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.7 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc.*), as well as IRB records and other regulatory documentation, will be retained by the investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), FDA regulations and guidances, and NIH requirements unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). QLHC will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the FDA. If any part of the study is done outside of the US, applicable regulatory requirements for the specific country participating in the study also apply.

12.8 Clinical Trials Agreement

Investigational agents are provided under a Clinical Trials Agreement (CTA) between Agent Manufacturer and the QLHC.

13. I-SPY COVID-19 STATISTICAL CONSIDERATIONS

13.1 Introduction and definitions

For inclusion in statistical analysis subjects must meet the inclusion criteria and have no exclusion criteria. To be included in the interim and final analyses the following additional criteria must be met:

- Randomized and has a consent and date completed
- At least one daily form is entered
- Baseline COVID is greater than or equal to 5

Baseline COVID:

- Screening COVID ordinal scale will be used if consent date and screening date are the same
- First daily CRF COVID ordinal scale will be used if consent date is after screening date

Primary endpoint:

- **Recovered:** The participants are defined as "Recovered" when there are two consecutive days of COVID scale less than 5. The recovery date is determined as the earliest second date among those two consecutive days.
- **Death:** The participants are defined as "Death" if they are not recovered by date of death. The date of death is determined as the earliest date between the date when daily COVID scale is 8 or the date of death recorded in the death form.
- **Censored:** The participants are defined as "Censored" if they are not recovered nor died. The date censored is determined as the last date where the daily COVID scale is entered.

Participant outcomes may change across different interim analysis as data collection is ongoing and incomplete data is being filled. Subjects will be excluded from the analysis for incomplete or inconsistent data entry

13.1 Sample Size/Accrual Rate

For any given agent, a minimum of 40 (for futility) or 50 (for graduation) and a maximum of 125 participants will accrue prior to the agent being dropped or graduated from the trial. The target maximum is 125 participants per arm, but given biweekly randomization updates may approach 150. Under a Master IND, new agents will replace the agents that leave the trial. Up to 100 sites will participate with an anticipated weekly accrual of 50 patients until COVID-19 prevalence decreases. We anticipate that I-SPY COVID-19 will be a standing trial constantly replacing agents leaving the trial with new agents.

13.2 Randomization and Stratification

The trial will use fixed randomization. With two experimental therapies, randomization will be 1.2:1:1, and with one experimental therapy it will be 1:1, where the first number in the randomization proportions denoting the control arm. We will use concurrent control for the primary analysis and decisions on graduation and dropping for futility, such that any experimental arm is always compared with controls accrued during the same time period. This means that any differences in the SOC will be reflected in the comparison. (Cumulative controls can be used as well in sensitivity analyses and to analyze temporal trends.)

Since we will use concurrent controls and since the control arm is used for multiple comparisons, it makes sense to make the control arm bigger than the experimental arm to reduce any stochastic noise within that arm (which affects all comparisons). For example, with 2 experimental arms the probability weight of 1.2 to the control increase statistical efficiency as each experimental arm is compared to control while maximizing the efficient use of enrolled patients.

Any number of experimental regimens may be considered simultaneously in the trial, however, for practical purposes the maximum number of experimental regimens considered at any given time will be limited to 2. Regimens may be added over time by the I-SPY COVID Agent Selection Committee. In deciding whether to add a regimen, the I-SPY COVID Agent Selection Committee will consider the trial's accrual rate and the number of regimens currently being considered. If the present and projected accrual and the current number of regimens in the trial mean that this goal will not be met, a new regimen should not be added.

13.3 Primary Endpoint and Probability of Success

Participant assignment to one of the regimens in the trial will be randomized. Regimens that perform sufficiently poorly will be dropped from the trial, as described below.

Continuously throughout the trial, each regimen's (Bayesian) probabilities of being successful (HR>1 for time to recovery OR HR<1 for time to death) will be calculated for each treatment. These probabilities will be used in making trial decisions (recommendations to the trial's DMC), as follows:

- A regimen will be recommended to be dropped from the trial for futility if the posterior probability of offering no more than a 50% benefit vs. backbone (on the hazard ratio scale) exceeds 90%: $Pr(HR_{r,d} < 1.5) \geq 0.9$, where $HR_{r,d}$ is the hazard ratio for recovery for treatment *d*, AND if the posterior probability of not offering a mortality benefit vs. backbone exceeds 90%: $Pr(HRm < 1 \mid data) \leq 0.5$, where $HR_{m,d}$ is the hazard ratio for mortality for treatment *d*. A minimum of 40 participants will be necessary before a regimen can be dropped for futility.
- A regimen will be recommended to graduate if the posterior probability of offering a benefit in time to recovery vs. backbone alone exceeds 0.975: $Pr(HR_{r,d} > 1.0) \geq 0.975$ OR if the posterior probability of offering a mortality benefit exceeds 90%: $Pr(HR_{m},d < 1 \mid data) \geq 0.9$. A minimum of 50 participants will be necessary before a regimen can be recommended for graduation.

- The DMC may consider terminating randomization to a treatment if $Pr(HR_{m,d} > 1.3) \geq 0.7$, where $HR_{m,d}$ is the hazard ratio for mortality for treatment *d*. If this criterion is met, it will trigger a DMC review of data for treatment *d* against controls to decide whether it is regarded as acceptable to continue enrolling patients to this arm.
- The DMC will terminate randomization to a treatment after 125 patients if it fails to graduate.

The DMC will during the review process also evaluate mortality data. This will be done by studying key mortality statistics (in addition to the hazard ratio for mortality): (1) Proportion of patients alive in the treatment and control arm at day 7, 14, 21, and 28; and (2) The cumulative incidence function for mortality. If these statistics are favorable, i.e. do not indicate increased mortality, the drug will graduate. The proportion of patients alive (i.e. alive and still at risk of both outcomes of the study, recovery and death) is given by the survival function, which we will also report to the DMC and in publications.

13.4 Design Algorithm Overview

The primary endpoint family in the trial consists of: time to recovery (reaching a durable WHO Covid-19 level 4) after randomization, and time to death. For this trial, a durable WHO Covid level is defined as at least 48 hours at COVID level 4 or less (nasal prongs oxygen) without returning to high flow oxygen or intubation. We will use Bayesian time-to-event models (with noninformative prior) to model the relationship between the primary outcomes and the treatments, adjusted for baseline COVID-19 level. The models will provide hazard ratios for each treatment for time to recovery and time to death. Death is a competing event to experience recovery, because patients who die are prevented from reaching WHO COVID-19 level 4. Our approach to calculate the cumulative probabilities over time (ie, the cumulative incidence functions) for the 2 events of interest is based on combining the cause-specific hazard functions estimated by 2 parametric survival models (Putter et. al). Simply treating failure times from competing events as censored and calculating the cumulative probabilities as one minus the survival function would be incorrect, since the 2 events are dependent (Lee et al.). We note that, consequently, survival percentiles (e.g., median time to reaching WHO COVID-19 level 4) would be incorrect too, unless competing events are modelled.

We model the survival data parametrically using the Weibull model which can be parametrized either as a proportional hazard or accelerated failure time model.

Let's consider the first parametrization and let Z be the time to recovery, which we assume to follow a Weibull distribution with shape parameter v and scale parameter u. In the proportional hazard Weibull regression model, the scale parameter is defined as a linear function of the covariates, i.e. treatment (X) and additional prognostic variables (C): $\log(ui) = \alpha + \beta Xi + \gamma Ci$. The β and γ parameters represent the effects of the treatments and of the prognostic variables. For those regression coefficients we have adopted uninformative flat prior distributions, i.e. $\beta \sim Unif(-\infty, +\infty)$. For the intercept term α and shape parameter v of the Weibull we have selected vague prior distributions as they are not related to either treatment or prognostic variables. In particular, we assumed $\alpha \sim Student3(0,10)$ and $v \sim Gamma(0.01, 0.01)$ as the shape parameter is always positive. The prior parameters will be updated continuously throughout the trial while decision on early stop (futility or graduation) will be based on the posterior distribution of the $\exp(\beta)$ parameters, which can be interpreted as the Hazard Ratio for the event of interest (note that death is the other competing event) for the treatment ($X = 1$) versus the control ($X = 0$).

It is also possible to convert the coefficients from the proportional hazard model to the accelerated failure time model, where $AF = \exp(\beta/-v)$ is the accelerator factor (also known as event time ratio), which communicates how much faster (or slower) the time endpoint occurs.

Throughout the trial, decisions must be made for each experimental treatment to either "graduate" it and recommend progressing to phase 3, terminate it for futility, or continue it in the trial to accrue more information.

13.5 Original Assessing Operating Characteristics of the Design via Simulation

To test the trial's operating characteristics we simulate the trial under a variety of scenarios, including the true efficacy of the backbone therapy, true efficacy of candidate treatments, and various accrual rates.

Below, we describe some of the simulations performed to investigate the operating characteristics of the trial. Several updates have been done to the trial. Specifically, we have made changes to the randomization probabilities, the number of patients required for dropping an arm for futility, and updates to the decision criteria for graduation and dropping for futility (from being based only on time to recovery to include a family of endpoints consisting of time to recovery and time to death). For the descriptions of the simulations, we start be describing simulations for the original specifications of the trial, and then describe the simulations supporting the updates to the trial protocol.

We consider a variety of possible scenarios ranging from very pessimistic, in which the null hypothesis of no benefit holds for every treatment, to optimistic cases in which several of the regimens are truly effective. For each scenario, and following the design we built, we enter participants into a virtual trial and simulate outcomes for them. When the "trial" is over we record various summaries of the trial results, including the duration of the period in which participants were randomized to each experimental regimen, whether the treatment graduated, reached futility, or ran to the maximum number of patients. We repeat this trial simulation procedure at least 500 times to find the operating characteristics of the design.

Base simulations
The data simulations consist of virtually creating multiple instances of how the trial will evolve and end under different assumptions using the following simulation algorithm:

In the virtual trial we repeat daily the following steps (max trial duration 200 days):

1. *Enrollment and simulation of data events of new patients*
   Every day, we enroll 7 new patients and simulate their time-to-event and event type (recovery or death). In particular, we sample both the times-to-recovery and time-to-death from Weibull distributions, using the approach described in Beyersmann, Jan, et al. "Simulating competing risks data in survival analysis." *Statistics in medicine* 28.6 (2009): 956-971. We let the cause-specific hazard functions for the 2 event types to depend both on Randomization Arm (Control or one of 4 Active agents) and baseline COVID level (level 5 vs 6/7).

   At the beginning of the trial, we set the randomization ratio to 2:1:1:1:1. We assume that the probability of enrolling a patient with a COVID level 6/7 is 35%.

   The parameters used to simulate the time-to-event depend on the scenario considered. The corresponding cause-specific Hazard Ratios for the 4 Active agents vs Control and for Covid level 6/7 vs 5 are reported at the bottom of Figure 2, which illustrate the Cumulative Incidence Functions for the two event types, conditional on Randomization Arm and baseline COVID level. These curves are largely consistent with Grasselli et al. JAMA. 2020;323(16):1574-1581,

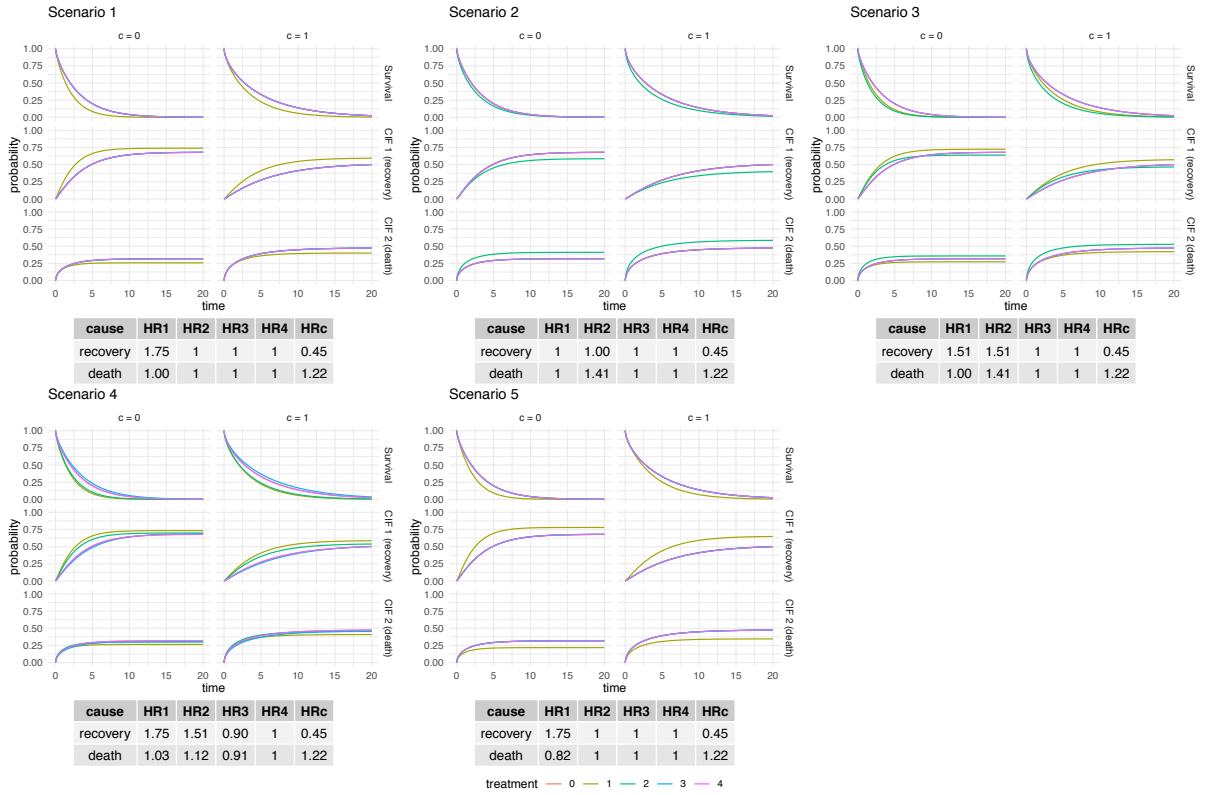
Richardson et al. JAMA. 2020;323(20):2052-2059, and the UK Intensive Care National Audit & Research Centre (ICNARC), which repeatedly publishes patient characteristics and outcomes of COVID-19 patients in the UK (https://www.icnarc.org/Our-Audit/Audits/Cmp/Reports).

2. *Update the data events (time and event)*
Every day in the virtual trial, we update the information available at that moment, i.e. how many patients are still alive and under treatment (censored), how many died (competing event), and how many are recovered (main outcome), and the corresponding time-to-event.

3. *Evaluate the accumulating data*
After having observed at least 10 recovered patients, we fit the two described Bayesian Weibull survival models (one per outcome) and update the prespecified prior distributions to obtain the posterior distribution for all models' parameters. The prior distributions for the coefficients of the linear predictor used to model the log-scale parameter of the Weibull distributions are Normal(0, 0.5), while the prior distribution for the shape parameter is Exponential(1).

4. *Decision rules*
After having enrolled at least 50 patients to a specific treatment, we decide either to continue or early terminate the randomization to the specific treatment based on threshold rules decided in the simulation study:
Graduation: $P(HR > 1) \geq 0.975$ (DMC will make sure treatment safety in terms of mortality)
Futility: $P(HR > 1.5) \leq 0.1$ or, indicating HR_2 the HR for mortality, $P(HR_2 > 1.3) \geq 0.7$
Stop: if the maximum number of 125 patients have been randomized to a specific treatment

5. *Update randomization probabilities*
Randomization probabilities are updated based on the remaining available treatments, by making sure they sum up to 1. The algorithm is then repeated from 1).



cause	HR1	HR2	HR3	HR4	HRc
recovery	1.75	1	1	1	0.45
death	1.00	1	1	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
recovery	1	1.00	1	1	0.45
death	1	1.41	1	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
recovery	1.51	1.51	1	1	0.45
death	1.00	1.41	1	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
recovery	1.75	1.51	0.90	1	0.45
death	1.03	1.12	0.91	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
recovery	1.75	1	1	1	0.45
death	0.82	1	1	1	1.22

Figure 2. *Survival and cumulative incidence curves for the five scenarios of the base simulations. The cause-specific hazard ratios for the four Active agents vs Control (HR1, …, HR4) and for baseline COVID level 6/7 vs 5 HRc) are reported in the tables below the survival and cumulative incidence curves.*

The results of the base simulations are presented in Table 3, where we report the probabilities of graduation, futility and safety stopping.

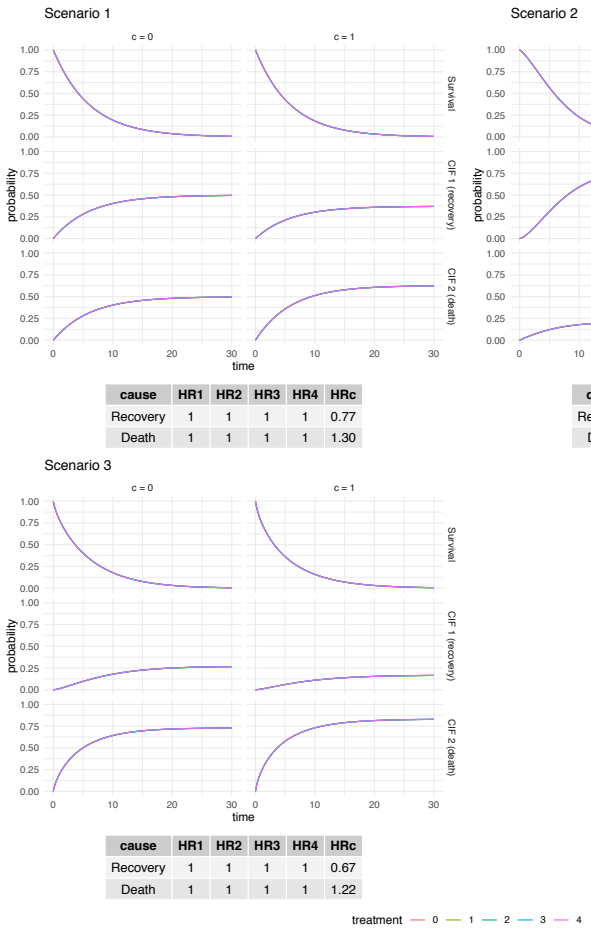
scenario	var	trt_1	trt_2	trt_3	trt_4
1	graduation	0.888	0.033	0.03	0.031
1	futility	0.1	0.963	0.968	0.969
1	safety	0.013	0.006	0.007	0.007
1	futility or safety	0.112	0.967	0.97	0.969
2	graduation	0.037	0.038	0.038	0.031
2	futility	0.959	0.908	0.958	0.968
2	safety	0.008	0.163	0.007	0.005
2	futility or safety	0.963	0.962	0.962	0.969
3	graduation	0.639	0.544	0.022	0.034
3	futility	0.356	0.299	0.978	0.965
3	safety	0.008	0.189	0.004	0.006
3	futility or safety	0.361	0.456	0.978	0.966
4	graduation	0.883	0.63	0.008	0.029

4	futility	0.109	0.338	0.992	0.969
4	safety	0.01	0.035	0.002	0.004
4	futility or safety	0.117	0.37	0.992	0.971
5	graduation	0.903	0.033	0.043	0.032
5	futility	0.095	0.963	0.955	0.962
5	safety	0.002	0.012	0.011	0.015
5	futility or safety	0.097	0.967	0.957	0.968

Table 3. *Probabilities for graduation, or dropping for futility or hitting criterion for DMC safety review for the five scenarios of the base simulations.*

Simulations of scenarios covering the null space

To investigate the robustness of simulations results under the null, we simulated scenarios using different shapes for the cumulative incidence and survival functions for the two outcomes (Figure 3). In these simulations, the Active agents have no effect on all the cause-specific Hazard Ratios (they're set equal to 1). The results of these simulations are shown in Table 4. The conclusion of these results is that type 1 error rates are consistent across different specifications of the null.



cause	HR1	HR2	HR3	HR4	HRc
Recovery	1	1	1	1	0.77
Death	1	1	1	1	1.30

cause	HR1	HR2	HR3	HR4	HRc
Recovery	1	1	1	1	0.67
Death	1	1	1	1	1.30

cause	HR1	HR2	HR3	HR4	HRc
Recovery	1	1	1	1	0.67
Death	1	1	1	1	1.22

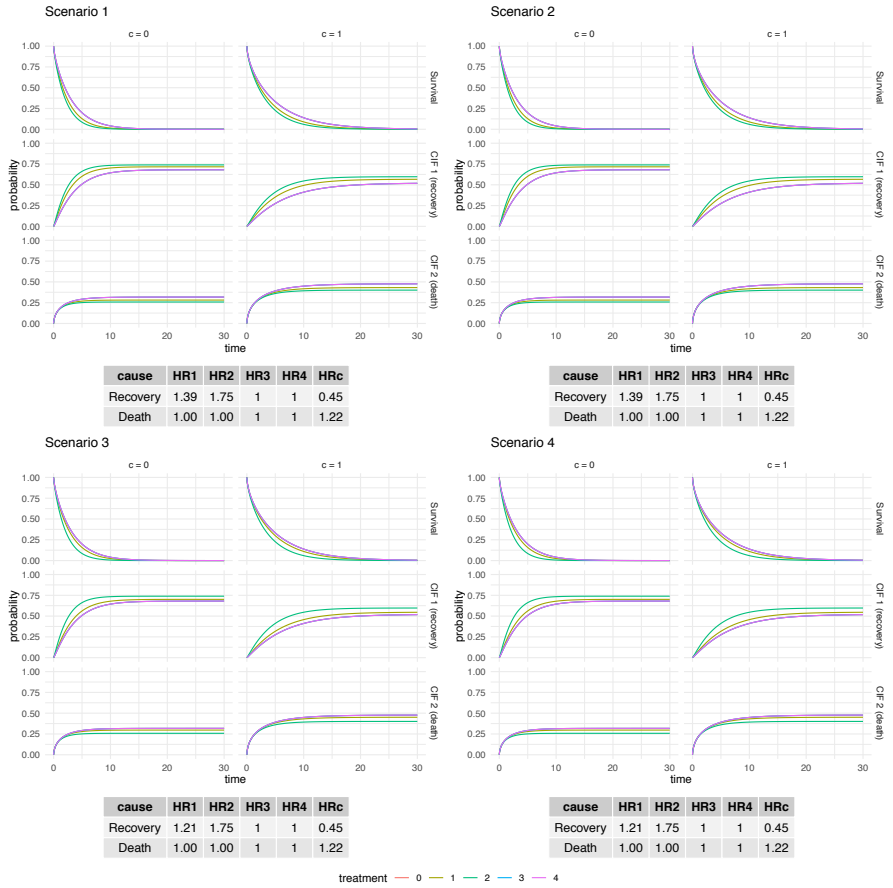
Figure 3. *Survival and cumulative incidence curves for the three scenarios of the simulations used to explore the null space. The cause-specific hazard ratios for the four Active agents vs Control (HR1, …, HR4) and for baseline COVID level 6/7 vs 5 HRc) are reported in the tables below the survival and cumulative incidence curves.*

scenario	var	trt_1	trt_2	trt_3	trt_4
1	graduation	0.052	0.039	0.043	0.046
1	futility	0.933	0.95	0.946	0.945
1	safety	0.025	0.032	0.031	0.028
1	futility or safety	0.947	0.961	0.957	0.954
2	graduation	0.041	0.05	0.058	0.065
2	futility	0.948	0.945	0.931	0.922
2	safety	0.044	0.026	0.039	0.038
2	futility or safety	0.959	0.95	0.942	0.935
3	graduation	0.033	0.035	0.043	0.038
3	futility	0.874	0.858	0.852	0.862
3	safety	0.004	0.013	0.01	0.011
3	futility or safety	0.878	0.868	0.857	0.868

Table 4. *Probabilities for graduation, or dropping for futility or hitting criterion for DMC safety review for the three scenarios of the simulations used to explore the null space.*

Simulations varying the randomization ratio, the number of subjects before evaluation and the maximum number of enrolled patients.

In these simulations, we explored the consequences on early stopping probabilities (for graduation, futility, and safety) of changing the randomization ratio (from 2:1:1:1:1 to 1.4:1:1:1:1) and of decreasing the number of patients needed before an Active agent could be evaluated (from 50 to 40). Furthermore, we explored if enrolling 200 patients instead of 125 would improve the operating characteristics of the trial. The four scenarios considered are illustrated, in terms of Cumulative Incidence and Survival functions, in Figure 4. In scenarios 1 and 3 a maximum of 125 patients can be enrolled in each of the Active agents' arms, while in scenarios 2 and 4, the maximum number of patients is set to 200.



cause	HR1	HR2	HR3	HR4	HRc
Recovery	1.39	1.75	1	1	0.45
Death	1.00	1.00	1	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
Recovery	1.39	1.75	1	1	0.45
Death	1.00	1.00	1	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
Recovery	1.21	1.75	1	1	0.45
Death	1.00	1.00	1	1	1.22

cause	HR1	HR2	HR3	HR4	HRc
Recovery	1.21	1.75	1	1	0.45
Death	1.00	1.00	1	1	1.22

Figure 4. *Survival and cumulative incidence curves for the three scenarios of the simulations used to explore the operating characteristics of the trials varying the randomization ratio, the number of subjects before evaluation and the maximum number of enrolled patients. The cause-specific hazard ratios for the four Active agents vs Control (HR1, …, HR4) and for baseline COVID level 6/7 vs 5 HRc) are reported in the tables below the survival and cumulative incidence curves.*

Results for these simulations are shown in Table 5.

scenario	var	trt_1	trt_2	trt_3	trt_4
1	graduation	0.42	0.85	0.02	0.03
1	futility	0.572	0.142	0.978	0.968
1	safety	0.014	0.01	0.01	0.008
1	futility or safety	0.58	0.15	0.98	0.97
2	graduation	0.448	0.876	0.03	0.018
2	futility	0.534	0.114	0.964	0.98
2	safety	0.02	0.01	0.01	0.01
2	futility or safety	0.552	0.124	0.97	0.982
3	graduation	0.168	0.864	0.022	0.018

3	futility	0.818	0.122	0.972	0.978
3	safety	0.022	0.014	0.008	0.014
3	futility or safety	0.832	0.136	0.978	0.982

Table 5. *Probabilities for graduation, or dropping for futility or hitting criterion for DMC safety review for the three scenarios of the simulations used to explore the operating characteristics of the trials varying the randomization ratio, the number of subjects before evaluation and the maximum number of enrolled patients.*

13.5.1 **Results from individual simulated trials**

Interactive results from individual simulated trials are available at:
http://alecri.github.io/downloads/example_simulations.html

13.6 Statistical Plan Updated 12.1.2020

The algorithm started running from day 1 (July 30, 2020) of enrollment and the status is reviewed every two weeks by the DMC, unless otherwise advised by study PIs or the biostatisticians.

The following adjustments to the statistical plan were made on 12.1.2020:

- Today, the randomization proportions are 2:1:1:1:1 between controls and the active arms. We are changing the randomization to 1.4:1:1:1:1. from 2:1:1:1:1 between controls and the active arms (based on simulations, this has the least impact on reducing power).
- We are reducing from 50 to 40 the minimum number of patients randomized to an active arm before it is dropped for futility.

13.7 Simulations update

The data simulations consist of virtually creating multiple instances of how the trial will evolve and end under different assumptions using the simulation algorithm described below.

In the virtual trial we repeat daily the following steps (max trial duration 200 days):

1. ***Enrollment and simulation of data events of new patients (base case)***
   Every day, we enroll 7 new patients and simulate their time-to-event and event type (recovery or death). In particular, we sampled the times- to- recovery from a Weibull distribution with parameters shape parameter = 1.15 and scale parameter = -1.725. The cause-specific HRs for patient COVID level 5 and 6 at baseline versus at level 7 at baseline was set to 0.45. The competing event death (level 8) was simulated from a Weibull distribution with shape parameter = 0.5 and scale parameter = -1.5. These parameter choices are largely consistent with Grasselli et al. JAMA. 2020;323(16):1574-1581, Richardson et al. JAMA. 2020;323(20):2052-2059, and the UK Intensive Care National Audit & Research Centre (ICNARC), which repeatedly publishes patient characteristics and outcomes of COVID-19 patients in the UK (https://www.icnarc.org/Our-Audit/Audits/Cmp/Reports).
2. ***Update the data events (time and event)***

Every day in the virtual trial, we update the information available at that moment, i.e. how many patients are still alive and under treatment (censored), how many died (competing event), and how many are recovered (main outcome), and the corresponding time-to-event.

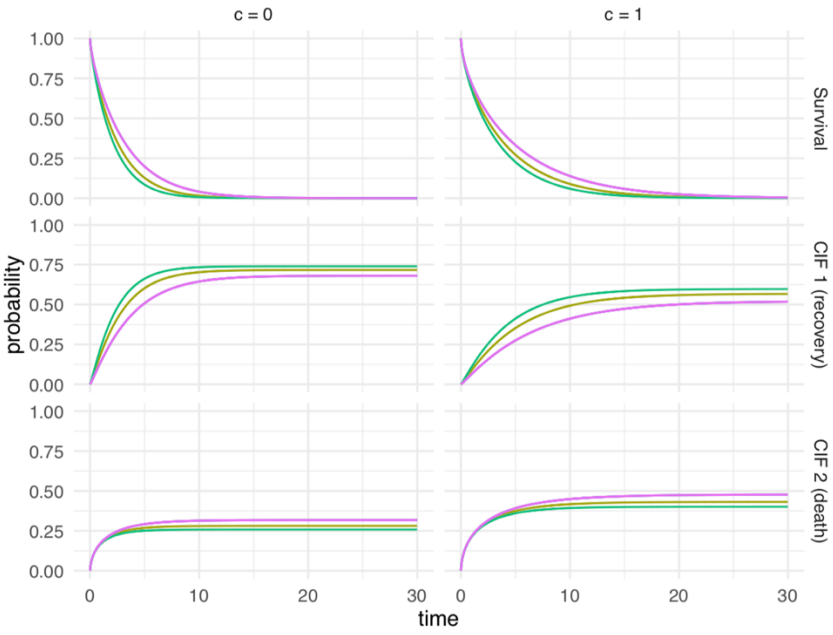
3. ***Evaluate the accumulating data***
   After having observed at least 10 recovered patients, we fit to the described Bayesian Weibull survival models (one per outcome) and update the prespecified prior distributions
4. ***Decision rules***
   After having enrolled at least 50 patients to a specific treatment, we decide either to continue or earlier terminate randomization to the specific treatment based on threshold rules decided in the simulation study:
   Graduation: $P(HR > 1) \geqslant 0.975$
   Futility: $P(HR > 1.5) \leqslant 0.1$ or, indicating HR_2 the HR for mortality, $P(HR_2 > 1.3) \geqslant 0.7$
   Stop: if the maximum number of 125 patients have been randomized to a specific treatment
5. ***Update randomization probabilities***
   Randomization probabilities are updated based on the remaining available treatments. The algorithm is then repeated from 1.

Within this framework, we compared the current operating characteristic of the trial with the ones we would get by introducing the proposed changes. Specifically, we simulated:

- Four investigational agents, two with a hazard ratio for recovery of 1 (non-effective treatments, one effective treatment with a hazard ratio of 1.75, and one with a hazard ratio of 1.4). See Figure 1.
- Maximum number of subjects enrolled in each 125.
- No investigational agent was associated with death (HR for death = 1).
- 7 patients/day accrual, and no limits to the length of the trial.
- 1000 virtual trials per scenario

Comparing power for the previous trial specifications with the new changes described above gives the following changes in power:

Effective drug HR	**Power current specs**	**Power updated specs**
HR 1.75	90%	87%
HR 1.4	47%	45%



cause	HR1	HR2	HR3	HR4	HRc
Recovery	1.39	1.75	1	1	0.45
Death	1.00	1.00	1	1	1.22

Figure 1. *Survival curves and cumulative incidence functions for recovery and death for patients with baseline covid level 5/6 (c=0) vs. 7 (c=1).*

13.7.1 **Statistical Plan Updated 3.16.2021**

This update to the statistical analysis plan concerns the update of the original primary endpoint (time to recovery) to a family of primary endpoints (time to recovery and time to death).

Updated simulations with the new stopping rules corresponding to the endpoint family

In these simulations, we explored the consequences of introducing new graduation and futility rules. These new stopping rules do not just involve the cause-specific HRs for the "Recovery" endpoint (HRr) and for the competing event "Death w/o recovery" (HRd), but also the HR for the "Mortality" endpoint (HRm).

The new stopping rules are the following and supersede those used so far (see "Base simulations"):

- Graduation: $\Pr(HRr > 1 \mid data) \geqslant 0.975$ or $\Pr(HRm < 1 \mid data) \geqslant 0.9$
- Futility: $\Pr(HRr > 1.5 \mid data) \leqslant 0.1$ and $\Pr(HRm < 1 \mid data) \leqslant 0.5$
- Safety: $\Pr(HRd > 1.3 \mid data) \geqslant 0.7$

We used a Bayesian Weibull survival model to model time-to-death, ignoring potential Recovery and censoring subjects still at risk at the time of the analyses. From this model, we obtained the posterior

distribution for HR_m. This model was fitted alongside the Weibull models described in "Base simulations". The same priors are specified for this third model's parameters.

The randomization weights (1.4:1:1:1), the maximum number of patients enrolled to each Investigational Agent arm (n=125), and the minimum number of patients needed before an Agent could be evaluated (n=40) have not been changed from the last set of simulations (simulations described above for varying the randomization ratio).

The scenario here considered is similar to Scenario 1 of §8 "Simulations varying the randomization ratio…", with the addition of a third effective Investigational Agent (IA 4) on both Recovery (though to a lesser extent than IA 2 and IA 3) and Mortality (see table below).

Endpoint	*IA 1*	*IA 2*	*IA 3*	*IA 4*	*Covid Status*
Recovery (HRr)	1.00	1.75	1.39	1.13	0.45
Death w/o recovery (HRd)	1.00	1.00	1.00	0.50	1.22
Mortality (HRm)	1.00	0.80	0.90	0.50	1.60

The results of these simulations are reported in the table below.

	IA_1	IA_2	IA_3	IA_4
graduation	0.042	0.878	0.474	0.866
futility	0.9	0.102	0.466	0.046
safety	0.016	0.016	0.026	0
futility or safety	0.904	0.118	0.488	0.046

Based on these simulation (in addition to results from other explored scenarios), the operating characterstics of the new decision rules seem reasonable. We have run simulations under the null space using the same scenarios as used for the base simulations. In these simulations, all hazard ratios were set to 1. The type 1 error rate varied from about 3% to 5% in these simulations of the null space.

13.7.2 **Interim Analysis**

The probability analyses will update continuously. The DMC will review every other week or sooner at the request of the study statistician if a decision about dropping for futility or graduation is imminent. The DMC may convene if a treatment arm is recommended for graduation or futility to discuss the decision or for any reason a member desires.

13.7.3 **Final Analyses:**

Decisions may occur before all patients randomized to a treatment have achieved their endpoint (death or recovery). In this case, the patients yet to reach their endpoint will continue to be followed and results reported after all patients have reached their endpoint.

Results will be provided overall and stratified by the following subgroups: On/off ventilator at study entry, gender, 0 vs. 1. vs. 2+ comorbidities, age <40, 40-65, 65-79, 80, on and off vasopressors at time of

enrolment. As drugs may be differentially effective in different stages of the disease, we will also test for an interaction effect between treatment and COVID-19 status level at baseline (following the best practices described in (Wang et al.). We will as a sensitivity analysis also run fully adjusted models, where age, sex, BMI, and 0, 1, or 2+ comorbidities (0, 1, or 2+) including COPD, smoker, asthma, diabetes, prior MI, CHF, kidney failure, or liver failure will be adjusted for (to investigate the impact on any stochastic imbalance in the randomization).

Due to the nature of the disease and the trial, we expect missing data will be minimal. If missing data does occur in the trial, we will use multiple imputation to account for the missingness.

14. ETHICAL AND REGULATORY CONSIDERATIONS

Regulatory documents are essential to clinical research. They serve to demonstrate the regulatory approval(s) and compliance of the Sponsor, Investigator, Monitor, and IRB with the current federal and state regulations and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. This study is funded in part by the United States Department of Defense, Defense Threat Reduction Agency and therefore subject to all applicable Department of Defense (DoD) regulatory requirements. Regulatory documents satisfy the regulatory requirements for the protection of human subjects and adherence to ethical standards in DoD supported research per DoD Instruction 3216.02.

Study sites selected for participation in the I-SPY COVID-19 Trial will be responsible for submitting essential regulatory documents to the DCC. The collection of regulatory documents will take place in accordance with applicable ICH GCP guidelines, state and federal regulations. Regulatory documents must be maintained per all applicable institutional and federal regulations. Any and all questions related to regulatory document submission should be directed to the attention of DCC Regulatory as outlined in protocol §14.5. Please see the I-SPY COVID Trial Manual of Operations, §*Essential Regulatory Document Collection Process*, for more detailed information and links for downloading required forms from the I-SPY COVID TRIAL website.

The following documents comprise the essential regulatory document packet required for agent shipment authorization and study site activation.

14.1 Form FDA 1572

Prior to initiating this study at any site, the Principal Investigator will provide an original signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing all the investigators at the organization at each site that will participate in the protocol.

14.2 Other Required Documents

Signed and dated current (within two years) CV/Biosketch for the site Principal Investigator and all sub investigators listed on Form FDA 1572.

Current professional licenses (where applicable) for the site Principal Investigator and all sub investigators listed on Form FDA 1572.

Original signed and dated I-SPY COVID-19 Financial Disclosure Form for the site Principal Investigator and all sub investigators listed on Form FDA 1572.

Certification of Human Subjects Protection Training (NIH or institution-based training program certificate) for the site Principal Investigator and all sub investigators listed on Form FDA 1572.

Delegation of Responsibilities Log signed by the site Principal Investigator which lists the names and responsibilities of all study staff, including all sub investigators listed on Form FDA 1572.

Lab certifications (CLIA and CAP) and lab normal ranges for all labs listed on each site's Form FDA 1572.

Documentation of Federalwide Assurance (FWA). A print-out of the institutional FWA number may be accessed *via* the OHRP website as follows: http://ohrp.cit.nih.gov/search/fwasearch.aspx?styp=bsc (click the button for FWAs [FWA number]).

Investigator's Brochure Acknowledgment Form signed by the site Principal Investigator.

IRB approval for all QLHC-approved protocol versions, Informed Consent versions (Screening, Treatment, and Supplemental), Investigator's Brochure versions (if applicable) and recruitment materials.

14.3 IRB Approval

Prior to initiating the study and receiving the drug agent(s), the investigators at the organizations must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to QLHC according to sponsor Amendment Guidelines. QLHC-approved amended protocol must be approved by the IRB prior to implementation. Quantum Leap Healthcare Collaborative must obtain written approval from the U.S. Army Medical Research and Development Command (USAMRDC), Office of Human Research Protections (ORP), Human Research Protections Office (HRPO).

As investigational agents move in and out of the trial, protocol amendments will be issued; see §6 for more detail.

14.3.1 IRB Approval Timeline Guidelines

Participating institutions will be notified of the allowable time frame permitted to get each amendment approved by their institutional IRB. For amendments that include new investigational agents in the trial, see §14.3.1.1.

14.3.1.1 Amendment Containing New Investigational Agent(s)

Major Modification (see §6 for definition): Participating institutions have a maximum 45 calendar days to submit and obtain IRB approval. If an institution's IRB approval letter is not received by the sponsor or their authorized designee ≤45 calendar days, accrual at that institution will be placed on hold until institutional IRB approval is obtained and approval letters have been received and processed by the sponsor or their authorized designee.

Minor Modification (see §6 for definition): Participating institutions have a maximum 30 calendar days to submit and obtain IRB approval. If an institution's IRB approval letters are not received by the sponsor or their authorized designee ≤30 calendar days, accrual at that institution will be placed on hold until institutional IRB approval is obtained and approval letters have been received and processed by the sponsor or their authorized designee.

14.4 Informed Consent

For the observational part of the study, there is a waiver of informed consent from the IRB. The purpose of the observational component is to gather information on outcomes in this severely ill COVID-19 population. Information for the observational portion will be collected from the medical record, patients will not be approached unless eligible for the therapeutic portion. Therefore, the observational component should be considered no more than minimal risk, and the waiver will not adversely affect the rights and welfare of the patients observed. Requiring consent for the observational component also has the potential to systematically bias the observational component towards less severely ill patients. As the observational component is for collecting patient outcomes - the daily form, drug form will be collected, but not biospecimens nor ePRO. The research is conducted in compliance with applicable laws and regulations.

Participants who are eligible for the treatment phase of the study will be be asked if they want to participate in the study. If they are interested in the study, a legally authorized representative (LAR, surrogate) will be sought to provide consent for the therapeutic component if the subject lacks capacity due to age, condition, or other reason to make a decision regarding consent to participate in research. The subject (or their surrogate) will be given a copy of the IRB-approved Universal Informed Consent to review, along with a supplement to that consent that describes the two specific active investigational agents and the remdesivir backbone. The investigator and study coordinator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the treatment phase of the study, he or she will be asked to electronically and or use a paper version to sign and date the Informed Consent document(s). The patient will then be randomized to one of the two treatment arms or the control arm.

Subjects will be informed that the study sponsor receives funding in part from the United States Department of Defense, Defense Threat Reduction Agency as part of an initiative to enhance science and technology capability. Subjects will be informed that by signing an Informed Consent Form, they or their LAR allow representatives from the Department of Defense, Defense Threat Reduction Agency to review their research records and their Protected Health Information (PHI) that is created or collected while they are in this study. In compliance with 10 USC 980, all participants randomized to any arm of the treatment component have the potential for direct benefit through additional monitoring, additional follow-up, and treatment with remdesivir that may not have otherwise been available to all subjects.

The Informed Consent documents must be reviewed and approved by QLHC or their authorized designee, HRPO and the IRB at each organization at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by QLHC and or their authorized designee and the Project Team, and then submitted to each organization’s IRB and HRPO for approval prior to initiation.

14.5 Submission of Regulatory Documents

All regulatory documents may be transmitted *via* email (preferred method) or facsimile with the exception of the following documents for which signed originals must be sent via traceable courier:

- Form FDA 1572
- Financial Disclosure Form

Please refer to the I-SPY COVID-19 Trial Manual of Operations for completion and submission guidelines.

14.6 Other

This trial will be conducted in compliance with the protocol, GCP, and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

Please refer to study Informed Consents.

16. REFERENCES

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Appendix A
Remdesivir Therapy

Participants who receive remdesivir as part of their standard of care will be administered remdesivir by IV for up to ten days. The participant can begin or even finish their remdesivir regimen prior to starting their investigational agent arm in this trial. For more information please see the label at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/214787Orig1s000lbl.pdf

Appendix A
Remdesivir Therapy

Participants who receive remdesivir as part of their standard of care will be administered remdesivir by IV for up to ten days. See the Fact Sheet for Healthcare Providers Emergency Use Authorization (EUA) of remdesivir:

https://www.fda.gov/media/137566/download

Appendix B
Overview of Investigational Agents and Biomarkers

1. Overview of Investigational Agents Study Status

Table 1.1 Investigational Agents IRB Approved, Pending Activation for Randomization

There are no Investigational Agents IRB approved, pending activation for randomization at this time.

Table 1.2 Investigational Agents IRB Approved, Activated for Randomization

Agent	Mechanism	Manufacturer
Cenicriviroc Mesylate	CCR2/5 Antagonist	Allergan/Abbvie
FIRAZYR® (Icatibant)	Bradykinin B2 Antagonist	Takeda
Razuprotafib	Tie2 Activator	Aerpio Pharmaceuticals
OTEZLA® (Apremilast)	PDE4 Inhibitor	Amgen

Table 1.3 Investigational Agents Graduated or Dropped, No Longer Activated for Randomization

There are no investigational agents graduated or dropped, no longer activated for randomization

2. Summary of All Additional Eligibility Criteria Required for All Investigational Agents

Table 2.1 Additional Active Investigational Agents Eligibility Criteria

Active Eligibility Criteria	
Amendment Added	**Criterion**
Inclusion	
A1	Willing to use highly effective methods of contraception during and for 1 month after treatment with study agent
Exclusion	
A1	ALT or AST > 5 times the upper limit of normal

Appendix D
Cenicriviroc mesylate (CVC)

I-SPY-COVID-19 Investigational Agent Information

INVESTIGATIONAL AGENT INFORMATION SUMMARY

Agent Chaperone:	Dr. D. Clark Files, Wake Forest, Clark.Files@wakehealth.edu
Agent Co-Chaperone:	Dr. Sheetal Gandotra, UAB, sgandotra@uabmc.edu

General Information:

Agent Class:	Inhibitor of ligation of C-C-Chemokine receptor type 2 (CCR2) and C–C Chemokine Receptor type 5 (CCR5). CVC has both anti-inflammatory and anti-fibrotic activity.
Structural Class:	Small molecule (MW 793.05)
Manufacturer:	Allergan, a Division of AbbVie Inc.

Pharmaceutical Information:

Dosage Form:	Single-dose tablet for oral administration
Physical Description:	The Cenicriviroc mesylate drug product formulation used for clinical trials is DP-7A it is supplied as an immediate release tablet for oral administration containing 150 mg of Cenicriviroc as Cenicriviroc mesylate (CVC) drug substance. The 150 mg tablets are yellow-coated. The formulated tablet contains the following excipients: fumaric acid (solubility enhancer), microcrystalline cellulose (filler), croscarmellose sodium (disintegrant), colloidal silicon dioxide (glidant), magnesium stearate (lubricant), and Opadry® II yellow (film coating).
Strengths to be used in trial:	150 mg immediate release tablets
Packaging Unit	Study drug will be provided in a HDPE bottle containing desiccant, and induction sealed.
Storage Conditions:	Store at 15°C to 30°C (59°F to 86°F) with transient excursions permitted to -20°C (-4°F) to 60°C (140°F).

Administration Information:

Route:	Oral (subjects should take CVC twice daily at approximately 12-hour intervals with food orally or through a feeding tube).
Standard Regimen:	150 mg twice daily (BID)
Agent Preparation:	No agent preparation is required. See Section 3.5
Pre-medication:	Specific pre-medication is not required for routine treatment. See Section 2.1.1

Administration: Treatment will be administered to subjects for 28 days or until the subject is discharged from the hospital, whichever occurs first. The subject will receive a minimum of 14 days of treatment whether or not the patient is discharged from the hospital during that period. If a patient is discharged from the hospital prior to receiving 14 days of treatment, the treatment will continue post-discharge to complete a 14-day course. CVC should be administered twice daily at approximately 12-hour intervals in fed condition and at approximately the same time each day (±2 hours). Patients may receive a larger dose (450 mg total) for their first day of treatment as a loading dose administered as a morning dose of 300 mg and an evening dose of 150 mg. See Section 2.1 for further details. Instructions for G-tube CVC administration are in the Pharmacy Manual.

Concomitant Medications: Following precautions and warning should be given:

- Antiretroviral agents (See the table below [Table 2.2] for other antiretroviral agents):
    - There are no current concerns in utilizing CVC in combination with Remdesivir
    - Dolutegravir, Tenofovir each has no significant impact on CVC exposure and would be acceptable to co-administer with study intervention in this study.
    - Ritonavir or any combination of a protease inhibitor with ritonavir should not be co-administered with a drug product in this study due to a significant drug interaction between ritonavir and CVC.
- Gastric acid-reducing agents should be administered at least 2 hours after CVC intake (4 hours for fast-acting antacids) and per specific protocol requirements (See Table 2.2).

CVC is a substrate of CYP3A4, CYP2C8 and P-gp. CVC is also a weak inhibitor of CYP3A4 and an inhibitor of BCRP and P-gp. The following classes of medications are disallowed at any time during CVC treatment:

- Potent CYP3A4 inhibitors and CYP3A4 inducers will be excluded
- Potent CYP2C8 inhibitors will be excluded
- Some drugs with narrow therapeutic windows that are sensitive CYP3A4 substrates will be excluded (ie, drugs which should not be co-administered with weak CYP3A4 inhibitors such as CVC) (Table 2.2)
- Appropriate dose adjustments of BCRP substrates are recommended

Refer to Section 2.6 for side-effect management and dose reduction plans.

The above is intended as a summary only; please see the complete appendix for additional investigational agent information

1. RATIONALE FOR TESTING

In December 2019, the Wuhan Municipal Health Committee (Wuhan, China) identified an outbreak of viral pneumonia cases of unknown cause. Coronavirus RNA was quickly identified in some of these patients. This novel coronavirus has been abbreviated as SARS-COV-2, and the disease caused by this virus has been designated COVID-19. Global efforts to evaluate novel antivirals and therapeutic strategies to treat COVID-19 have intensified. There is currently no vaccine to prevent SARS-CoV-2 infection or therapeutic agent to treat COVID-19. Therefore, there is an urgent public health need for rapid development of novel interventions.

The pathophysiology of SARS-CoV-2 is not fully investigated yet. Current findings show that SARS-CoV-2 binds to cells of the respiratory tract via ACE-2 receptor and, after fusion of the virus with the cell membrane, replicates in these cells, causing apoptosis and cytopathic damage. Immediate cytopathic effects do not play a major role in SARS pathophysiology, in contrast to the influenza pneumonia. Therefore, inflammation is considered important for the sequelae of the respiratory tract injury from SARS-CoV infection (Van den Brand 2014). Host specific factors in pathophysiology of SARS-CoV induced pneumonia include inflammatory cells, immune cells and cytokines. Infiltration of activated inflammatory cells like lymphocytes, blood-monocytes, and neutrophils induces the production of even more cytokines and enhances the inflammation (Yen 2006).

A variety of chemokines and cytokines have been found in the blood of SARS-patients like interleukin (IL)-1, IL-6, IL-8, IL-12, IFN-γ, monocyte chemotactic protein (MCP)-1 = CC-motif ligand 2 (CCL2), monokine induced by IFN-γ (MIG), IFN-inducible protein (IP-10 = chemokine C-X-C motif ligand 10, CXCL10) and transforming growth factor (TGF)-β (17). Production of chemokines and cytokines like IP-10, MCP-1, IL-6 and IL-8 are important for recruitment of neutrophils and monocytes. Infiltration with these cells correlates with severity of lung disease observed in patients (Van den Brand 2014, Jiang 2020, Cameron 2007). Therefore, we hypothesize that inhibition of inflammatory cells via inhibiting their recruitment (based on antagonism of chemokine receptors) would reduce cytokine release, inflammation-related tissue damage and ultimately improve the disease severity and clinical course of COVID-19.

Severe pneumonia caused by coronaviruses such as SARS-CoV and MERS-CoV is often associated with rapid virus replication, massive inflammatory cell infiltration and elevated pro- inflammatory cytokine and chemokine responses resulting in acute lung injury, and acute respiratory distress syndrome (ARDS). Patients with this acute cytokine storm have high levels of MCP1 (CCL2) and RANTES (CCL5), likely caused by feedback loop from SARS-COV upregulating CCR2 and CCR5 (Law 2009).

Very high levels of RANTES may lead to severe lung inflammation, ARDS and death in patients with SARS. Ng et al. demonstrated that the RANTES -28 G allele, which is associated with high levels of RANTES, was a risk factor that associated with severe clinical outcomes and death in both Hong Kong and Beijing Chinese SARS patients. The authors noted that many cytokines/chemokines released from activated immune cells not only take part in the process of anti-viral immune response, but are also involved in cell damage and organ dysfunction. The same group demonstrated that there is significant induction of CCR-1, CCR-3 and CCR-5 mRNA expression in SARS-CoV infected dendritic cells (DCs), suggesting the possibility of an autocrine loop in facilitating the trafficking of DCs (Ng 2007). Moreover, presence of the CCL2 G-2518A allele, which leads to high CCL2 levels, made patients more susceptible to infection with SARS-CoV (Tu 2015). Thus, clinical data demonstrate that both CCL5 and CCL2 play key roles in the inflammatory sequelae and poor clinical outcomes of SARS-CoV infection. Additionally,

in patients with ARDS, CCL2 is up-regulated, inducing the migration of circulating CCR2+ inflammatory cells into alveoli (Zemans 2017). Patients with ARDS also have elevated levels of CCL2 in BAL fluid compared with controls and greater neutrophil recruitment (Williams 2016).

Both CCR2 and CCR5 play roles in ARDS and the consequences of SARS-CoV. Rationale of dual inhibition includes: redundancy among these pathways and dual inhibition preventing a spill over that may occur with blocking only one; and, these two routes have complementary roles in infection-associated inflammation and tissue damage. For example, CCR2 targeted action is more critical for inhibition of circulating immune cells (eg, monocytes, memory T cells), their trafficking, and migration into tissue; CCR5 action is more related to inhibiting tissue-based immune cells, and therefore a somewhat later time window (eg, macrophages, activated T effector cells, dendritic cells). Both CCR2/5 paths have been shown to be operative, and effects off-set by Dual antagonism of CCR2/5 has been shown to be operative in: CoV infection; ARDS; and influenza-caused lung inflammation, tissue destruction, and fibrosis. Moreover, specifically with CVC, dual CCR2/5 has been shown to be an effective mechanism in pre-clinical models of liver inflammation/fibrosis and viral encephalitis.

Importantly, shown in a murine model of SARS-CoV infection, there are 2 time windows: 1) before the onset or worsening of pneumonitis when the administration of a CCR2 inhibitor has the highest likelihood of being beneficial to patients with COVID-19 and 2) during the pneumonitis when both CCR2 and CCR5 inhibition may be beneficial, reinforcing the potential role for dual targeting of CCR2/5 (Figure 1.1, Chen et al).

Figure 1.1: Pathogenesis of SARS-CoV Infection in Senescent Mice

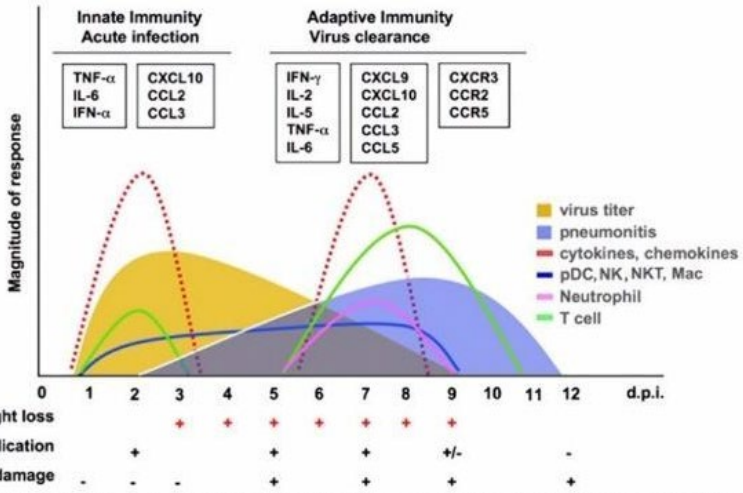


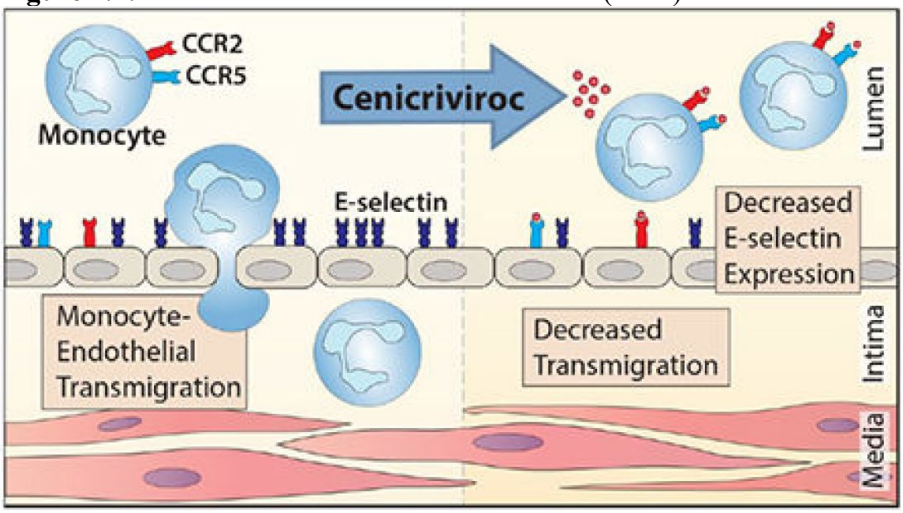
FIG. 7. Time course of host responses to primary SARS-CoV infection in senescent mice. A biphasic expression of inflammatory mediators associated with cellular infiltration into the lungs of infected mice, coincident with peaks in viral replication and clearance, respectively, is seen. Clinical illness such as weight loss was observed coincident with pulmonary viral replication, while lung pathology (pneumonitis) was associated with T-cell infiltration when virus was cleared from the lung.

Cenicriviroc (CVC) mesylate is an orally active, potent inhibitor of ligation of CCR2 and CCR5. Initially developed as an entry-inhibitor of HIV, it showed in a first study a dose dependent increase of CCL2 in the blood. This indicated that CVC efficiently blocks a CCR2 and CCR5 in vivo.

Currently, CVC is being evaluated for participants with nonalcoholic steatohepatitis (NASH) and fibrosis (Cenicriviroc Investigator Brochure). In vitro data with CVC have demonstrated that it blocks the binding of C-C motif chemokine ligand 2 (CCL2; also known as monocyte chemotactic protein 1

[MCP-1]) to CCR2, and also blocks the binding of CCR5 ligands, CCL3 (also known as macrophage inflammatory protein [MIP]-1α), CCL4 (also known as MIP-1β) and CCL5 (also known as regulated on activation normal T-cell expressed and secreted [RANTES]), to CCR5. Ex vivo experiments showed that nanomolar concentrations of CVC achieved 98% receptor occupancy of CCR2 on human monocytes and ~90% receptor occupancy for CCR5 on human CD4+ and CD8+ T-cells. Additionally, CVC was an efficient inhibitor of monocyte and human lymphocyte (primarily T-cells) migration in vitro. CVC treatment decreases the recruitment and migration of CCR2-expressing monocytes to the site of liver injury, mainly via CCR2 antagonism, thereby reducing the infiltration of pro-inflammatory, monocyte-derived macrophages into the liver (Mossanen 2016, Puengel 2016).These assumptions were supported by animal studies and first results from clinical trials, including a Phase 2b, two-year clinical trial on 289 participants with NASH. Results from the Phase 2b CENTAUR study showed that CVC treatment over 2 years resulted in a clinically meaningful, antifibrotic benefit, which was sustained in the majority of participants, and the drug was generally well tolerated.

Figure 1.2: Mechanism of Action for Cenicriviroc (CVC)



While NASH involves chronic inflammation, COVID-19 has consequences including an acute inflammation of the lung. Thus, the following is relevant: that data from animal studies support a positive action of CVC in inflammation associated with severe infection. In mouse models of peritonitis as well as carbon tetrachloride (CCl4) and acetaminophen induced acute liver failure, the application of CVC attenuates the infiltration with blood monocytes and blood derived macrophages, ultimately inhibiting inflammation and improving liver failure (Mossanen 2016, Lefebvre 2016, Ratziu 2020). Moreover, immunomodulatory action from CVC has been shown to result in reductions of inflammation and in tissue preservation in murine models of viral encephalitis.

In a mouse model of acute liver injury, administration of CVC significantly decreased the numbers of monocyte-derived macrophages and associated inflammation and tissue damage. CVC also reduced the numbers of Kupffer cells or tissue-based macrophages with a KC-like phenotype peri-injury. Important for potential safety concerns, other immune cell populations such as neutrophils or lymphoid cells were not affected (Puengel 2017). In a 2nd mouse model of liver injury, CCR2 inhibition with CVC reduced the influx of proinflammatory monocytes into the liver, which significantly attenuated the early phase of tissue injury and later necrosis (Mossanen 2016). Of note, lung disease models demonstrating infiltrating

monocyte-derived interstitial macrophages have a similar inflammatory phenotype, including CCR2/5 based processes, as in the liver disease models, and alveolar macrophages share multiple features with Kupffer cells in the liver.

In an ex-vivo study of monocytes derived from HIV-infected patients, dual CCR2/5 inhibition with CVC was more effective in decreasing trans-endothelial migration of monocytes than single CCR2 or CCR5 blockade. Moreover, CVC was also associated with decreased expression of E-selectin on endothelial cells, a major receptor for monocyte recruitment (D'Antoni 2018).

By blocking CCR2 and CCR5, CVC is known to decrease the recruitment of infiltrating monocyte-derived interstitial macrophages, and this would result in the reduction in tissue-inflammation and -damage (Mossanen 2016, Puengel 2016). Moreover, the reduction of trans-endothelial vascular migration of monocytes could play a role in attenuating vascular-based injury. Therefore, we believe that treatment with CVC could prevent the cytokine storm that causes lung injury and is the cause of acute respiratory distress syndrome (ARDS) in patients with COVID-19.

1.1 Biological Actions

1.1.1 *In Vitro* and Mechanistic Studies

In vitro primary pharmacodynamic (PD) studies were undertaken to determine the chemokine receptor-binding properties of CVC and its metabolites, and to investigate the cellular effects, including CCR2 and CCR5 receptor occupancy and CCR2- and CCR5-dependent chemotaxis in monocytes and lymphocytes. See the Investigators Brochure for additional information.

1.2 Efficacy Studies

The development program for CVC has been focused on liver disease (non-alcoholic steatohepatitis, NASH), but has also been studied in other patient populations, such as patients with HIV. Efficacy data collected for this development effort has demonstrated that CVC has potent anti-inflammatory and antifibrotic activity in a range of animal fibrosis models. These drug activities are likely to be relevant to COVID-19 induced lung damage**.**

1.2.1 Nonclinical Data

CVC's anti-inflammatory and antifibrotic effects have been demonstrated in a range of nonclinical models of inflammation and fibrosis (Lefebvre 2016). See the Investigators Brochure for additional information.

1.2.2 Human Studies

CVC is currently in Phase 3 clinical development for the treatment of liver fibrosis in adults with NASH. As of January 2020, approximately 2000 participants have been exposed to either single or multiple doses of CVC in completed and ongoing clinical studies. A summary of completed clinical studies is provided in Appendix 7 of the Investigators brochure.

The CENTAUR study was a 2-year placebo-controlled Phase 2b study in 289 adult participants with liver fibrosis and NASH. CVC treatment over 2 years resulted in a clinically meaningful, antifibrotic benefit, which was sustained in the majority of participants, and the drug was generally well tolerated. Given that

severity of fibrosis stage was shown to be the only histological feature independently associated with clinical outcomes over the long term, these results provide additional evidence of the potential of CVC as a safe and efficacious pharmacologic treatment for liver fibrosis in adults with NASH. CVC had a generally comparable safety and tolerability profile to placebo over 2 years.

Based on the results of CENTAUR, the Phase 3 AURORA study is being conducted to confirm the antifibrotic benefit of CVC as compared with placebo in adults with NASH and Stage 2 or 3 fibrosis (non-alcoholic steatohepatitis clinical research network [NASH CRN]).

Also being conducted is a Phase 2 clinical study ROLLOVER to provide open-label treatment and to assess the long-term safety of continued treatment with CVC for eligible participants who have previously participated in CVC studies.

The ORION study was a proof of concept 24-week placebo-controlled study, conducted in 45 obese adult participants with suspected NAFLD and either prediabetes or T2DM. There was no observed therapeutic effect of CVC on peripheral and adipose tissue insulin sensitivity as compared to placebo. CVC was well tolerated and resulted in a lower incidence of AEs compared with placebo. No new safety signals were observed.

The PERSEUS study was a proof of concept, 24-week single arm, open-label, study, conducted in 24 adult participants with primary sclerosing cholangitis (PSC). Treatment with CVC for 24 weeks resulted in a modest decrease in the surrogate endpoint of ALP and no new safety signals were observed. Clinical development for this indication has been discontinued, not for safety reasons.

CVC has also been evaluated for the treatment of human immunodeficiency virus (HIV) type 1 infection and demonstrated antiviral effects due to inhibition of the CCR5 co-receptor that is required for HIV entry into T-cells. Clinical development for this indication has been discontinued for business reasons.

1.3 Toxicity and Safety

1.3.1 Animal and In Vitro Studies

The effects of CVC on the central nervous, cardiovascular, and respiratory systems were examined in a core battery of safety pharmacology studies. CVC at oral doses up to 1600 mg/kg had no effects on the general physical condition or behavior of rats. CVC caused slight (3.5%, 16% and 29%) inhibition of human ether-a go-go–related gene (hERG) current in HEK 293 cells at concentrations of 0.01, 0.1, and 1.0 μmol/L, respectively. CVC had no statistically significant effect on cardiac action potential parameters in sheep isolated Purkinje fibers at concentrations up to 0.208 μmol/L. CVC had no significant effects on blood pressure, heart rate, body temperature, or electrocardiogram (ECG) parameters (including PR interval, QRS duration, QT interval, and corrected QT interval) at oral doses up to 2000 mg/kg in cynomolgus monkeys. Intravenous (IV) administration of CVC did not elicit any observable effects on the cardiovascular system of anesthetized monkeys at doses up to 4.5 mg/kg. CVC at oral doses up to 2000 mg/kg in rats had no effects on the respiratory rate, tidal volume, minute volume, or enhanced pause up to 24 hours postdose. IV administration of CVC did not elicit any observable effects on the respiratory system of anesthetized monkeys at doses up to 4.5 mg/kg.

See the Investigators Brochure for additional information.

1.3.2 Human Studies

As of January 2020, CVC has been administered to over 2,000 participants in completed and ongoing studies in participants with HIV infection, non-alcoholic steatohepatitis (NASH), hepatic impairment, renal impairment, and healthy volunteers, with a safety profile similar to placebo. CVC doses range from 25 mg to 900 mg daily across all CVC studies. In a Phase 2b study in HIV, participants were exposed to CVC for 41 weeks (mean) and 48 weeks (median). In Phase 2b in NASH, participants received CVC for 99 weeks (mean) and 102 weeks (median). There was no apparent dose- or exposure-relationship for safety, and CVC demonstrated a safety profile similar to placebo. A global Phase 3 study in participants with NASH is ongoing.

In HIV studies, participants received 150 and 200 mg of CVC once daily. In the NASH trial, participants received 150 mg of CVC daily for two years. There were no additional adverse events reported in HIV participants in comparison to the standard treatment at this time, moreover, CVC was generally better tolerated than the comparator (ie, efavirenz). In particular, there was no increased rate of infections reported. In HIV participants, the main AEs were gastrointestinal symptoms reported by 3% and a skin rash reported in 2% of the participants. Serious AEs were reported in 2%; 3% showed Grade 3 and 0 Grade 4 AEs, which was below the control arm (Thompson 2016). In NASH studies, there was a similar picture. The rate of adverse events during the first year of treatment was not different to participants receiving placebo. In the second year of treatment, there was an increase in nasopharyngitis of 8.2 and 9.1% (CVC treatment arms) vs 3.3% (placebo), however, without an increase in upper respiratory tract infections, or AEs related to infection, in comparison to placebo. (Ratziu 2020).

1.4 Pharmacokinetics/Pharmacodynamics

1.4.1 Non-Clinical Studies

The absorption, distribution, metabolism, and excretion of [^{14}C]-CVC were studied in rats, dogs, and monkeys at doses of 3 mg/kg. Metabolite identification was performed in rats and dogs. In addition, in vitro metabolism and transporter studies were conducted to assess metabolite profiles and drug-drug interaction (DDI) potential.

The pharmacokinetics (PK) of CVC were evaluated following oral administration of [^{14}C]-CVC in rats, dogs, and monkeys. The bioavailability of 3 mg/kg CVC (dosed as [^{14}C]-CVC) in fasted rats, dogs, and monkeys was 10.2%, 88.5%, and 15.6%, respectively, after single-dose, oral administration. Food decreased absorption in rats and dogs (bioavailability of 6.4% and 22.8%, respectively), but not in monkeys. See the Investigators Brochure for additional information.

1.4.2 Clinical Studies

CVC was well absorbed following oral administration of single or repeated doses, achieving peak plasma concentrations about 3 to 6 hours post dose in healthy participants. A dose proportional increase in the exposure of CVC and its metabolites (M-I and M-II) was observed following single ascending dose administration of 75 mg to 450 mg. The t½ of CVC following single and repeated doses was approximately 34 to 42 hours across dose regimens. After repeated QD dosing of 150 mg, steady state levels of CVC were achieved between Day 5 to Day 7. At steady state, CVC and its metabolites accumulated 1.65- to 1.8-fold, which corresponds to an effective accumulation half-life of approximately 18 hours. Food increases exposure of CVC by up to 5.2-fold.

In clinical drug interaction studies, potent CYP3A4 inhibitors such as ritonavir (RTV), darunavir/RTV combination, and atazanavir/RTV combination increased the maximum plasma concentration (C_{max}) of CVC by 2.39-fold, 2.17-fold, and 2.55-fold, and the AUC of CVC by 3.55-fold, 3.13-fold, and 3.89-fold, respectively. The exposure of midazolam, a substrate of CYP3A4, was increased by 1.84-fold when co-administered with 150-mg CVC. CVC increased the exposure of rosuvastatin (BCRP substrate) by 3.52-fold and of atorvastatin and simvastatin (CYP3A4 substrates) by 1.37-fold and 2.48-fold, respectively. However, no significant increase in the exposure of digoxin (P-gp substrate) and caffeine (CYP1A2 substrate) was observed.

Additional CYP interaction details are denoted in the Investigators Brochure.

1.5 Analysis plan

Please see analysis plan in the Master Protocol for the I-SPY COVID-19 Trial.

2. INVESTIGATIONAL STUDY AGENT ADMINISTRATION IN THE I-SPY COVID-19 TRIAL

Reported clinical SAEs and potential risks are described in Section 3.2.

2.1 Dose Regimen and Dose Groups

Study intervention within the CVC arm should be administered based on one of the three dosing scenarios listed in Table 2.1.

CVC will be administered as a 450 mg loading dose on Day 1 (split in 300 mg and 150 mg 12 hours apart), though ”Dose 2” on Day 1 will be skipped in Dosing Scenario 3 (ie, participant is randomized in the late afternoon or early evening).

Then, for all patients and starting on Day 2, there will be a 150 mg twice-daily oral maintenance until discharge from the hospital and for a minimum of 14 days of dosing. If a patient is discharged before completing a minimum of 14 days of dosing, dosing will continue out-patient.. Doses should be administered with food (or tube feeds). For participants requiring mechanical ventilation, Investigators will need to determine the most appropriate means for providing the drug product with meals (ie, crushed tablet within feeding tube with adequate flush); preparation instructions for administration of CVC using a G-tube or other enteral feeding tube can be found in the study Pharmacy Manual. If a feeding tube is not available for >24 hours, the participant will discontinue study drug intervention, but will remain in the study for endpoints and safety observations (Master Protocol Section 9). Missed doses are not made up

Table 2.1 Dosing of CVC Depending on Time of Day of Randomization (Scenarios)

	Scenario 1		**Scenario 2**		**Scenario 3**	
	Participant is randomized and entered into the system for first-dose of Day 1 with breakfast		Participant is randomized and entered into the system for first-dose of Day 1 with lunch or early afternoon snack/meal		Participant is randomized and entered into the system for first-dose of Day 1 with dinner; 2nd dose of Day 1 is N/A	
BID Dose	*Dose 1*	*Dose 2*	*Dose 1*	*Dose 2*	*Dose 1*	*Dose 2*
Day 1 (Loading Dose, mg)	*300*	*150*	*300*	*150*	*300*	N/A
Time of dosing	8:00 AM	8:00 PM	11:30 AM	11:30 PM	6:00 PM	N/A
Acceptable Time Range	6 - 10 AM	6 - 10 PM	9:30 AM - 1:30 PM	9:30 PM - 1:30 AM	4 - 8 PM	N/A
Days 2-28 (Dose, mg)*	*150*	*150*	*150*	*150*	*150*	*150*
Time of dosing	8:00 AM	8:00 PM	8:00 AM	8:00 PM	8:00 AM	8:00 PM
Acceptable Time Range	6 - 10 AM	6 - 10 PM	6 - 10 AM	6 - 10 PM	6 – 10 AM	6 - 10 PM

* Note: Subjects who are discharged from the hospital and have received at least 14 days of CVC will not have drug therapy through Day 29. Patients will receive CVC for 28 days or until hospital discharge, which one occurs first; a minimum of a 14-day course will be given, potentially the latter part post-discharge should that occur pre-Day 14.

2.1.2 Dose Rationale

To ensure rapid onset of action of CVC, a loading dose of 450 mg will be administered on Day 1 (300mg first dose in the morning and 150mg 12 hours later); patients enrolled in the trial in the latter portion of the day may receive only the 300 mg component of the Day 1 loading dose (see Table 2.1 for details on dosing scenarios). Starting Day 2, patients will receive 150 mg of CVC twice per day (Total dose of 300 mg per day) for 28 days or until the subject is discharged, depending on which occurs first. The subject will receive a minimum of 14 days of treatment whether or not the patient is discharged from the hospital.

Prior clinical data, clinical pharmacological data, and PK/PD modelling have indicated that CVC inhibited CCR2 (measured as MCP-1 increase) and CCR5 (measured as MIP-1b increase) with an IC_{50} of 25 ng/mL and 43 ng/mL, respectively (Study 3152-102-002). This model predicts maximal increase in MCP-1 (~6-fold) and MIP-1b (~2-fold) achieved at steady state by once daily dosing of 150 mg CVC, which reflects complete receptor occupancy. In order to achieve complete receptor occupancy at an earlier time, a loading dose of 300 mg CVC in the morning and 150 mg CVC after 12 hours will be administered: patients enrolled in the trial in the latter portion of the day may receive only the 300 mg component of the Day 1 loading dose (see Table 2.1 for details on dosing scenarios). This dosing regimen predicts achievement of ~90% receptor occupancy by end of Day 1. Thereafter, continued dosing of 150 mg CVC twice daily is expected to maintain effective receptor occupancy for the duration of the treatment.

Moreover, the proposed total dose of 450 mg CVC on Day 1 (split into 300 mg and 150 mg), depending on which dosing scenario the patient has started in (see Table 2.1 for details on dosing scenarios), is justified based on clinical trials in which multiple doses of CVC up to 900 mg QD for 7 days were administered to healthy volunteers with no safety findings observed. Furthermore, other safety margin data also give support for this dosing regimen. In GLP toxicology studies, the NOAEL was determined to be 2000 mg/kg/day in mice, 100 mg/kg/day in rats, and 1000 mg/kg/day in monkeys. Based on the most sensitive NOAEL dose of 100 mg/kg/day in rats, a HED of 960 mg was estimated. The most commonly observed AEs (≥ 2%) with CVC in multiple-dose studies evaluating daily doses up to 900 mg for ≥ 7 days were headache and constipation.

Furthermore, CVC has been shown to be safe and well-tolerated following multiple-dose administration of 450 mg and 900 mg once daily for 7 days (Study 3152-107-002). In study 3152-107-002, steady state C_{max} of CVC at doses of 450 mg and 900 mg was 2977 ng/mL and 4025 ng/mL, respectively, and the corresponding $AUC_{0-\tau}$ values were 37724 and 72905 ng.h/mL, respectively. Since the Day 1 dose of 450 mg in the current study is being split (as 300 mg in the morning and 150 mg to be administered after 12 hours) and from day 2 onwards at a dose of 300 mg (split as 150 mg BID), the maximal CVC concentrations (Cmax) as well as steady state AUC (AUC_t) in this study are expected to be less than those observed in earlier clinical studies (3152-107-002 and 3152-106-002). Note: patients enrolled in the trial in the latter portion of the day may receive only the 300 mg component of the Day 1 loading dose (see Table 2.1 for details on dosing scenarios).

Since food has been shown to have significant effect on CVC exposure, all doses are recommended to be administered within 30 minutes of consumption of a meal (or feeds), which is also how CVC has been administered in other clinical trials.

2.1.2.1 Pharmacokinetic Modelling Assessments for Dose Rationale

CVC is a dual antagonist of CCR2 and CCR5 receptors. Increase in serum concentrations of ligands of CCR2 (MCP-1) and CCR5 (MIP-1β) is used as a surrogate marker (biomarker response) to determine

CVC receptor occupancy in clinical studies. In an earlier Phase 1 clinical study (Study 3152-105-002), exposure response relationship between plasma CVC concentrations and biomarker response (MCP-1 or MIP-1β) were explored using a sequential PK/PD model (Figure 2.1.1). The structural PK model was a two-compartment model with first-order absorption and with a lag time. The exposure response for each biomarker was modelled by an indirect pharmacodynamic response model. The kinetic parameter estimates from the indirect response model are summarized in Table 2.1.1.

Figure 2.1.1: Sequential approach of population PK and Indirect response model utilized to determine exposure response

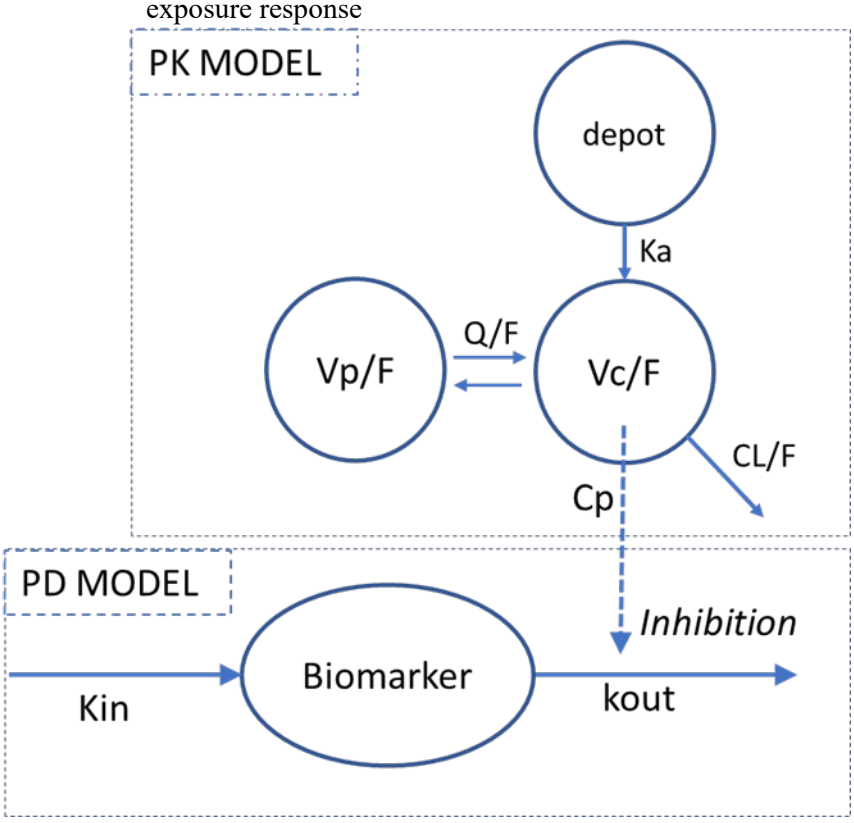


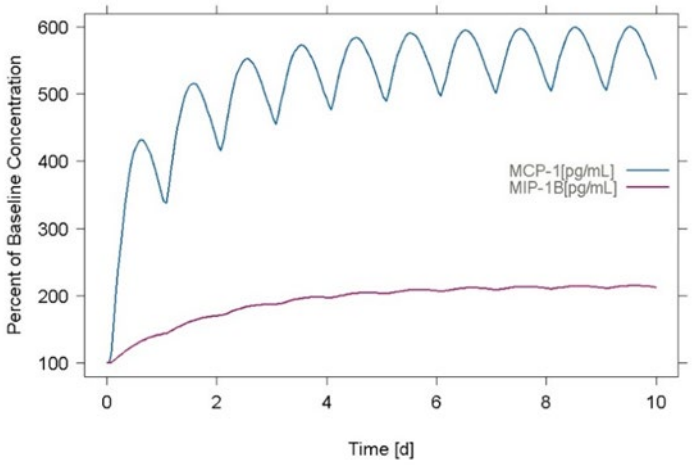
Table 2.1.1: Parameter Estimates for Indirect Response Model

	MCP-1 [pg/mL]			MIP-1B [pg/mL]		
	Typical Value	RSE(%)	IIV	Typical Value	RSE(%)	IIV
Baseline [pg/mL]	339	3.8	18%	98	7.8	38%
kout [1/h]	0.60	fixed	n.e.	0.055	9.8	n.e.
Imax [-]	0.90	fixed	n.e.	0.62	fixed	n.e.
IC50 [ng/mL]	25	63	78%	43	31	117%
Proportional Residual Error	19%	12	n.e.	12%	5.7	n.e.

RSE – relative standard error for estimated typical value; IIV – inter-individual variability; n.e. – not estimated; Kin=Baseline*kout; %inhibition of kout = Cp*Imax/(Cp+IC50); "fixed" - to aid a stable estimation, some parameter values were fixed in final model based on values obtained in iterative model runs and evaluated through numerical and visual goodness-of-fit.

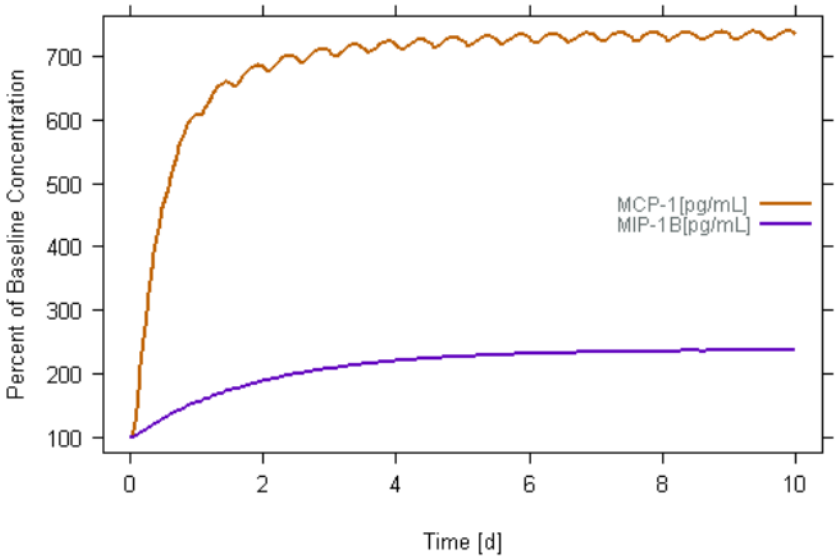
Based on these parameters, the biomarker response (% change from baseline of MCP-1 or MIP-1β) was predicted following once daily dosing of 150 mg CVC (Figure 2.1.2). These simulations suggest maximal change in MCP-1 levels (~5.5-fold increase) and MIP-1β (~2-fold) upon once daily dosing of CVC for at least 5 days and 10 days, respectively.

Figure 2.1.2: Predicted biomarker response following once daily dosing of 150 mg CVC



Since treatment of fibrosis in NASH patients require chronic administration of CVC (AURORA study duration: 5 years of once daily treatment of 150 mg CVC) and the earliest change in endpoint (reduction in fibrosis) is expected after once daily treatment for 1 year, achieving maximal biomarker change after 5 to 10 days of once daily treatment is acceptable in NASH indication. However, COVID-19 is a severe acute infectious disease that requires rapid onset of action and relatively short duration of treatment (planned duration of treatment 28 days or until hospital discharge, which one occurs first, with a minimum of 14 days of treatment). Therefore, dosing regimen containing a total loading dose of 450 mg CVC (divided as 300 mg in the morning + 150 mg in the evening) was proposed on Day 1 of this study followed by dose of 300 mg/day (divided as 150 mg in the morning + 150 mg in the evening). For the proposed dosing regimen, the biomarker response (% change from baseline of MCP-1 or MIP-1β) was predicted as shown (Figure 2.1.3). As per simulations, loading dose is expected to increase MCP-1 levels by 6-fold and MIP-1β levels by 1.5-fold on Day 1. Following twice-daily administration of 150 mg CVC, maximal increase in MCP-1 (~7-fold) and MIP-1β (~2-fold) were sustained during the duration of treatment beginning Day 2. These simulations suggest effective blockade of activation of CCR2 and CCR5 receptors by respective ligands could be achieved rapidly and sustained by following the proposed dosing regimen.

Figure 2.1.3 Predicted biomarker response following proposed dosing regimen of CVC (Day 1: 300 mg in the morning + 150 mg in the evening, Day 2 to Day 29: 150 mg twice daily)



As of January 2020, approximately 2000 participants have been exposed to either single or multiple doses of CVC in completed and ongoing clinical studies. CVC doses explored in these clinical studies range from 25 mg to 900 mg across all CVC studies. There was no apparent dose- or exposure-relationship for safety observed. Furthermore, splitting the daily dose is expected to limit the plasma peak concentrations of CVC corresponding to 300 mg dose on Day 1 and 150 mg dose from Day 2 onwards. In addition, food effect on the bioavailability of CVC formulation is significant (up to 5.2-fold increase with standard breakfast). The CVC dose can be administered to an intubated and severely ill COVID-19 patient via G-tube and with liquid food. In such a case, if there were to be reduced enteral absorption, then any lowered plasma concentrations/exposure of CVC may be partially offset by the twice-daily dosing of CVC.

In summary, these data provide support and rationale for the proposed dosing being well within appropriate parameters and safety. The sponsor believes the proposed loading dose (450 mg on Day 1) and twice daily administration of 150 mg CVC are clearly justified based on the following: safe use of CVC doses up to 900 mg in prior clinical studies; immediate need for optimal plasma exposure of CVC in patients with COVID-19 infection, ensure full antagonistic activity against CCR2 and CCR5 receptors in target tissues; and maintenance of such exposure throughout the duration of treatment without compromising patient safety.

2.1.3 Cenicriviroc Premedication Regimen and Prophylactic Medications

No specific premedication is required for CVC treatment.

2.2 Eligibility

Eligibility criteria listed in the I-SPY COVID-19 TRIAL Master Protocol apply to all participants. In addition, the following criteria apply to those patients receiving CVC:

Inclusion:

- No additional inclusion criteria
- Willing to use highly effective methods of contraception during and for 1 month after treatment with CVC.

Exclusion:

- ALT or AST > 5 times the upper limit of normal
- Use of medications that are contraindicated with CVC and that could not be replaced or stopped during the study period (See Table 2.2 for Disallowed Medications)

2.3 Contraindications

Pregnancy should be avoided in women receiving this compound. Women of childbearing potential should undergo pregnancy testing prior to initiation of CVC and periodically during treatment. In addition, adequate contraceptive methods should be consistently used by males and females during and for 1 month after treatment with CVC.

CVC is contraindicated in nursing mothers. The extent to which CVC or any of its metabolites are excreted in human breast milk is not known.

CVC is also contraindicated in participants with clinically significant hypersensitivity to any components of its formulation.

Subjects must use highly effective methods of contraception during and for 1 month after treatment with CVC.

The following methods have been determined to achieve a failure rate < 1% per year, when used consistently and correctly, are considered highly effective birth control methods and are permitted under this protocol:

Complete abstinence from sexual intercourse if this is the subject's usual and preferred lifestyle.*

- Dual method of contraception including but not limited to:
    - Condom with spermicide (if available) in conjunction with use of combine (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation:
        - Oral
        - Intravaginal
        - Transdermal
    - Condom with spermicide (if available) in conjunction with use of progestogen-only hormonal contraception associated with inhibition of ovulation:
        - Oral
        - Injectable
        - Implantable
    - Condom with spermicide (if available) in conjunction with use of an intrauterine device (IUD)
    - Condom with spermicide (if available) in conjunction with use of a diaphragm
    - Condom with birth control patch or vaginal ring**
    - Condom with oral, injectable, or implanted contraceptives*
    - Condom with intrauterine-hormone-releasing system (IUS)

- For the purposes of this study, surgical sterilization includes:
    - Hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy, vasectomy.
    - Bilateral tubal occlusion
    - Vasectomized partner: a highly effective birth control method provided that partner is the sole sexual partner of the female participant who is of childbearing potential and that the vasectomized partner has received medical assessment of the surgical success**

* Considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

**Subjects who are using hormonal contraceptives should be instructed to use an additional contraceptive measure during the study (see above for other methods).

2.4 Concomitant Medications

At Screening, all medications taken up to 30 days prior to the screening visit will be recorded.
In addition, supportive therapies given during the course of the study should be collected and recorded.
At each study visit, the site will capture any and all medications taken by the subject since the last visit or during the visit (as applicable).

Caution should always be exercised when administering concomitant medications based on the individual medication profile and clinical risk-benefit assessment. A complete list of prohibited medications is provided in Table 2.2. Concomitant therapies of particular note include:

- Antiretroviral agents (See the table below [Table 2.2] for other antiretroviral agents)
    - There are no current concerns in utilizing CVC with Remdesivir
    - Dolutegravir, Tenofovir each has no significant impact on CVC exposure and would be acceptable to co-administer with study intervention in this study.
    - Ritonavir or any combination of a protease inhibitor with ritonavir should not be co-administered with drug product in this study due to a significant drug interaction between ritonavir and CVC.

Any medication or vaccine (including over-the-counter, prescription medicines, vitamins, herbal supplements, and/or cannabis or other specific categories of interest) that the participant is receiving at the time of screening, has received in the 30 days prior to screening, or is anticipated to receive during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The Sponsor's Medical Safety Physician or equivalent representative should be contacted if there are any questions regarding concomitant or prior therapy.

Due to potential interactions the following medications are not allowed. If there is a medical need to utilize any of the medications below, then the Investigator should discuss appropriate steps with the Sponsor.

Table 2.2. Medications of Interest

Disallowed Medications

Antibacterials	rifampin, nafcillin, clarithromycin, erythromycin, telithromycin
Anticonvulsants	carbamazepine, phenytoin
Antidepressants	Nefazodone
Antifungals	voriconazole, itraconazole, ketoconazole, posaconazole
Antihistamines	Astemizole
Anti-inflammatory drugs	Sulfasalazine
Antimetabolite drugs	Methotrexate
Antipsychotics	Pimozide
Antivirals	Efavirenz, etravirine, boceprevir, dasabuvir/ombitasvir/paritaprevir/ritonavir, indinavir, lopinavir/ritonavir, nelfinavir, ombitasvir/paritaprevir/ritonavir, ritonavir, saquinavir, telaprevir, glecaprevir/pibrentasvir
Ade Ergot Alkaloids	dihydroergotamine, ergonovine, ergotamine, methylergonovine
Lipid-lowering agents	Gemfibrozil
Other	Cisapride, hydroxychloroquine or chloroquine

Allowed Medications Requiring Dose Adjustments	
Opioids: **Fentanyl, Alfentanil**	The preference for opioid use for analgesia is to employ a non-fentanyl based strategy (eg, dilaudid). **Note: fentanyl and alfentanil are CYP3A4 substrates, thus use of either with CVC may increase fentanyl and alfentanil exposure.** If needed, fentanyl/alfentanil should be administered with close surveillance and a dose-titration strategy: ● If feasible, it is recommended that the initial dose be decreased by 50% and up-titrated to the desired effect. ● If a customary starting-dose is needed in the Investigator's judgment, should be down-titrated after initiation

Sedative/hypnotics: midazolam, triazolam	The preference for sedation is to employ a non-midazolam-based strategy (eg, propofol). **Note: the exposure of midazolam (a substrate of CYP3A4) can be increased 1.84 fold when co-administered with CVC.** If needed, midazolam or triazolam should be administered with close surveillance and a dose-titration strategy: ● If feasible, it is recommended that the initial dose be decreased by 50% and up-titrated to the desired effect. ● If a customary starting-dose is needed in the investigator's judgement, should be down-titrated after initiation
Immunosuppressants: cyclosporin, tacrolimus	In patients actively receiving cyclosporine or tacrolimus, CVC should be given as 150 mg QD instead of 150 mg BID.
Gastric acid-reducing agents (H2 receptor antagonists, antacids, proton-pump inhibitors [PPIs])	Gastric acid-reducing agents should be administered at least 2 hours after CVC dosing (4 hours after dosing is ideal for fast-acting antacids). When possible, an H2 receptor antagonist (except cimetidine) or antacid is preferred over a proton-pump inhibitor (PPI). It is recommended to start with the lowest dose of these agents and titrate appropriately. ● H2 receptor antagonists (eg, famotidine or ranitidine) should not exceed a dose comparable to famotidine 40 mg daily. ● Antacids (eg, aluminum hydroxide, calcium carbonate, magnesium carbonate, magnesium hydroxide, or bismuth subsalicylate) should be given at least 4 hours after administration of study drug. ● PPIs (eg, omeprazole, lansoprazole, esomeprazole, pantoprazole, raberprazole, dexlansoprazole) are not recommended, however if needed, administer approximately 3 hours after study drug at a dose that does not exceed doses comparable to omeprazole 20 mg daily. Note: due to the prolonged acid-reducing effect of PPIs (~16-24 hours), it is advised to follow these recommendations to reduce their potential impact on absorption of the subsequent CVC dose.
Lipid-lowering agents: atorvastatin, simvastatin, lovastatin, pravastatin, rosuvastatin	The maximum recommended daily doses are as follows: ● atorvastatin 40 mg, ● simvastatin 20 mg, ● lovastatin 40 mg, ● pravastatin 40 mg, and ● rosuvastatin 20 mg; ● pitavastatin use is allowed without dose restriction The medical monitor or equivalent representative must be consulted prior to use of higher doses of statins than those recommended above.
PDE5 enzyme inhibitors: sildenafil, tadalafil, vardenafil	The recommended starting doses for these medications are as follows: ● sildenafil 25 mg, ● tadalafil 2.5 mg, ● vardenafil 2.5 mg
Anticoagulants	The recommended instructions for these medications are as follows: ● rivaroxaban - if required, do not exceed 10 mg

	● apixaban and edoxaban – may increase CVC exposure, use with reduced doses and close clinical monitoring

2.5 Clinical Evaluation and Procedures

Laboratory evaluations for general safety monitoring are described in the Master Protocol, Sections 8.1─8.3; additional evaluations/procedures necessary for this arm include (below Table is in addition to Table of Assessments in Section 8 of Master Protocol, Table 8-1):

Study Phase	Screening (D-1 or D1)	Baseline (D1) [6]	Study Intervention Period, inpatient	Post Hospitalization Period,
Study Blood Draw[1, 2]		x	x (D3W1 & D7W1)	
Liver biochemical tests[3, 4]		x	x (D3W1, D7W1, and weekly until discharge)	x (Day 15 +3 days) [5]
Hematology (cbc)		x	x (D7W1 and weekly until discharge)	
Pregnancy test [8]	x		x (D7W1 and weekly until discharge)	
Safety Follow-up Call or Visit				x (Day 28 +/- 2 days) [7] x (Day 60 +/- 5 days) [7]

1. Re-statement of row from Table 8-1 from Master Protocol. Collect blood for plasma and PBMC at time of enrollment, at day three, and at day 7. Plasma collected on both days will be assessed for SARS CoV-2 viral and Ab titer, pharmacodynamic markers and exploratory biomarkers.
2. Levels of CCL2, 3, 4, 5, and CCR2, 3, 4, 5 will be measured for biomarker analyses.
3. This is for routine care of all participants; this is separate from testing that may need to occur in follow-up for abnormal values (see Appendix Section 2.6.1)
4. To include: ALT, AST, ALP, TBL, and direct bilirubin
5. Only if patient is on study drug post-discharge and assessment not made within prior 3 days. Note, this time window allows testing on or after the day of last drug-dose but not before.
6. Assessments must occur prior to dosing
7. To serve as visit (or telephone/telemedicine visit) 30 days after last dose of study drug; potential AE/SAE collection to be performed and ePRO
8. Pregnancy can be tested using either serum or urine assessments, per institutional policy. Only in female patients of child-bearing potential.

2.5.1 CVC Pharmacokinetic Testing

During this study, pharmacokinetic (PK) sampling will be conducted to assess the concentrations of CVC in COVID-19 patients.

PK testing will be performed only if feasible. Below are the recommended days and times for PK sampling:

- Day 1 (post 300-mg loading dose). One PK sample between 3 – 6 hours post 300-mg dose (rationale: measure CVC concentrations after loading dose)
- Day 4 (pre-dose; 3-6 hours post-dose; 8-12 hours post-dose). Three PK samples collected (rationale: measure CVC concentrations during steady state)
- Three days post-feeding-tube placement; only for patients receiving CVC through a feeding tube (pre-dose; 3-6 hours post-dose; 8-12 hours post-dose). Three PK samples collected (rationale: measure CVC concentrations during steady state in patients receiving CVC through a feeding tube)

2.6 Dose Modifications and Management of Potential Toxicity

Any potential dose modifications need to be discussed with the Sponsor.

2.6.1 Liver Assessment: Suggested Actions and Follow-up

Close monitoring should be initiated for the following participants:

- Participants with abnormal LFTs (ie, AST, ALT, alkaline phosphatase and/or total bilirubin) which are > ULN at the time of discharge and whose discharge LFTs > baseline.

The participant should be evaluated for liver biochemistry elevation potentially meeting Hy's law criteria as soon as possible, preferably within 24 to 48 hours from the time the investigator becomes aware of the abnormal results. Evaluation should typically include repeat testing of all 4 of the usual serum biochemical measures (ALT, AST, ALP, and TBL) to confirm the abnormalities and to determine if they are increasing or decreasing. Testing should be repeated until the levels decrease or stabilize.

If it is difficult for the participant to return to the study site promptly, the participant should be retested locally, but normal laboratory ranges should be recorded, results should be made available to sponsor's study physician and the investigator immediately, and the data should be included in the eCRF. If repeat testing within this time frame is not possible, the study intervention should be discontinued.

It is critical to initiate close monitoring immediately upon detection and confirmation of signals of liver biochemistry elevation potentially meeting Hy's law criteria as early as possible and not to wait until the next scheduled visit or monitoring interval. Close monitoring of the participant should be initiated in conjunction with the sponsor and consideration given to the following:

- Obtain a more detailed history of symptoms and prior or concurrent diseases.
- Obtain a history of concomitant drug use, including nonprescription medications, herbal products and dietary supplements, alcohol and recreational drug use, and special diets.
- Obtain a history of exposure to environmental chemical agents.
- Initiation of appropriate evaluations including applicable laboratory tests (eg, direct bilirubin, INR), physical assessments, and other assessments (eg, imaging)
    - Rule out other potential causes of biochemical abnormalities, eg, acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; NASH; hypoxic/ischemic hepatopathy; and biliary tract disease.
- Consider gastroenterology or hepatology consultations.

If any of the following criteria are met, discontinuation of study intervention should be considered (if indicated, prior to receipt of confirming retest biochemistry laboratory test results) and the sponsor notified of the discontinuation:

- ALT or AST ≥ 3 × ULN and the participant is symptomatic with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia (> 5%)
- ALT or AST ≥ 3 × ULN and total bilirubin > 2 × ULN or INR > 1.5
- ALT or AST ≥ 5 × ULN for more than 2 weeks
- ALT or AST ≥ 8 × ULN

All participants showing liver biochemistry elevation meeting potential Hy's law criteria should be followed until all abnormalities return to normal or to the baseline state.

Reporting of Potential Hy's Law Cases

Potential Hy's law cases are defined by biochemical test results of hepatocellular injury and impaired hepatic function. They should be evaluated and followed further (ie, close monitoring initiated) to determine whether these laboratory abnormalities are indicative of liver biochemistry elevation meeting potential Hy's law criteria. As indicated above, discontinuation of study intervention should also be considered. Criteria that identify a potential Hy's law case are as follows:

- ALT or AST ≥ 3 × ULN AND
- Total bilirubin ≥ 2 × ULN AND
- Alkaline phosphatase < 2 × ULN

Sites must report every participant who meets the Hy's law criteria if this occurs within the time the participant signs the ICF until 30 days after the last dose of study intervention.

A laboratory alert for a liver biochemistry elevation potentially meeting Hy's law criteria case will be sent immediately to the sponsor and investigators when the above criteria have been met, even if no clinical symptoms have been experienced. An Adverse Event of Interest Abnormal Liver Function Reporting Form should be completed as soon as possible (within 24 hours of notification) for liver biochemistry elevations potentially meeting Hy's law criteria cases and submitted to the Sponsor. The eCRF pages associated with the potential Hy's law cases must be completed within 7 calendar days. Potential Hy's law cases will be evaluated by a hepatologist with expertise in drug-induced liver injury (DILI) and reviewed by the DSMB.

Adverse Events of Special Interest (AESI)

An Adverse Event of Interest Abnormal Liver Function Reporting Form should be completed as soon as possible (within 24 hours of notification) for liver biochemistry elevations potentially meeting Hy's law criteria cases and submitted to the Sponsor. The eCRF pages associated with the potential Hy's law cases must be completed within 7 calendar days. Potential Hy's law cases will be evaluated by a hepatologist with expertise in drug-induced liver injury (DILI) and reviewed by the DSMB. DILI would be considered an AESI

2.6.2 Kidney toxicity or impairment

Stage 4 chronic kidney disease or requiring dialysis is currently an exclusion criterion for this arm. If a patient's kidney function decreases to a level which requires dialysis during the study, it is recommended to discontinue CVC (but to continue following the patient).

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2.6.2 Overdose

No specific information is available on the treatment of overdose of CVC. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

An overdose of CVC, regardless of the presence of an associated SAE, is considered an ECI and must be documented and reported.

Additionally, an SAE associated with an overdose of CVC must be documented and reported according to the requirements for SAEs.

Please refer to the I-SPY COVID-19 MOP for instructions on reporting overdose.

3. INVESTIGATIONAL AGENT PHARMACEUTICAL INFORMATION

3.1 Investigational Study Agents (IND 150378, IND Sponsor: QLHC)

The CVC drug product used for clinical trials is supplied as ready-to-use 150 mg yellow-coated, immediate release tablets for oral administration. The formulated tablets contains the following excipients: fumaric acid (160 mg) and other excipients, including microcrystalline cellulose, croscarmellose sodium, colloidal silicon dioxide, magnesium stearate, and Opadry® II yellow (film coating). Study drug will be provided in a HDPE bottle containing desiccant, and induction sealed. All labels for CVC will meet all applicable requirements of the US FDA and Annex 13 of Good Manufacturing Practices and/or all local regulations, as applicable.

Confidential pharmaceutical information for investigational study agents supplied by pharmaceutical partners is available through an FDA IND cross-reference letter.

3.2 Reported Clinical AEs and Potential Risks of CVC

As of January 2020, CVC has been administered to over 2,000 participants in completed and ongoing studies in participants with HIV infection, non-alcoholic steatohepatitis (NASH), hepatic impairment, renal impairment, and healthy volunteers, with a safety profile similar to placebo. CVC doses range from 25 mg to 900 mg daily across all CVC studies. In a Phase 2b study in HIV, participants were exposed to CVC for 41 weeks (mean) and 48 weeks (median). In Phase 2b in NASH, participants received CVC for 99 weeks (mean) and 102 weeks (median). There was no apparent dose- or exposure-relationship for safety, and CVC demonstrated a safety profile similar to placebo. A global Phase 3 study in participants with NASH is ongoing.

3.2.1 Potential Risks

Contraindications

No data currently exists for the effects of CVC on reproduction and development in humans. Pregnancy should be avoided in women receiving this compound. Women of childbearing potential should undergo pregnancy testing prior to initiation of CVC. In addition, adequate contraceptive methods should be consistently used by males and females during and for 1 month after treatment with CVC.

CVC is contraindicated in nursing mothers. CVC and its metabolites were detected in milk secreted from rats administered CVC. CVC milk to plasma ratios ranged from 0.7 to 0.8 in dams indicating that milk represents a potential route of exposure to CVC in rat pups. The extent to which CVC or any of its metabolites are excreted in human breast milk is not known.

CVC is also contraindicated in participants with clinically significant hypersensitivity to any components of its formulation.

Warnings and Precautions

Treatment-emergent elevations in liver transaminases have been observed in participants receiving CVC treatment. Most elevations were transient, mild to moderate in severity, and resolved upon continued treatment with CVC. Some elevations were severe in intensity and resulted in dose interruption or permanent discontinuation of CVC. CVC has been administered to >2000 patients, including patients with non-alcoholic fatty liver disease (NASH) and a separately to those with HIV, in clinical trials. In NASH patients with pre-existing liver disease, 2 cases of possible autoimmune hepatitis (1 participant in the CVC arm and 1 participant in the placebo arm) have been observed.

Hepatic and Renal Impairment

Based on clinical data (studies 652-1-121 and 3152-102-002), CVC exposures did not increase in participants with mild hepatic impairment (Child-Pugh A), increased in participants with moderate (Child-Pugh B) (AUC0-tau 55%), and significantly increased in severe (Child-Pugh C) (AUC0-t 40%) hepatic impairment. relative to corresponding matched healthy participants. Of note, these clinical studies allowed inclusion for ALT or AST abnormalities up to 5x ULN. Based on the PK and safety results for these studies, no CVC dose adjustment will be required in patients with hepatic impairment. Based on results from the human ADME study (Study 3152-103-002), renal excretion of CVC and its metabolites was negligible (< 2% of dose). Consistent with this observation, the renal excretion of CVC (non-radiolabel study) was observed to be < 0.00011% (Study 01-03-TL-652-001). The impact of end stage renal disease on the PK of CVC is under evaluation (Study 3152-104-002).

Drug Interactions

CVC is a substrate of CYP3A4, CYP2C8 and P-gp. CVC is also a weak inhibitor of CYP3A4 and an inhibitor of BCRP and P-gp. The following classes of medications are disallowed at any time during CVC treatment:

- Potent CYP3A4 inhibitors and CYP3A4 inducers will be excluded
- Potent CYP2C8 inhibitors will be excluded
- Some drugs with narrow therapeutic windows that are sensitive CYP3A4 substrates will be excluded (ie, drugs which should not be co-administered with weak CYP3A4 inhibitors such as CVC). See Table 2.2.
- Appropriate dose adjustments of BCRP substrates are recommended

Co-administration of CVC and remdesivir is allowed.

When required, acid-reducing agents should be administered at least 2 hours after the CVC dose to ensure that adequate CVC concentrations are maintained. When possible, use of an H2 receptor antagonist (except cimetidine) or antacids is preferred over a proton pump inhibitor. It is recommended to start with the lowest dose of these agents and titrate according to clinical response. See Table 2.2.

If lipid lowering medications are used, clinical monitoring and dose titration are recommended to achieve the desired clinical response. See Table 2.2 for specific guidance.

Overall Risk Assessment

In summary, the risks of CVC are low and similar to placebo

3.2.2 Previous Experience with Products of Same Class

N/A

3.2.1 Observed AE's in Human Trials

In HIV studies, participants received 150 and 200 mg of CVC once daily. In the NASH trial participants received 150 mg of CVC daily for two years. There were no additional adverse events reported in HIV participants in comparison to the standard treatment at this time, moreover, CVC was generally better tolerated than the comparator (ie, efavirenz). In particular, there was no increased rate of infections reported. In HIV, main AEs were gastrointestinal symptoms reported by 3% and a skin rash reported in 2% of the participants. Serious AEs were reported in 2%; 3% showed Grade 3 and 0 Grade 4 AEs, which was below the control arm (Thompson 2016).

In NASH trial participants, overall, the safety profile of CVC (150 mg QD) was comparable to that in participants treated with placebo and was well tolerated over 2 years in the Phase 2 CENTAUR study. The types, severity, and frequency of TEAEs reported after 2 years of CVC treatment was consistent with those reported after 1 year of treatment. The overall incidence of TEAEs during the study was similar across the treatment groups (≥ 95.0% of participants in each group). No deaths occurred during the study. The frequency and types of TEAEs reported were comparable between treatment groups during Year 1 and Year 2. The incidence of treatment-emergent Grade 3 or 4 laboratory abnormalities during Year 2 was generally similar across the treatment groups. In the second year of treatment, there was an increase in nasopharyngitis of 8.2 and 9.1% (CVC treatment arms) vs 3.3% (placebo), however, without an increase in upper respiratory tract infections, or AEs related to infection, in comparison to placebo (Ratziu 2020).

See the Investigators Brochure for additional information.

3.3 Investigational Agent Availability

The agent product is provided as tablets in a HDPE bottle containing desiccant, and induction sealed. Dose strengths of 150 mg are to be used in this trial. The investigator or designee will record the lot number, expiration date and the amount of study medication dispensed to each participant.

3.4 Investigational Agent Distribution

Shipment of investigational agents to a participating site will not be approved until documentation of IRB approval of the sponsor-approved protocol and consent is available, and the collection of all essential documents is complete.

3.5 Investigational Agent Preparation and Handling

Treatment will be administered on an inpatient and outpatient basis. Only authorized site staff may supply or administer study intervention. CVC tablets (150 mg) will be provided by Allergan, a division of AbbVie. No preparation is required for CVC tablets, unless patients are on a feeding tube, in which case refer to the Pharmacy Manual.

Briefly, the procedure for administration through a feeding tube is:

1. Study drug should be administered with food or feeds. Administer the study drug immediately after feeding the patient.
2. After administering tube feeding, flush the tube with water prior to study drug administration.
3. Transfer one tablet to a clean pill crusher and grind it to a powder. Transfer the powder to a clean medicine cup.
4. Add 30 ml of room temperature water to the medicine cup and stir vigorously to suspend the material.
5. Draw the suspension into a large syringe (eg, 60 mL syringe) and administer via the feeding tube.
6. After this first administration, flush the tube with room temperature water.
7. To ensure all study drug has been delivered, add 30 ml of room temperature water to medicine cup and stir to include any potentially remaining material.
8. Draw the fluid into the same large syringe (eg, 60 mL syringe) and transfer to the patient via the feeding tube.
9. After this final administration of the study drug, flush the tube with room temperature water.
10. Document the date and time, the patient's response (if any response occurred), and your assessment of study drug delivery.
11. Document all liquids administered, including feed and flushes, on the patient's intake and output record.

3.6 Investigational Agent Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all investigational agents. The investigator is required to maintain adequate records of receipt, dispensing, and final disposition of study agent. On the receipt, record from whom study agent was received and to whom study agent was provided to, date, quantity, and batch or lot number. On the dispensing record, note quantities and dates study agent was dispensed to and returned by each participant. Participants will be required to maintain an Investigational Agent Log (pill diary).

3.7 Investigational Agent Packaging and Labeling

CVC is packaged and labeled by Allergan, according to GCP, FDA, and national requirements. Labels are printed and attached to the study agent bottle or other packaging container prior to shipping to the site. Each is labeled with a single panel label within local labeling requirements.

Each label must remain affixed to the bottle.

3.8 Investigational Agent Storage

Bottles of CVC tablets should be stored Store at 15°C to 30°C (59°F to 86°F) with transient excursions permitted to -20°C (-4°F) to 60°C (140°F). Do not shake or freeze bottles. Do not use drug product if tablets are broken, cracked, or otherwise not intact.

Detailed descriptions of the storage and handling instructions for CVC tablets are provided in the MOP.

3.9 Investigational Agent Destruction/Disposal

Once agent accountability is performed, the participating sites should use local/institutional procedures for disposal of returned/used/unused study agent and bottles. Copies of all certificates of destruction of any used/unused study agent must be provided to the Sponsor. Prior to destruction, the pharmacist should contact the assigned study monitor.

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Appendix E
Icatibant (Firazyr)

I-SPY-COVID-19 Investigational Agent Information

INVESTIGATIONAL AGENT INFORMATION SUMMARY

Agent Chaperone:	Kathleen Liu, UCSF, Kathleen.Liu@ucsf.edu
Agent Co-Chaperone:	Derek Russell, UABMC, dwrussell@uabmc.edu

General Information:

Agent Class:	Competitive antagonist selective for the bradykinin (BK) type 2 receptor (BKR2)
Structural Class:	Small molecule, synthetic decapeptide (MW 1304.6 as free peptide base)
Manufacturer:	Shire HGT, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited
Phase of Development:	Approved for the treatment of attacks of hereditary angioedema (HAE) since 2008 in EU and 2011 in US (https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/022150s000lbl.pdf).

Pharmaceutical Information:

Dosage Form:	A sterile, single-use, prefilled syringe solution for subcutaneous administration.
Physical Description:	Each syringe contains 3 mL of a sterile solution of icatibant 30 mg (as icatibant acetate). The excipients include: acetic acid glacial 1.32 mg, sodium hydroxide 0.64 mg, sodium chloride 7.45 mg, and water for injection q.s. to 1 mL. The prefilled syringe is clear (type I glass) with gray plunger stopper (bromobutyl coated with fluorocarbon polymer) with a LUER-LOK with a screw tip cap and white polypropylene backstop. A hypodermic needle (25 G) is included in the pack.
Strengths to be used in trial:	30 mg in sterile, single-use syringe.
Packaging Unit	Single-dose, single-use prefilled syringe with a hypodermic needle (25G) included in the package.
Storage Conditions:	Store between 2°C to 25°C (36°F to 77°F). Do not freeze.

Administration Information:

Route:	Subcutaneous (SC) injection in the abdominal area over at least 30 seconds.
Regimen:	30 mg every 8 hours daily for three (3) days (total of 9 doses), or for six (6) days (total of 18 doses) pending safety assessment (Section 2.6.3 Ten Patient Safety Reviews)

Agent Preparation:	No agent preparation is required. See Section 3.5
Pre-medication:	Specific pre-medication is not required for routine treatment. See Section 2.1.1
Administration:	Treatment will be administered to subjects while hospitalized as inpatients. See Section 2.1 for further details.

Concomitant Medications: Following precautions and warning should be given:

Icatibant metabolism is not mediated by CYP450 enzymes. Therefore, metabolic drug interactions between icatibant and CYP450 substrates, inhibitors and inducers are not expected.

Icatibant is a bradykinin B2 receptor antagonist and thereby has the potential to have a pharmacodynamic interaction with ACE inhibitors where icatibant may attenuate the antihypertensive effect of ACE inhibitors.

Refer to §2.6 for side-effect management and dose reduction plans.

The above is intended as a summary only; please see the complete appendix for additional investigational agent information

1. RATIONALE FOR TESTING

Rationale for targeting the kallikrein-kinin system (KKS)

Acute respiratory distress syndrome (ARDS) is a severe and life-threatening condition characterized by pulmonary edema, severe hypoxemia and reduced lung compliance. The pathogenesis of ARDS involves inflammatory cytokines/mediators, disruption of the endothelial/epithelial barrier integrity, infiltration of inflammatory cells, procoagulant actions and decreased anti-fibrinolytic activities. The contact activation system plays an important role in the modulation of these lung inflammatory responses.

The contact activation system is comprised of coagulation factor XII (FXII), plasma prekallikrein (PPK) and high molecular weight kininogen (HK). FXII binds to negatively charged substances at cell surfaces (endothelial cells, platelets, leukocytes, LPS) leading to activated FXIIa, which activates the intrinsic coagulation pathway via activation of factor XI (FXI) and activates the kallikrein-kinin system by enzymatically cleaving PPK to active plasma kallikrein (PK). FXII has been shown to upregulate the expression of pro-inflammatory mediators IL-8, IL-1β, IL-6 and TNF-α2. PK in turn reciprocally activates FXII in a positive feedback loop, and enzymatically liberates bradykinin (BK) from HK. BK is a potent proinflammatory and vasodilatory peptide that contributes to several of the deleterious consequences seen in ARDS. BK facilitates recruitment of inflammatory mediators, inflammatory cells, vascular permeability (leading to pulmonary edema), vasodilation (leading to ventilation/perfusion mismatch) and systemic hypotension. High levels of FXII are observed in broncho-alveolar lavage fluid in ARDS patients, and consumption of FXII, PPK, HK and BK in plasma has also been observed, highlighting the overwhelming and persistent activation of the contact activation system and KKS in ARDS and suggests that targeting this system may be an important and rational therapeutic approach. While the molecular mechanisms inciting ARDS are not well-elucidated, there is evidence of activation of the contact activation system and KKS early in lung injury (Roche & Roche, 2020; van de Veerdonk et al., 2020).

Analyses of COVID-19 course in both survivors and fatal cases showed that, in most of the cases, onset of first signs and symptoms is followed by a phase with milder respiratory symptoms (dry cough, dyspnea) before progression to more severe clinical manifestations and acute respiratory distress. This pre-ARDS phase generally lasts few days (reported median approximately 3 4 days) and represents a window of opportunity for interventions that may interfere with the inflammatory cascade responsible for tissue injury as well as support ventilatory function while endogenous mechanisms and treatments promote recovery versus further deterioration to life-threatening conditions (Wang et al., 2020; Zhou et al., 2020).

Icatibant is a competitive antagonist selective for the BK type 2 receptor (BKR2). It is a synthetic decapeptide with a structure similar to BK, but with 5 non-proteinogenic amino acids. Icatibant SC injection, approved in the US, EU, and many other countries, is indicated for the treatment of acute attacks of HAE in adults 18 years of age or older and, in some geographies, in adolescents and children aged 2 years or older. Blocking BKR2 with icatibant may reduce the pathologic consequences downstream of this receptor, such as vascular permeability, induction of the inflammatory cascade and recruitment of inflammatory cell infiltrate.

Icatibant is characterized by rapid onset of action at the level of BK2R receptors and of clinical symptoms in hereditary angioedema as well as by a short half-life of approximately 1.4 hours (1.48 ± 0.35 hours) after a single subcutaneous dose of 30mg (Leach et al., 2015). Thus, administration of multiple doses at given intervals is necessary to ensure continuous exposure over multiple days.

In HAE, the option to repeat administration up to 3 doses in a 24 hour-period is allowed if a single subcutaneous injection of 3 mL of icatibant 10 mg/mL (total of 30 mg) is not sufficient to induce

complete control of signs and symptoms (FIRAZYR® Prescribing Information). Although in clinical trials no more than 8 injections of icatibant per month were administered, analysis of post-marketing data obtained from 557 patients with HAE using icatibant to treat a total of 3025 attacks in the Icatibant Observational Survey (IOS) program yielded no evidence that administration of >8 doses of icatibant in a month or of repeated administrations with intervals between consecutive doses <6 hours was associated with additional risks (Zanichelli et al., 2017). Moreover, repeated subcutaneous administrations of icatibant 30 mg at 6-hour intervals in healthy volunteers did not result into appreciable drug accumulation (Leach et al., 2015).

Anecdotal experience from experimental use of icatibant in patients with COVID-19 showed that use of up to 9 doses over 3 days was well-tolerated and associated with improvement of respiratory function (unpublished data).

It appears therefore appropriate that during this critical window, administration of icatibant is scheduled to allow continuous exposure and biological activity of treatments supporting respiratory function and reducing pulmonary inflammatory.

1.1 Non-Clinical Studies

The nonclinical program characterized icatibant in pharmacological, pharmacokinetic (PK), and toxicology studies. Absorption, distribution, metabolism, and excretion studies were performed in mice, rats, and dogs; the PK of icatibant was characterized in single and repeat-dose experiments. An assessment of the potential toxicity of icatibant was performed with single-dose toxicity, repeat-dose toxicity in adult and juvenile animals, genotoxicity, carcinogenicity, and reproductive toxicity studies.

Icatibant is a potent competitive antagonist of the human B2 receptor with an affinity similar to bradykinin. Icatibant engages its target in vitro and in vivo and exerts pharmacodynamic (PD) responses in models of inflammation and bronchoconstriction. The selectivity of icatibant for the B2 compared with the B1 receptor was evaluated in receptor binding assays in vitro; the half maximal inhibitory concentration (IC50) of icatibant was determined to be 4.3 nM for B2 and 6 μM for B1, with an inhibition constant (Ki) of 2.0 nM for B2 and 1.2 μM for B1.

Icatibant did not elicit any cardiac conduction change in vitro (human ether-á-go-go–related gene channel), nor did it have any effect in vivo in dog models of heart failure (ventricular pacing, physical exertion, coronary ligation). There was no evidence of corrected QT interval (QTc) prolongation, and no hemodynamic changes were seen following dosing of icatibant in these in vivo models.

Following SC administration, there was a biphasic decline of radioactivity in blood, initially rapid (1 to 2 hours post dose), followed by a second phase lasting days. Two metabolites, M1 and M2, have been isolated and identified across several species, including human. Excretion of radiolabeled icatibant was mainly renal, and the absolute bioavailability of icatibant following SC administration is approximately 100%.

The principal findings in the repeat-dose toxicity studies were effects on hormone levels, reproductive organs, and sexual maturation in adult and juvenile/immature rats and dogs. Measures of hormone levels in dogs showed reductions in testosterone and follicle stimulating hormone (FSH); luteinizing hormone (LH) levels in female animals also showed a general trend to decrease. Similar findings were noted on hormone levels in rats. In mature animals, decreases in hormone levels occurred within the first 24 hours of dosing (dosed twice weekly, 3 times a day [TID]); however, these levels had recovered to control values prior to the next day of dosing. Decreased fertility was observed in untreated females that were paired with high-dose males. All other findings (microscopic and organ weight) were completely or

partially reversed following the dose-free recovery period. Bradykinin, acting through the B2 receptor, is recognized to have a role in control of hormone secretion within the hypothalamus. Therefore, these effects on hormone secretion, with consequent effects on sexual organs, were not unexpected.

Genotoxicity assessments (in vitro and in vivo) of icatibant did not show any indication of genotoxic potential. In 2-year carcinogenicity studies in mice and rats, SC administration of icatibant had no effects on the incidence or morphology of tumors to indicate any carcinogenic potential.

Injection site reactions seen in dogs are consistent with the consequences of mast cell activation and peptide release by the local high concentrations of icatibant at these sites and were transient.

Due to the pharmacological activity of icatibant as a bradykinin B2 receptor antagonist, it has the potential to lower levels of circulating gonadotropins. In nonclinical evaluations of fertility and embryofetal development, icatibant was nonteratogenic but effects on implantation and parturition in female rats were observed.

The icatibant-related effects that were noted to be nonclinical were largely related to the known bradykinin (B2) pharmacology. Hormonal and reproductive tract findings (including effects on sexual maturation in juvenile animals) were generally reversible and were less marked when icatibant was administered on an intermittent, clinically relevant dosing regimen. In considering the relationships between the plasma exposures to icatibant in nonclinical studies and those achieved in clinical use, exposures of icatibant at the lowest-observed-adverse-effect levels (LOAELs) and no-observed-adverse-effect levels (NOAELs) (see Table 2 in the investigators brochure) in the repeat-dose toxicity studies are adequate to cover maximum systemic exposures in humans. Furthermore, clinical data utilizing an exaggerated dose regimen demonstrated no clinically significant changes from baseline in reproductive hormones, sperm counts, or menstrual cycle length. This suggests that adult patients treated with icatibant are unlikely to experience adverse reactions affecting sexual organs, given the acute, intermittent nature of Sars-Cov-2 induced ARDS and potential use of icatibant.

1.2 Clinical Studies

Phase 1 to Phase 3 clinical studies have been performed to evaluate the efficacy, safety and tolerability, and PK/PD properties of icatibant. The efficacy of SC icatibant as measured by the time to onset of symptom relief in hereditary angioedema (HAE) subjects with moderate to severe acute cutaneous and/or abdominal angioedema attacks was compared to placebo or tranexamic acid in controlled Phase 3 studies (223 patients). In addition, 2 open-label, single-arm, Phase 3 studies were completed in HAE subjects; an open-label Phase 3b study to evaluate the safety of self-administration with icatibant and a Phase 3b study to evaluate the effectiveness of icatibant for acute attacks of HAE in adult Japanese subjects. Additionally, a randomized, double-blind, 2-armed, placebo-controlled Phase 3 study of SC icatibant in adult subjects with angiotensin converting enzyme inhibitor (ACE-I)-induced angioedema was conducted to assess the safety and efficacy of icatibant versus placebo in resolving attacks of angioedema caused by ACE-I use. A summary of completed clinical studies with SC icatibant for the indications of HAE and ACE-I-induced angioedema is presented in Table 3 of the investigator's brochure.

1.2.1 Efficacy

The controlled Phase 3 studies (Cicardi et al. 2010; Lumry et al. 2011) were similar in design; each had a controlled phase to assess efficacy and safety in a randomized, controlled, double-blind setting and an open-label extension phase to assess repeated treatment over time for subsequent HAE attacks. Collectively, across the 3 controlled Phase 3 studies, subjects on icatibant had a faster median time to onset of symptom relief (2.0, 2.5, and 2.0 hours, respectively) compared to tranexamic acid (12.0 hours)

and placebo (4.6 hours and 19.8 hours). The treatment effect of icatibant was confirmed by secondary efficacy endpoints. In subgroup analyses of data pooled across these 3 controlled Phase 3 studies, time to onset of symptom relief and time to onset of primary symptom relief were similar regardless of age group, sex, race, weight, or whether or not the subject used androgens or antifibrinolytic agents. A consistent treatment response was observed across repeated attacks in multiple studies, during which a total of 237 subjects were treated with 1383 doses of SC icatibant for 1278 acute attacks of HAE. Most HAE attacks treated in the controlled Phase 3 studies were managed successfully with a single SC injection of icatibant. Of the 1278 icatibant-treated attacks, 1149 were treated during the open-label extension and were, therefore, eligible for multiple (up to 3) injections of icatibant; 91.5% (1051/1149) of these attacks were treated with 1 injection. It is noteworthy that a total of 66 subjects with attacks of HAE affecting the larynx were treated with icatibant across several studies. The treatment response for subjects with laryngeal attacks was similar to that of subjects with nonlaryngeal attacks with respect to time to onset of symptom relief.

In a separate study, the efficacy of icatibant as a treatment for ACE-I induced angioedema in adults showed that there was no difference between icatibant and placebo in the treatment of ACE-I-induced angioedema.

1.2.2 Safety

In completed Phase 1 to Phase 3 registrational studies, icatibant SC was administered by a health care professional (HCP) to 189 healthy subjects and to 304 subjects with HAE and to 60 subjects with ACE-I-induced angioedema. An additional 101 subjects self-administered SC icatibant for management of HAE attacks. Icatibant has been well tolerated in all clinical studies. Nonserious reactions (erythema, swelling, burning sensation, itching, warmth, and pain) at the injection site were the most commonly reported adverse effects in clinical studies, and occurred transiently in almost all subjects after SC injection of icatibant. These reactions were self-resolving and typically mild to moderate in severity. Icatibant was generally nonimmunogenic. Over repeated treatment for multiple attacks in the 3 controlled Phase 3 studies, transient positivity in test results for the presence of anti-icatibant antibodies was observed in rare cases. No hypersensitivity or anaphylactic reactions were reported with use of icatibant in the clinical program. Self-administered icatibant was also well tolerated by HAE subjects, with a profile similar to that seen in controlled Phase 3 studies of icatibant administered by an HCP. No new safety issues were identified in association with self-administration of icatibant for acute HAE attacks.

As summarized in the non-clinical section, high repeated doses of icatibant were associated with effects on sexual organs and sexual maturation in nonclinical studies in rat and dog. These findings are consistent with the role of bradykinin action at B2 receptors in the control of reproductive hormone release. A completed Phase 1 study (HGT-FIR 062) evaluated the effect of repeated administration of SC icatibant 30 mg on reproductive parameters (serum reproductive hormone levels in males and premenopausal females, and seminal fluid analysis in males) in healthy adults. There were no clinically significant changes from baseline in basal and gonadotropin-releasing hormone (GnRH)-stimulated concentrations of reproductive hormones (testosterone, dehydroepiandrosterone [DHEA], dehydroepiandrosterone-sulfate [DHEA-S], sex hormone binding globulin [SHBG], FSH, LH, and inhibin-B in males, and estradiol, progesterone, prolactin, DHEA, DHEA-S, SHBG, FSH, and LH in females) in subjects exposed to icatibant. Additionally, there were no significant effects of icatibant on the concentration of luteal phase progesterone, an indicator of ovulation status and luteal function, or on menstrual cycle length in females, and there were no significant effects of icatibant on semen parameters in males. Likewise, no clinically significant changes in reproductive hormones were observed with icatibant treatment in pediatric subjects (> 2 years of age).

Based on Icatibant Outcome Survey (IOS) (2009-2015), safety data from 3025 icatibant-treated attacks in 557 patients demonstrated icatibant as generally well tolerated, and incidence and severity of AEs was similar to expected in HAE population. No major differences were noted in on-label vs off-label icatibant users; only 3 SAE were considered related to icatibant and no SAEs occurred in patients with cardiovascular disease using icatibant (Zanichelli et al., 2017).

1.2.3 Pharmacodynamics and Pharmacokinetics

Following bradykinin challenge, development of bradykinin-induced hypotension, vasodilation, and reflex tachycardia was prevented in healthy young subjects who received doses of 0.8 mg/kg over 4 hours, 1.5 mg/kg/day or 0.15 mg/kg/day for 3 days. Doses of 0.8 mg/kg inhibited the response to bradykinin challenge for 6 to 12 hours after initiation of infusion.

A thorough QT study was conducted to determine whether administration of the proposed single SC dose (30 mg) and the supratherapeutic SC dose (90 mg) of icatibant had the potential to cause QT interval prolongation in healthy adults. Exposure-response analysis was also conducted. No significant QTc prolongation effect of icatibant (30 and 90 mg) was detected in this study. Evaluation of ΔΔQTcI versus icatibant concentration indicated no evidence of exposure-response relationship. The supratherapeutic dose (90 mg) produced mean C_{max} values of 2.8-fold the mean C_{max} for the therapeutic dose (30 mg).

The PK of icatibant has been extensively characterized in healthy volunteers and subjects in studies using both IV and SC administration. The PK profile of icatibant in subjects with HAE is similar to that in healthy volunteers. Following SC administration of a single 30 mg dose of icatibant to healthy subjects (N = 96), a mean ± SD C_{max} of 974±280 ng/mL was observed after approximately 0.75 hours. The mean $AUC_{0-\infty}$ after a single 30 mg dose was 2165±568 ng·hr/mL, with no evidence of accumulation of icatibant following three 30 mg doses administered 6 hours apart. Following SC administration, plasma clearance was 245±58 mL/min with a mean elimination half-life of 1.4±0.4 hours and volume of distribution at steady state (V_{ss}) of 29.0±8.7 L.

Icatibant is extensively metabolized by proteolytic enzymes to inactive metabolites that are primarily excreted in the urine, with less than 10% of the dose eliminated as unchanged drug.

Icatibant is not degraded by oxidative metabolic pathways, is not an inhibitor of major CYP isoenzymes (CYP 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4), and is not an inducer of CYP 1A2 and 3A4.

Clinical PK studies demonstrate that for mild to moderate impairment of renal or hepatic function no dose adjustment is necessary. In 10 subjects with hepatorenal syndrome (GFR 30-60 mL/min), clearance of icatibant was not dependent on renal function. Icatibant clearance in subjects with a wide range of hepatic impairment (Child-Pugh score ≥7 and ≤15) was similar to that in healthy subjects.

The PK profile of icatibant in healthy adult Japanese subjects was in line with findings observed previously in adult non-Japanese subjects. However, exposure to icatibant in Japanese subjects with HAE was generally lower than that observed in Japanese healthy subjects residing in the US, non-Japanese healthy subjects, and non-Japanese patients with HAE. The lower exposure in Japanese HAE subjects did not influence the efficacy of icatibant in this population.

2. INVESTIGATIONAL STUDY AGENT ADMINISTRATION IN THE I-SPY COVID-19 TRIAL

Intervention will be administered on an inpatient basis. Reported clinical SAEs and potential risks are described in §3.2.

2.1 Dose Regimen and Dose Groups

Icatibant is administered subcutaneously in the abdominal area at a dose of 30 mg every 8 hours daily for three (3) days (total of 9 doses), or for six (6) days (total of 18 doses) pending safety assessment (see Section 2.6.3 Ten Patient Safety Reviews)

2.1.1 Icatibant Premedication Regimen and Prophylactic Medications

No specific premedication is required for icatibant routine treatment.

2.2 Eligibility

Eligibility criteria listed in the I-SPY COVID-19 TRIAL Protocol apply to all participants. In addition, the following criteria apply to those patients receiving icatibant.

Inclusion:

- Refer to master protocol; no additional criteria for icatibant.

Exclusions:

- Refer to master protocol; no additional criteria for icatibant.

2.3 Contraindications

- Known hypersensitivity reaction to Firazyr or any of its ingredients.

2.4 Concomitant Medications

At Screening, all medications taken up to 30 days prior to the screening visit will be recorded.
In addition, supportive therapies given during the course of the study should be collected and recorded.

Icatibant metabolism is not mediated by CYP450 enzymes. Therefore, metabolic drug interactions between icatibant and CYP450 substrates, inhibitors and inducers are not expected.

Icatibant is a bradykinin B2 receptor antagonist and thereby has the potential to have a pharmacodynamic interaction with ACE inhibitors where icatibant may attenuate the antihypertensive effect of ACE inhibitors.

Caution should always be exercised when administering concomitant medications based on the individual medication profile and clinical risk-benefit assessment.

2.5 Clinical Evaluation and Procedures

Laboratory evaluations for general safety monitoring are described in master protocol §8.1─ 8.3; no additional evaluations/procedures are necessary for this arm.

2.6 Dose Modifications and Management of Toxicity

Any potential dose modifications need to be discussed with the Sponsor. No additional follow-up assessments (other than ongoing safety assessment as described in master protocol) are suggested at this time.

2.6.2 Overdose

In a clinical study evaluating a 90 mg dose (30 mg in each of 3 SC sites), the AE profile was similar to that seen with 30 mg administered in a single SC site. In another clinical study, a dose of 3.2 mg/kg IV (approximately 8 times the therapeutic dose) caused transient erythema, itching, or hypotension in healthy subjects. No therapeutic intervention was necessary.

In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

An overdose of icatibant (administration not per specified regimen dose and frequency), regardless of the presence of an associated SAE, is considered an adverse event of clinical interest (ECI) and must be documented and reported. Additionally, an SAE associated with an overdose of icatibant must be documented and reported according to the requirements for SAEs.

Please refer to the I-SPY COVID-19 MOP for instructions on reporting overdose.

2.6.3 Ten-Patient Safety Reviews

Safety Review Cohort: The first 10 patients randomized to icatibant and who have begun treatment will be considered part of the First Phase Safety Review Cohort. The First Phase Safety Review window will encompass the time period from the time of randomization through study day 7 for the last participant in the First Phase Safety Review cohort, to be followed by a 1 week hold on accrual.
If unacceptable toxicities (as defined below) are observed during the First Phase Safety Review window, randomization will be paused until attribution has been determined, and review by the Safety Committee and DSMB has occurred. After 10 patients are enrolled to the icatibant arm, randomization to that arm will be paused until the 10^{th} participant has completed the First Safety Review window and a final toxicity review of all 10 patients has occurred to ensure the safety profile of the agent is acceptable.

Monitoring by the DSMB, the I-SPY 2 COVID Safety Committee, and the Study Statisticians will be continuous for adverse events and severe adverse events, as well as dose reductions, delays and discontinuations. During the pause in randomization, trends in AEs and dose delays will be compared by the DSMB and the I-SPY COVID Safety Committee to the distribution of events in the appropriate historical comparator arm, the control arm of remdesivir. Excess events will be noted for severity and impact on the risk benefit ratio for the COVID-19 patient population. Recommendation for continuation at the 3 day dosing duration, advancement to a 6 day dosing duration, or termination of randomization to the icatibant arm by the Safety Committee, and the DSMB, will be documented in a memo, which will be discussed with and approved by the company prior to sending to the study sites, their IRBs and the FDA before any action is taken.

If a recommendation is given to advance the icatibant arm to 6 day dosing, then a Second Phase Safety Review will be established in which the next 10 patients randomized to icatibant will receive the study drug at the same dose and schedule but for a duration of 6 days. The Second Phase Safety Review process will otherwise proceed in precisely the same manner as above, with the sole exception that the DSMB and

the I-SPY COVID Safety Committee will recommend to either continue at the 6 day duration regimen, return to the 3 day dosing duration, or terminate the randomization to the icatibant arm.

Participants in the Safety Review who receive whichever icatibant dosing duration is ultimately chosen (i.e., the final recommended icatibant dosing duration for subjects enrolled after the Safety Review process) will be included in the primary efficacy analysis. Any participants who receive an icatibant dosing duration other than the duration ultimately chosen will not be included in the primary efficacy analysis.

2.6.4. Definition of Unacceptable Toxicity of Icatibant (10 patient safety cohort)

The occurrence of any of the following toxicities during the first 1 week of treatment will be considered unacceptable toxicity if assessed by the investigator to be at least possibly related to study treatment. Occurrence of an unacceptable toxicity will result in a pause in randomization to the icatibant arm whilethe I-SPY COVID Safety Committee further evaluates the event and determines whether it is safe to proceed.

1. Any life-threatening event occurring during the 3 days of icatibant dosing or the 24 hours following the final dose, which is judged to be likely or probably related to icatibant (Note: the term life-threatening refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).

2. Important icatibant related medical events that may not result in death, be life-threatening, or require hospitalization, but may be considered serious when, based upon the appropriate medical judgement, they may jeopardize the participant or subject and may require medical or surgical intervention to prevent a life-threatening event as defined above.

The I-SPY COVID Safety Committee and DSMB can decide to halt accrual and randomization into the icatibant arm at any time based on toxicity observed in the 10-patient safety review.

3. INVESTIGATIONAL AGENT PHARMACEUTICAL INFORMATION

3.1 Investigational Study Agents (IND 150378, IND Sponsor: QLHC)

The icatibant drug product used for clinical trials is supplied as a sterile, isotonic, and buffered solution of icatibant acetate in a single-use, prefilled syringe for subcutaneous administration. Each mL of the solution contains 10 mg of icatibant (free base). Each prefilled syringe delivers 3 mL of solution equivalent to a 30 mg icatibant dose. The solution is clear and colorless.
The solution also contains sodium chloride, glacial acetic acid, sodium hydroxide and water for injection with a pH of approximately 5.5. The solution does not contain preservatives.

Study drug will be provided as a pack size of 1 prefilled syringe with 1 needle. All labels for icatibant will meet all applicable requirements of the US FDA and Annex 13 of Good Manufacturing Practices and/or all local regulations, as applicable.

3.2 Reported Clinical AEs and Potential Risks of Icatibant

3.2.1 Potential Risks

The only identified risk is localized injection-related reactions. In general, injection site reactions were mild or moderate in severity, and resolved within 4-6 hours of icatibant administration without the need for intervention.

3.2.2 Previous Experience with Products of Same Class

None available at this time.

3.2.1 Observed AEs in Human Trials

The most frequently reported adverse reactions (occurring in greater than 1% of patients and at a higher rate with icatibant versus placebo) were injection site reactions (97%) (e.g., bruising, hematoma, burning, erythema, hypoesthesia, irritation, numbness, edema, pain, pressure sensation, pruritus, injection site swelling, urticaria, and warmth), pyrexia (4%), dizziness (3%), and elevated transaminases (4%). Other adverse reactions include rash, nausea and headache.

3.2.2 Adverse Events of Special Interest

There are no suggested adverse events of special interest for Icatibant.

3.3 Investigational Agent Availability

Icatibant is manufactured by Shire HGT, Inc. The investigational agent product is provided as sterile solution for injection. Dose strengths of 30 mg in a 3 mL single use syringe are to be used in this trial. The investigator or designee will record the lot number, expiration date and the amount of study medication dispensed to each participant.

Icatibant is provided under a CTPA between Shire HGT, Inc. and QLHC.

3.4 Investigational Agent Distribution

Shipment of investigational agents to a participating site will not be approved until documentation of IRB approval of the sponsor-approved protocol and consent is available, and the collection of all essential documents is complete.

Investigational agents may be requested by the investigator (or their authorized designees) at each organization. Investigational agents will be shipped directly to the institution or site where the agent will be prepared and administered. The transfer of agents between institutions is not permitted (unless prior approval from the sponsor is obtained). Agents are requested by completing the Investigational Agent Request Form (to include complete shipping contact information) and submitting the form to the sponsor-designated DCC, see I-SPY COVID MOP for additional details

Once QLHC or their designee establishes that the requesting site is authorized to receive investigational agents, the order will be forwarded to the manufacturer, who will ship the investigational agent directly to the study site. Instructions for ordering investigational agents are available in the I-SPY COVID MOP.

3.5 Investigational Agent Preparation and Handling

Icatibant for injection (30 mg) will be provided by Shire HGT, Inc. No preparation is required for Icatibant for injection. Icatibant should be inspected visually for particulate matter and discoloration prior to administration. The drug solution should be clear and colorless. Do not administer if the product

contains particulates or is discolored. Treatment will be administered on an inpatient basis. Only authorized site staff may supply or administer study intervention.

3.6 Investigational Agent Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all investigational agents. The investigator is required to maintain adequate records of receipt, dispensing, and final disposition of the study agent. On the receipt, record from whom study agent was received and to whom study agent was shipped, date, quantity, and batch or lot number. On the dispensing record, note quantities and dates study agents were dispensed to and returned by each participant.

3.7 Investigational Agent Packaging and Labeling

Icatibant is packaged and labeled by Shire HGT, Inc., according to GCP, FDA, and national requirements. Labels are printed and attached to the outer carton prior to shipping to the site. Each is labeled with a single panel label that will include the name "I-SPY COVID". Additional information pre-printed on the carton includes, but is not limited to, the following:

- Blank spaces to write the study number and investigator name
- Agent identification
- Lot number
- Dosing instructions (package insert)
- Blank spaces to write the participant's identification number, initials, and date dispensed

Additional information may be attached to a label on the product carton.

3.8 Investigational Agent Storage

Icatibant syringes should be stored at 2°C to 25°C (36°F to 77°F). Do not freeze. Store in carton until time of administration.

Detailed descriptions of the storage and handling instructions for Icatibant for injection are provided in the MOP.

3.9 Investigational Agent Destruction/Disposal

Once agent accountability is performed, the participating sites should use local/institutional procedures for disposal of returned/unused study agent and bottles/containers. Copies of all certificates of destruction of any unused study agent must be provided to DCC. **Prior to destruction,** the pharmacist should contact the assigned study monitor.

Unused investigational agents shall be returned to the designated facility. Please contact DCC for instructions.

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Appendix F

Razuprotafib (AKB-9778)

I-SPY-COVID-19 Investigational Agent Information

INVESTIGATIONAL AGENT INFORMATION SUMMARY

Agent Co-Chaperone:	Carolyn Calfee, UCSF
Agent Co-Chaperone:	Nuala Meyer, Penn
Agent Co-Chaperone:	Michael A. Matthay, UCSF

General Information:

Agent Class:	First in class activator of Tie-2, which maintains the stability of the quiescent, adult vasculature, via inhibition of vascular endothelial-protein tyrosine phosphatase (VE-PTP)
Structural Class:	Small molecule (MW 586.70 as Free Acid Form)
Manufacturer:	Aerpio
Phase of Development:	Completed three phase 2 studies as a subcutaneous systemic treatment for multiple indications relating to diabetic retinal disease (IND 113322), including diabetic macular edema (DME), non-proliferative diabetic retinopathy (NPDR), and retinal vein occlusion (RVO).

Pharmaceutical Information:

Dosage Form:	Ready to dose sterile solution for subcutaneous injection.
Physical Description:	Razuprotafib (AKB-9778) Sterile Solution is supplied as a clear, colorless to slightly yellow isotonic, sterile, unpreserved solution. Razuprotafib ready to dose solution (20 mg/mL or 40 mg/mL) is provided in a 3 mL borosilicate serum vial fitted with a 13mm Teflon septum and 13mm red flip top seal. A dose volume of 0.5 mL of 20 mg/mL solution will deliver a dose of 10 mg. and a dose volume of 0.5 mL of 40 mg/mL solution will deliver a dose of 20 mg.
Strengths to be used in trial:	10 mg and 20 mg.
Packaging Unit	Individual vials are provided to sites in a cardboard tray, with an inserted divider, that can hold up to 49 vials. Each tray is contained within a shipper that can hold up to 4 nested trays. The package will include a temperature logger. The shipment will be according to current Good Manufacturing Practice. Upon receipt the vials are removed from the trays for storage in a secure (locked) and temperature monitored drug storage room.
Storage Conditions:	Store between 15°C to 25°C (59°F to 77°F), with transient excursions of up to 30°C (86°F) permitted for up to 24 hours.

Administration Information:

Route:	Subcutaneous (SC) injection in the 4 quadrants of the abdominal area is preferred.
Standard Regimen:	20 mg q8h for 7 days once the safety run-in confirms tolerability.
Agent Preparation:	Aseptic filling of syringes to desired dose is required. See Section 3.5
Pre-medication:	Specific pre-medication is not required for routine treatment. See Section 2.1.1
Administration:	Treatment will be administered to subjects while hospitalized as inpatients. See Section 2.1 for further details.

Concomitant Medications: Following precautions and warning should be given:

Caution should be used with drugs that are substrates of CYP2C8 as razuprotafib may increase blood levels of these drugs.

Refer to §2.6 for side-effect management and dose reduction plans.

The above is intended as a summary only; please see the complete appendix for additional investigational agent information

1. RATIONALE FOR TESTING

Aerpio Pharmaceuticals, Inc. (Aerpio) has developed razuprotafib (AKB-9778), a unique small molecule approach to the prevention and treatment of COVID-19 related acute respiratory distress syndrome (ARDS), as well as ARDS related to other conditions, including sepsis, pneumonia, aspiration of orogastric contents, trauma, and burns. Other causes may also include pancreatitis, smoke inhalation, circulatory shock in the absence of sepsis, transfusion of blood products, cardiothoracic surgery, and drug toxicity.

Razuprotafib is a novel Tyrosine kinase with Immunoglobulin-like and Epidermal growth factor-like domains 2 (Tie2) activator with potential to stabilize the pulmonary vasculature preventing the progression of COVID-19 associated pulmonary pathology, decreasing the need for ventilator support, and reducing mortality. Tie2 is the receptor for the Angiopoietin (Angpt) family of secreted proteins and is expressed on vascular endothelial cells, including pulmonary endothelial cells. Its activation is required for maintaining the stability of the quiescent, adult vasculature (Saharinen *et al*., 2017; Peters *et al*., 2004). Over the past decade, vascular destabilization due to reduced Tie2 activation has been implicated in the pathophysiology of a variety of acute and chronic conditions characterized by endothelial dysfunction, vascular injury, and inflammation, including ARDS (Saharinen *et al*., 2017; Sack *et al*., 2020; Parikh *et al*., 2017; Higgins *et al*., 2018; Leligdowicz *et al*., 2018).

Razuprotafib activates Tie-2 via inhibition of vascular endothelial-protein tyrosine phosphatase (VE-PTP) which is a receptor tyrosine phosphatase that is also expressed on vascular endothelial cells and is the most downstream negative regulator of Tie2 activation (Shen *et al*., 2014; Souma *et al*., 2018). Importantly, in conditions associated with chronic endothelial dysfunction and vascular injury such as diabetes and hypertension, VE-PTP expression is increased and Tie2 activation is decreased possibly explaining the predisposition of patients with these conditions for increased severity of COVID-19 (Carota *et al*., 2019; Huang *et al*., 2020; Driggin *et al*., 2020; Clerkin *et al*., 2020). Moreover, VE-PTP expression is increased and Tie2 activation is decreased by hypoxia, perhaps contributing to the rapid deterioration and multiorgan failure that occurs in COVID-19 patients with severe respiratory failure (Shen *et al*., 2014; Carota et al., 2019). Notably, angiotensin converting enzyme 2 (ACE2), a functional receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, is expressed in pulmonary endothelium indicating that the pulmonary vasculature is a direct target in the development of COVID-19 pulmonary pathology (Hoffmann *et al*., 2020; Kuba et al., 2005; Shenoy *et al*., 2011). Thus, restoring Tie2 activation in the pulmonary vasculature represents a promising host-directed approach to treating COVID-19 associated pulmonary pathology.

Among all organs in the body, the lung possesses the highest fraction of endothelial cells and largest cross-sectional area of vasculature, thus making it a prime site for therapeutic Tie2 modulation. The Tie2 receptor is largely endothelial-specific, essential for vascular maturation, and may play a role in the defense of microvascular breach in ARDS; therefore, as a Tie2 activator, razuprotafib is a promising candidate for the treatment of COVID-19 related ARDS

1.1 Non-Clinical Studies

Please refer to the Investigator's Brochure for details on the nonclinical safety program conducted in support of Razuprotafib Sterile Solution for Subcutaneous Injection.

The nonclinical safety program conducted with razuprotafib included safety pharmacology studies; pharmacokinetic (PK), metabolism, and *in vitro* drug-drug interaction studies; toxicology studies in rats, dogs, and monkeys; embryo-fetal development studies in rats and rabbits, fertility and early development

evaluation in rats, and genotoxicity and phototoxicity studies. While razuprotafib was developed as an inhibitor of the human form of the tyrosine phosphatase VE-PTP, observations in the rodent (mouse and rat) pharmacology studies and the high conservation across species of the razuprotafib binding site on VE-PTP (from molecular modeling studies based on crystal structures of razuprotafib with VE-PTP) demonstrate that razuprotafib is also a potent inhibitor of the murine, rat, dog, and monkey orthologues of the phosphatase.

Razuprotafib is a potent and selective small molecule inhibitor of the catalytic domain of VE-PTP. In enzyme assays, razuprotafib showed sub-nanomolar potency (50% inhibitory concentration [IC50] = 0.017 nM) with reversible, competitive inhibition kinetics. In a broad phosphatase selectivity panel, razuprotafib showed excellent selectivity for VE-PTP *versus* serine/threonine phosphatases, dual specificity phosphatases, and multiple other canonical protein tyrosine phosphatases (5- to ≥5000-fold selectivity).

Razuprotafib has excellent SC bioavailability in all nonclinical species tested. Plasma concentrations of AKB-9778 (razuprotafib) increase rapidly, with a time to achieve maximal plasma concentration (Tmax) of approximately 0.5 hours post-dose, and then rapidly decline with elimination half-life (T1/2) of approximately 1 hour post-dose following SC administration. Razuprotafib demonstrates linear, dose-dependent increases in exposure in mice, rats, dogs, and monkeys. There are generally minimal sex differences in exposure and no apparent accumulation upon repeat dosing.

Razuprotafib is highly bound to plasma proteins in mouse, rat, dog, and human plasma (97.4% to 98.7%). Razuprotafib does not bind to melanin. Radiolabel mass balance studies in rats, dogs, and monkeys demonstrated that razuprotafib (as total radioactivity) was predominantly eliminated in the feces (84% to >94%) and less so in the urine (<5% to 11%) following a single SC administration. The excretion profile in monkeys was similar to that for razuprotafib administered SC to human. A radiolabel quantitative whole-body autoradiography (QWBA) study in pigmented rats demonstrated rapid tissue distribution and elimination.

In vitro cytochrome P450 (CYP) reaction phenotyping in human microsomes demonstrated razuprotafib to be predominantly metabolized by CYP2C8. Incubation of [14C]-AKB-9778 in hepatocytes for multiple species suggested the presence of up to nine potential metabolites in rat, dog, and monkey, and up to five potential metabolites in human; however, [14C]-AKB-9778 was the primary component (by total radioactive peak area) detected for all species. Similarly, the predominant [14C]-AKB-9778 component detected in plasma following SC administration was AKB-9778 (razuprotafib).

In *in vitro* metabolic enzyme and transporter interaction studies, potentially clinically relevant razuprotafib interactions included metabolism-dependent inhibition of CYP2C8 and as a substrate of organic anion transporting polypeptide 1B1 (OATP1B1) and OATP1B3 transporters. Razuprotafib did not have notable interaction with other key CYP enzymes and efflux and influx transporters.

The *in vitro* safety pharmacology assessments demonstrated very limited interactions with razuprotafib. *In vivo* data from conscious beagle dogs indicated lack of potential for QT prolongation following SC administration and at high razuprotafib plasma exposures (68 µM total; 1.7 µM free). Razuprotafib did not have effects on the respiratory function and neurobehavioral functional observational battery evaluations in conscious rats following SC administration. Additionally, *in vivo* safety pharmacology assessments demonstrated a consistent finding in both rat and dog of a non-adverse decrease in blood pressure with a compensatory increase in heart rate. Changes in blood pressure in both species were transient (4 to 6 hours), correlated with the drug plasma profile, and appeared to plateau with increasing dose, consistent with a pharmacological effect. The generation of NO via eNOS activation downstream of Tie2 activation supports a pharmacologic basis for vasodilation and decreased blood pressure.

In the *in vivo* GLP nonclinical general, genetic, and reproductive toxicity studies, razuprotafib formulated as a solution in 10% hydroxypropyl-beta-cyclodextrin (HPβCD) demonstrated an excellent safety profile and was well tolerated by SC administration for up to 6 months in rats and up to 9 months in dogs and monkeys. The primary target tissues for razuprotafib were identified as injection site and liver in rat, dog and monkey; skin/subcutis (vascular) in dog and monkey; and gastrointestinal (GI; vascular) in dog.

In rat, liver findings observed in all pivotal rat toxicity studies included elevations in aspartate transaminase (AST) and alanine transaminase (ALT) that correlated with microscopic observations of individual, randomly-scattered, minimally necrotic hepatocytes, that were demonstrated to be reversible at the end of a 28-day recovery period. The NOAEL was 90 mg/kg/day in the rat 28-day toxicity study. Similar liver findings were not observed in dog or monkey.

In the 9-month dog study, non-adverse, reversible, broadly distributed dermal-localized nodules were observed on all dogs at 15 mg/kg/day and at the high dose NOAEL of 30 mg/kg/day, and one nodule was observed on one male dog at 6 mg/kg/day. The majority of nodules were identified microscopically as focal angiectasis that were observed to rapidly resolve upon drug withdrawal. In addition, largely GI tract-localized, non-adverse, minimal to mild angiectasis was observed by microscopic examination at all dose levels but not in the vehicle control group. The vascular findings were not associated with any clinical observations, serum chemistry, or other microscopic changes. In the rat 6-month study, minimal focal angiectasis was observed in the uterus with cervix of only one high-dose rat; and in the 9-month monkey study, a solitary dermal nodule was observed on the tail of one high dose animal and characterized as angiectasis with resolving thrombosis. These observations suggest an increased sensitivity of the dog to vascular effects of razuprotafib.

No adverse systemic findings in monkey were observed up to the high dose of razuprotafib in the 28-day (45 mg/kg/day) and 6-month (22.5 mg/kg/day) studies.

No liver findings or unusual or unexpected dermal findings have been observed in clinical studies with razuprotafib. For guidance on assessment and monitoring of potential liver findings or vascular effects, refer to Section 6.0 in the investigators brochure, Guidance for Investigators.

Razuprotafib is not considered to be mutagenic or clastogenic based on the negative findings in the pivotal battery of genotoxicity studies.

Definitive embryo-fetal development toxicity studies in rat and rabbit showed no evidence of external fetal teratogenic effects or of skeletal or visceral malformations. In a definitive fertility and early development study in male and female rats, there were no effects on fertility and reproductive outcome, mating, estrous cycles, and male reproductive parameters at any dose. A decreased number of corpora lutea was observed in females at the high dose of 90 mg/kg/day. Therefore, the fertility study NOAEL was 90 mg/kg/day in male rats and 30 mg/kg/day in females.

1.2 Clinical Studies

Please refer to the Investigator's Brochure for details on the clinical program conducted in support of Razuprotafib Sterile Solution for Subcutaneous Injection. To date, Razuprotafib (AKB-9778) for Subcutaneous Injection has been evaluated in six completed phase 1 and 2 studies which are summarized in Table 1-1 of the investigator's brochure

1.2.1 Efficacy

The efficacy of razuprotafib in humans has not been tested in clinical trials for the prevention and treatment of COVID-19 related ARDS. As an indicator of target engagement consistent with eNOS activation downstream of Tie2, there was a dose dependent reduction in blood pressure in study AKB-9778-CI-2002 which corresponded with the plasma concentration profile. In TIME2 (AKB-9778-CI-2003) study, the bulk of the reduction in blood pressure occurred in patients with baseline systolic pressures greater than 140 mmHg versus very little change in patients with baseline systolic pressures less than 140 mmHg consistent with enhanced endothelial function in these patients.

Other end-organ readouts of vascular stability in the context of diabetes are also improved by razuprotafib. In TIME2 (AKB-9778-CI-2003) in patients with DME, SC razuprotafib 15 mg BID combined with monthly intravitreal injections of the anti-VEGF compound Lucentis resulted in a highly statistically significant reduction in retinal thickness compared to either agent alone. This was the first demonstration of a clinical benefit in a randomized, placebo-controlled trial of any therapy in combination with anti-VEGF in diabetic macular edema (Campochiaro *et al*., 2016). Furthermore, among patients with evidence of diabetic nephropathy (urine albumin/creatinine ratio [UACR] ≥30 mg/g), razuprotafib reduced UACR by approximately 20% compared to an increase in UACR among patients treated with Lucentis alone, indicative of a potential beneficial effect on renal function. In a subsequent Phase 2 study (AKB-9778-CI-5001, TIME2b) in patients with NPDR, SC razuprotafib 15 mg BID for 48 weeks in patients with significant albuminuria (UACR > 30 mg/g) again reduced UACR by about 20% compared to an increase in patients receiving placebo. Thus, the evidence of target engagement indicated by the blood pressure effect and evidence of efficacy suggested by the beneficial effects in the diabetic retina.

1.2.2 Safety

Razuprotafib was shown to be safe and well tolerated in clinical studies. To date, razuprotafib has been evaluated in six completed early phase clinical studies and a total of 351 subjects have received SC razuprotafib, with over 100 subjects receiving razuprotafib treatment for up to 12 months. Some adverse events observed in clinical studies of razuprotafib are most likely due to exaggerated vasodilatory responses to the pharmacologic action of razuprotafib. A transient reduction in blood pressure, with a compensatory increase in heart rate, has been measured following SC dosing with razuprotafib. The mean drop in systolic blood pressure in the TIME2 study was 10 mm Hg at 30 minutes, which had recovered to 5 mm Hg below baseline by 90 minutes, for a 15 mg dose. Reductions in systolic blood pressure were primarily observed when patients were sitting or standing and were attenuated by keeping the patient in the supine position. Adverse events possibly related to the vasodilatory effect of razuprotafib include:

- Hypotension
- Orthostatic hypotension; and
- Dizziness

In general, events associated with the vasodilatory action of razuprotafib were mild, transient, occurred within the first hour after dosing, and generally resolved within several hours post-dose. Adverse events generally did not require adjustment to study medication or have subsequent associated sequelae. Other commonly observed adverse events in studies with razuprotafib include fatigue, headache, nausea, vomiting and injection site findings.

Among the 351 subjects that have received SC razuprotafib and 127 subjects that have received SC placebo, a single death has been reported in a subject on placebo in AKB-9778-CI-5001. The subject died from a myocardial infarction that was judged to be unrelated to treatment.

All serious adverse reactions to date have been considered unrelated to treatment by the Sponsor and no

serious adverse reactions were reported. The frequency and other characteristics of SAEs and events leading to discontinuations are generally consistent with those anticipated in the population evaluated in these studies.

In study AKB-9778-CI-2002, 1/7 subjects in the razuprotafib 22.5 mg BID dose group and 1/7 subjects in the razuprotafib 30 mg BID dose group withdrew after the first dose of study medication due to a syncopal and pre-syncopal event, respectively. Both subjects recovered within 30 minutes with no treatment other than oxygen and oral fluid intake.

No unusual dermal findings have been observed in human clinical studies with the razuprotafib HPβCD formulation.

Overall, razuprotafib was shown to be safe and well tolerated, with mild or moderate adverse events most likely due to exaggerated vasodilatory responses to the pharmacologic action of razuprotafib. Other adverse events are generally consistent with those anticipated in the population evaluated in these studies.

1.2.3 Pharmacokinetics and Pharmacodynamics

Razuprotafib administered by SC injection is highly bioavailable with a predictable, dose-related PK profile. The PK profile exhibited a rapid increase in plasma concentration after SC dose administration, with Tmax values achieved within 15 minutes. A dose-related increase in plasma concentrations has been observed across the dose range of 5 to 30 mg BID (as well as 5 to 80 mg as a single dose). Plasma concentrations declined rapidly with an elimination half-life ($T_{1/2}$) of approximately 1 hour; and the Day 1 and Day 14 concentration profiles were similar suggesting no significant accumulation is expected when administered on a BID or TID regimen.
The PK profile was similar in healthy adults and in subjects with diabetic macular edema, supporting the use of PK data in the latter to estimate safety margins for the proposed dosing regimen of 10 mg or 20 mg TID in the planned clinical study in subjects with moderate to severe COVID-19 requiring supplemental oxygen.

As an indicator of target engagement, there was a dose-dependent reduction in blood pressure in AKB-9778-CI-2002, which corresponded with the plasma concentration profile consistent with eNOS activation downstream of Tie2. The modest blood pressure reduction at the 15 mg dose in this study was reproduced in AKB-9778-CI-2003 and AKB-9778-CI-5001. The magnitude of blood pressure reduction with razuprotafib was dependent on baseline blood pressure in the three studies. In TIME2, the bulk of the reduction in blood pressure occurred in patients with baseline systolic pressures greater than 140 mmHg versus very little change in patients with baseline systolic pressures less than 140 mmHg consistent with enhanced endothelial function in these patients.

In patients with evidence of diabetic nephropathy in AKB-9778-CI-2003 (UACR ≥ 30 mg/g), razuprotafib reduced UACR by about 20% compared to an increase in patients treated with ranibizumab alone, indicative of a potential beneficial effect on renal function. As a marker of kidney glomerular hyperpermeability, the reduction in UACR demonstrates that razuprotafib reduces urinary protein leakage in diabetic patients. In the subsequent Phase 2 study (AKB-9778-CI-5001) in patients with non-proliferative diabetic retinopathy (NPDR), SC razuprotafib 15 mg QD or BID for 48 weeks failed to significantly improve the diabetic retinopathy severity score. However, in patients with significant albuminuria (UACR>30 mg/g), razuprotafib BID reduced UACR by about 20% compared to an increase in patients receiving placebo. Thus, the evidence of target engagement indicated by the blood pressure effect and evidence of efficacy suggested by the beneficial effects in the diabetic retina and kidney support the razuprotafib 10 mg or 20 mg TID dose in the proposed study of severe or critically ill COVID-19 patients.

2. INVESTIGATIONAL STUDY AGENT ADMINISTRATION IN THE I-SPY COVID-19 TRIAL

Intervention will be administered on an inpatient basis. Reported clinical SAEs and potential risks are described in §3.2.

2.1 Dose Regimen and Dose Groups

2.1.1 Rationale for Dose Regimen

Razuprotafib for subcutaneous (SC) injection has been evaluated in 3 sequential clinical trials in patients with diabetes in which razuprotafib, dosed up to 30 mg BID for 28 days and up to 15 mg BID for 3-12 months, was demonstrated to be well tolerated with evidence of target engagement and efficacy. As an indicator of target engagement, a dose-dependent reduction in blood pressure at 15 mg was observed and corresponded with the plasma concentration profile, consistent with eNOS activation downstream of Tie2 activation. Thus, the evidence of target engagement indicated by the blood pressure effect and evidence of efficacy suggested by the beneficial effects in the diabetic retina (reduced retinal edema in combination with Lucentis) and kidney (decreased UACR) support the razuprotafib 10 and 20 mg dose in the proposed study.

Razuprotafib administered by SC injection is highly bioavailable with a predictable, dose-related PK profile that includes a rapid increase in plasma concentration (T_{max} achieved within 15 minutes) that rapidly declines ($T_{1/2}$ ~1 hour). At doses of up to 30 mg BID, PK profiles on Day 1 and Day 14 were similar suggesting no significant accumulation is expected when administered at 10 or 20 mg q8h.

As opposed to BID administration in the outpatient setting, administering study medication on a q8h frequency in the hospital setting is feasible and provides additional safety mitigation by dividing the total daily dose into smaller individual doses. In particular, dividing the total daily dose in the q8h regimen would mitigate C_{max}-related blood pressure effects. In addition, the doses of 10 and 20 mg q8h do not exceed the highest daily dose that was evaluated in previous clinical trials in diabetic patients.

Although safety and efficacy of razuprotafib has been well characterized in patients with diabetic eye disease and associated comorbidities, the use of razuprotafib in COVID-19 patients is novel. Therefore, the study includes a safety run-in dose escalation (see section 2.1.2 below).

2.1.2 Initial 10 Subject Safety Run-in

To confirm safety with respect to the potential for hypotension with this agent, the first 10 subjects will undergo a 10 mg dose q8h for 24h, proceeding to 20 mg q8h for the remaining 6 days. To ensure safety and dose tolerability, specific rules are in place so that for each subject, several possible dosing regimens may apply, as detailed below. Subjects will be maintained in a supine position or the head of bed no higher than 30 degrees, for dose administration and one hour afterwards. Vital signs including blood pressure will be recorded q5 minutes x 15 minutes, then q15 minutes for an hour for the first dose, and every 30, 60, and 90 minutes following subsequent doses. A study physician will be present at bedside for each subject's first dose and one hour following.

1. Anticipated progression: 10 mg q8h for 24h, then 20 mg q8h for 6 days:

    - For subjects who are not in shock (no vasopressor use), the patient will be dosed 10 mg subcutaneously and monitored as described above. If clinically significant hypotension

occurs, then treatment will be at the discretion of the treating clinician. We will record administration of iv fluid boluses and vasoactive agents (vasopressors). If vasopressors are initiated in the two hours following study dosing then no further doses of Razuprotafib will be given to the patient. If the patient requires one or two fluid boluses (500 ml each), then the patient would be given 10 mg of Razuprotafib 24 hours later. If a fluid bolus is needed again after the second dose, then the study drug will be discontinued.

- For subjects who are in shock, they can receive 10 mg of Razuprotafib if they require only two vasopressors and the dose of levophed is less than 15 mcg/kg. If clinically significant hypotension occurs, then treatment will be at the discretion of the treating clinician. We will record administration of iv fluid boluses and vasoactive agents (vasopressors). If the dose of levophed increases more than 5 mcg/kg or an additional vasopressor is needed, then the study drug will be discontinued.

Following the first 10 subjects completing their 7 day regimen in one of the scenarios above, safety data will be reviewed by the DSM.

2.1.3 Razuprotafib Premedication Regimen and Prophylactic Medications

No specific premedication is required for razuprotafib routine treatment. Patients will be positioned supine (head of bed 30 degrees, per ICU routine) and blood pressure will be monitored q5 minutes x 15 minutes, then q15 minutes for an hour for the first dose. For subsequent doses, blood pressure will be monitored at 30, 60, and 90 minutes post-dose.

2.2 Eligibility

Eligibility criteria listed in the I-SPY COVID-19 TRIAL Protocol apply to all participants. In addition, the following criteria apply to those patients receiving razuprotafib.

Inclusion:

No additional eligibility requirements relative to the master iSPY COVID protocol

Exclusion:

None.

2.3 Contraindications

- None known at this time.

2.4 Concomitant Medications

At Screening, all medications taken up to 30 days prior to the screening visit will be recorded.
In addition, supportive therapies given during the course of the study will be collected and recorded.

- In *in vitro* DDI studies, razuprotafib was not a substrate for or inhibitor of substrate transport across common efflux and influx transporters. Razuprotafib was an irreversible, metabolism-dependent inhibitor of CYP2C8 (*R2* ≥1.25). However, in *in vivo* animal and human metabolism studies, razuprotafib was not extensively metabolized (*i.e*. no circulating metabolites were detected).

- *In vivo* clinical interaction studies have not been conducted; however, there has been no evidence of any unusual trend regarding adjustment of any medications in the clinical trials conducted to date.
- Concomitant administration of gemfibrozil, a potent CYP2C8 inhibitor, is prohibited.
- Caution should be used with drugs that are substrates of CYP2C8 as razuprotafib may increase blood levels of these drugs. Examples of these drugs are: amodiaquine, cerivastatin, dasabuvir, enzalutamide, imatinib, loperamide, montelukast, paclitaxel, pioglitazone, repaglinide, and rosiglitazone. Most of these drugs are not routinely used in the inpatient setting.

Caution should always be exercised when administering concomitant medications based on the individual medication profile and clinical risk-benefit assessment.

2.5 Clinical Evaluation and Procedures

Laboratory evaluations for general safety monitoring are described in master protocol §8.1─ 8.3; additional evaluations/procedures necessary for this arm include:

- At selected sites (TBD), PK samples will be collected on Day 3 of dosing at 30 and 90 minutes after the first daily dose
- Clinical chemistry including AST/ALT and total bilirubin on days 1, 3, 5 and 7 of dosing
- Change from baseline in high sensitivity CRP and D-dimer
- Change from baseline in systemic biomarkers of vascular leakage and inflammation (Angpt-1, Angpt-2, IL-6, IL-8, and sTNFR1). Assessments are optional as exploratory endpoints

2.6 Dose Modifications and Management of Toxicity

Any potential dose modifications need to be discussed with the Sponsor.

Dose delays and reductions may be implemented as necessary. Subjects will be withdrawn from the study if persistent or unacceptable treatment-related toxicity is observed.

For newly enrolled patients with hypotension requiring norepinephrine > 15 mcg/min (or equivalent) to maintain mean arterial blood pressure ≥ 60 mm Hg, irrespective of vasopressin dose they can receive 10 mg of Razuprotafib. If clinically significant hypotension occurs, then treatment will be at the discretion of the treating clinician. We will record administration of iv fluid boluses and vasoactive agents (vasopressors). If the dose of levophed increases more than 5 mcg/kg or an additional vasopressor is needed, then the study drug will be discontinued.

2.6.1 Liver Safety: Liver function tests will be monitored regularly during the dosing period. If LFTs indicate development of acute hepatic injury i.e. AST/ALT >5X ULN and bilirubin of >3X ULN, razuprotafib dosing will be discontinued. In clinical trials to date, there is no evidence of razuprotafib induced hepatotoxicity (for additional information see IB).

Dose reduce to 10 mg dose for Child-Pugh > 7 and resume 20 mg when Child-Pugh returns < 7, stop if Child-Pugh exceeds 10.

2.6.2 Dialysis: If hemodialysis is required, dosing on dialysis days will be held (dose prior to and two doses following dialysis). Dosing q8h will resume the day following dialysis. CRRT will be allowed at the discretion of the investigator.

2.6.3 Overdose

There is no known antidote for razuprotafib. In cases of suspected overdose, subjects should be treated per standard medical practice based on the Investigator's judgment.

An overdose of razuprotafib, regardless of the presence of an associated SAE, is considered an ECI and must be documented and reported.

Additionally, an SAE associated with an overdose of razuprotafib must be documented and reported according to the requirements for SAEs.

Please refer to the I-SPY COVID-19 MOP for instructions on reporting overdose.

3. INVESTIGATIONAL AGENT PHARMACEUTICAL INFORMATION

3.1 Investigational Study Agents (IND 150378, IND Sponsor: QLHC)

The razuprotafib drug product used for clinical trials is supplied as a sterile, isotonic, unpreserved solution, essentially free of particulates of razuprotafib (AKB-9778) sodium salt. Razuprotafib sterile solution (20 mg/mL or 40 mg/mL) is provided in a 3 mL borosilicate serum vial fitted with a 13mm Teflon septum and 13 mm red flip top aluminum seal. A dose volume of 0.50 mL of 20 mg/mL solution will deliver a dose of 10 mg. and a dose volume of 0.50 mL of 40 mg/mL solution will deliver a dose of 20 mg. The formulation also contains 15% (150 mg/mL) HPβCD (Betadex), used as a solubilizer, and 1.2% (12 mg/mL) mannitol, for tonicity modification. The formulation is controlled to pH 4.0 to 8.6.

All labels for razuprotafib solution for injection will meet all applicable requirements of Title 21 Code of Federal Regulations of the US FDA and the European Union Directive Annex 13 of Good Manufacturing Practices and/or all local regulations and guidance documents, as applicable.

3.2 Reported Clinical AEs and Potential Risks of razuprotafib

3.2.1 Potential Risks

Based on the nonclinical and clinical data available for razuprotafib, potential effects that could be encountered in subjects given razuprotafib *via* SC administration based on planned population and duration of treatment are hemodynamic effects, injection site reactions, vascular effects, and elevated liver function tests (See Section 6.1 of the investigators brochure for additional details)

3.2.2 Previous Experience with Products of Same Class

None available at this time.

3.2.1 Observed AE's in Human Trials

Please refer to the Investigator's Brochure, section 5.5 for details by individual clinical study of the observed adverse events conducted in support of Razuprotafib Sterile Solution for Subcutaneous Injection.

Adverse events possibly related to the vasodilatory effect of razuprotafib include hypotension, orthostatic hypotension and dizziness. In general, events associated with the vasodilatory action of razuprotafib were mild, transient, occurred within the first hour after dosing, and generally resolved within several hours post-dose. Adverse events generally did not require adjustment to study medication or have subsequent associated sequelae. In addition to the vasodilatory effect of razuprotafib, other AEs were typical of events observed for the patient population; i.e., patients with diabetes mellitus and/or diabetic macular edema. The most common treatment-related adverse events in subjects treated with razuprotafib were, nausea, headache, and injection site bruising.

All SAEs to date have been considered unrelated to razuprotafib treatment and no serious adverse reactions (SARs) have been reported.

3.3 Investigational Agent Availability

Razuprotafib drug substance and drug products are manufactured by Contract Manufacturing Sites on behalf of Aerpio Pharmaceuticals under executed Quality Agreements and associated Master Service Agreements and contracts. The investigational drug product is provided as sterile solution ready for injection. The investigator or designee will record the vial number, and the amount of study medication dispensed to each participant on a study drug log. The product lot number and expiration date is maintained by Aerpio, and is provided upon request.

Razuprotafib is provided under a CTPA between Aerpio Pharmaceuticals and QLHC.

3.4 Investigational Agent Distribution

Shipment of investigational agents to a participating site will not be approved until documentation of IRB approval of the sponsor-approved protocol and informed consent form is available, and the collection of all required essential documents as per ICH Good Clinical Practice Guidelines E6(R2) is complete.

Investigational agents may be requested by the investigator (or their authorized designees) at each organization. Investigational agents will be shipped directly to the institution or site, by Aerpio (or their authorized designee), where the agent will be prepared and administered. The transfer of agents between institutions is not permitted (unless prior approval from the sponsor is obtained). Agents are requested by completing the Investigational Agent Request Form (to include complete shipping contact information) and submitting the form to the sponsor-designated DCC, see I-SPY COVID MOP for additional details. Once provided to the site, the investigational product is under the control and of the investigator as required by ICH GCP and the FDA form 1572 which they signed.

Once QLHC or their designee establishes that the requesting site is authorized to receive investigational agents, the order will be forwarded to the drug distribution depot (as designated by Aerpio), who will ship the investigational agent directly to the study site. Instructions for ordering investigational agents are available in the I-SPY COVID MOP.

3.5 Investigational Agent Preparation and Handling

Razuprotafib 20 mg/mL (2.0% as the free acid) solutions will be provided by Aerpio (or their designee). Razuprotafib solutions should be inspected visually for particulate matter and discoloration prior to administration. The drug solution should be a clear colorless to slightly yellow solution, essentially free of particulates. Do not administer if the product contains particulates or is discolored. Notify Aerpio if a vial is not administered.

Syringes for subcutaneous injection are prepared using aseptic technique prior to administration and stored for not more than 24 hours. A 1.0 ml BD type plastic sterile syringe fitted with a 27 ga needle (or similar) is used for dose preparation and administration. Each vial contains sufficient volume to prepare three syringes of 0.5 mL. The three syringes should be prepared at the same time, with 3 consecutive insults to the septum, using 3 individual sterile syringes. The remaining contents of the vial not administered. Prepared drug product syringes can be stored at room temperature, 15ºC to 25ºC (59ºF to 77ºF), with excursions of up to 30ºC (86ºF) permitted for not more than 24 hours, pending administration. The used vial can be retained until study completion for proper drug accountability.

Treatment will be administered on an inpatient basis. Only authorized site staff may supply or administer study intervention. Following administration of each prepared syringe, the syringe is discarded into an approved biohazard sharps container. Any syringes that have exceeded 24 hours in syringe, are also discarded into an approved biohazard sharps container.

3.6 Investigational Agent Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all investigational agents. The investigator is required to maintain adequate records of receipt, dispensing, and final disposition of the study agent. On the receipt, the data from the logger enclosed with the shipment is downloaded from the device and stored with the receipt records. Additionally, record who received the study agent, date, and quantity. On the dispensing record, note vial and date the study agents were prepared and dispensed to each participant. The volume remaining in the vial, after preparation of 3 syringes of 0.75 mL as described above, is retained for final accountability but not administered.

3.7 Investigational Agent Packaging and Labeling

Razuprotafib (AKB-9778) is packaged and labeled by Aerpio, according to ICH GCP, FDA, and local requirements. Labels are printed and attached to the study agent vial or other packaging container prior to shipping to the site. Each is labeled with a single panel label that will include, but is not limited to, the following information:

- Protocol ID
- IND caution statement
- Agent identification
- Storage conditions
- Dosing instructions
- Sponsor Name (Aerpio Pharmaceuticals)
- Blank spaces to write the participant's identification number, initials, and date dispensed
- Caution statement indicating that the agent is for clinical trial use only

Each label must remain affixed to the vial. Additional information may be attached to a label on the exterior product carton.

Each individual prepared syringe barrel may be labeled as required by local and or site requirements, with a locally prepared label.

3.8 Investigational Agent Storage

Razuprotafib vials should be stored at 15°C to 25°C (59°F to 77°F), with transient excursions of up to 30°C 86ºF permitted. Store the vials in the original carton, or they may be removed and stored on a shelf or in a vial rack available at the site per standard process, until time of administration.

Detailed descriptions of the storage and handling instructions for razuprotafib (AKB-9778) sterile solution for injection are provided in the MOP.

3.9 Investigational Agent Destruction/Disposal

Once agent accountability is performed of the remaining used and unused vials, the participating sites should use local/institutional procedures for disposal of used/unused study agent and vials. Prepared syringes are discarded point of use, into an approved biohazard sharps container at the site. Copies of all certificates of destruction of any unused study agent must be provided to DCC. **Prior to destruction,** the pharmacist should contact the assigned study monitor, and confirm the final drug accountability has been performed and documented.

Unused investigational agents shall be returned to the designated facility, or destroyed locally as directed by Aerpio. Please contact DCC for instructions.

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Appendix G
Apremilast (Otezla)

I-SPY-COVID-19 Investigational Agent Information

INVESTIGATIONAL AGENT INFORMATION SUMMARY

Agent Chaperone:	Karl W. Thomas, MD, kwthomas@wakehealth.edu
Agent Co-Chaperone:	Paul Berger MD, paul.berger@sanfordhealth.org

General Information:

Agent Class: Selective immunosuppressant; an oral phosphodiesterase 4 (PDE4) inhibitor.
Structural Class: Small molecule (MW 460.5 g/mol)

Manufacturer: Amgen

Phase of Development: Apremilast was approved by the US FDA in 2014, for treatment of adults with active psoriatic arthritis and moderate to severe plaque psoriasis, and approved in 2019, for oral ulcers associated with Behçet's disease (https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/205437Orig1s007lbl.pdf). Apremilast was approved for use in the European Union in January 2015.

Pharmaceutical Information:

Dosage Form: Single dose, film-coated tablet for oral administration.

Physical Description: The Apremilast drug product used for clinical trials is supplied as a tablet for oral administration containing 30 mg of apremilast drug substance. The 30 mg tablets are beige-coated (Opadry II Beige), diamond shaped tablets with "APR" engraved on one side and "30" on the opposite side. The formulated tablet contains the following excipients: microcrystalline cellulose, lactose monohydrate, croscarmellose sodium, and magnesium stearate.

Strengths to be used in trial:	30 mg.
Packaging Unit:	The tablet formulation of apremilast will be provided in bottles.
Storage Conditions:	Store below 30°C.

Administration Information:

Route: Oral (subjects should take apremilast twice daily at approximately 12-hour intervals by swallowing or dissolved in water and administered through a feeding tube).
Standard Regimen: 30 mg twice daily (BID).
Agent Preparation: No agent preparation is required. See Section 3.5
Pre-medication: Specific pre-medication is not required for routine treatment. See Section 2.1.1

Administration: Treatment will be administered to subjects while hospitalized as inpatients for a 14 day course. Subjects should be administered apremilast twice daily at approximately 12-hour intervals without restriction of food or drink, and at approximately the same time each day (±2 hours). See Section 2.1 for further details.

Concomitant Medications: The following medications are prohibited:

- Hydroxychloroquine in combination with azithromycin
- CYP3A inducers (i.e., rifampicin, phenobarbital, carbamazepine)

Refer to §2.6 for side-effect management and dose reduction plans.

The above is intended as a summary only; please see the complete appendix for additional investigational agent information.

This study is being conducted to determine whether apremilast can safely and effectively be used to mitigate, treat, or cure COVID-19 or limit the harm of the COVID-19 pandemic in accordance with the Secretary of the Department of Health and Human Services' (HHS's) Declaration under the Public Readiness and Emergency Preparedness Act for medical countermeasures against COVID-19 (COVID-19 Declaration) effective February 4, 2020. The purpose of this study is to test whether apremilast results in clinical benefit in patients hospitalized with COVID-19. This study is authorized to proceed under an approved investigational new drug application (IND) in accordance with the public health and medical response of FDA, an Authority Having Jurisdiction as described under the PREP Act, to prescribe, administer, deliver, distribute or dispense this Covered Countermeasure as defined by and following the HHS's COVID-19 Declaration.

1. RATIONALE FOR TESTING

COVID-19

Since the outbreak of the SARS-COV2 infection (COVID-19) in December 2019, this rapidly evolving pandemic has infected at least 5.2 million people and has claimed 340,000 lives globally (John Hopkins COVID-19 data source, as of 23 May 2020). Compared to the typical, annual influenza epidemic, COVID-19 is associated with a mortality rate that is 10-fold higher, with a disproportionately higher rate of death among the elderly and individuals with underlying comorbidities (Wu and McGoogan, 2020). Although the vast majority of patients appear asymptomatic, or present with mild symptoms, experience from China has revealed that 14% of patients present with severe disease and 5% have a critical presentation associated with respiratory failure, shock, or multi-organ system dysfunction (Wu and McGoogan, 2020). The morbidity and mortality associated with this illness has been staggering; in the United States, 19% of COVID-19 patients with known disposition have required hospitalization, with 6% of all patients requiring ICU admission (United States Centers for Disease Control [US CDC], 2020b). The clinical course in severe cases of COVID-19 infection is characterized by a hyperinflammatory immune response and often a rapid progression to acute respiratory distress syndrome (ARDS), resulting in a high ICU mortality rate, ranging from 39% to 72% for those admitted to the ICU (US CDC, 2020a). These high ICU mortality rates from China were also observed in Italy, where the ICU mortality was 26% with worse outcomes for older patients (≥ 64 years of age, 36%) compared to younger patients (≤ 63 years of age, 15%) (Grasselli et al, 2020).

Currently, treatment options for COVID-19 are limited. Up to recently, clinical management of the disease has been limited solely to supportive measures, with frequent off-label use of unproven or experimental therapies. Therefore, there is a significant high unmet need for an effective therapy for management of COVID-19. Additionally, recent data from COVID-19 infections have demonstrated an increase in proinflammatory cytokines, such as IL-6 and tumor necrosis factor (TNFα), with higher levels being observed in patients with more severe disease (Chen et al, 2020; Huang et al, 2020). Characterization of the disease to date has identified this inappropriate hyperinflammatory immune response as a key driver leading to the clinical decompensation observed in COVID-19 patients; thus, a therapeutic that mitigates this immune response might be able to alter the clinical disease course.

Amgen Investigational Product Background: Apremilast

Apremilast is an oral small molecule phosphodiesterase 4 (PDE4) inhibitor that has been approved in adult patients for treatment of psoriatic arthritis (PsA), plaque psoriasis (PsO), and Behçet's disease (BD). PDE4 is expressed in both the innate and adaptive cellular components of the immune system. Inhibition of PDE4 results in elevation of intracellular cAMP levels, down regulating inflammatory responses through reduced expression of TNFα, IL-23, and other pro-inflammatory cytokines (Schafer et al, 2014, Schett et al, 2010). In addition, inhibition of PDE4 also increases anti-inflammatory cytokines such as IL-10 (Eigler, 1998). In clinical studies of PsA and PsO, compared to placebo, treatment with apremilast had a significant impact on changes in plasma cytokines, including reduction of inflammatory cytokines TNFa, IL-8, IL-6, IL-17, MCP-1, MIP-1β, and increase of anti-inflammatory mediators IL-10 and IL-1RA (Schafter et al, 2015; Apremilast Investigator's Brochure). Ibudilast, another PDE4 inhibitor, inhibited TNFα and IL-2 in a lipopolysaccharide-stimulation mouse model of neonatal ARDS, and reduced pulmonary injury in this context (Yang et al, 2020). This and other preclinical studies provide the rationale in support of evaluating apremilast in hospitalized COVID-19 patients. Since apremilast and other PDE4 inhibitors reduce proinflammatory cytokines such as IL-6 and TNFα in other inflammatory states, it is hypothesized that apremilast can suppress the hyperinflammatory response associated with COVID-19 and prevent progression of the morbid clinical course.

Benefit/Risk Assessment

Apremilast has not been evaluated in patients with COVID-19. However, there is some evidence from nonclinical studies that apremilast and another PDE4 inhibitor, ibudilast, may improve pulmonary manifestations in patients with COVID-19. Apremilast is generally well tolerated and does not have an increased risk of serious infections or other identified risks that could preclude its use in this patient population.

As of 20 March 2020, apremilast had been administered at daily doses ranging from 10 to 105 mg/day to more than 8,328 subjects in completed and ongoing clinical studies, approximately 7,235 of whom received apremilast in 30 phase 2, phase 3, and phase 4 studies in multiple indications including PsA, PsO, and BD.

In pivotal phase 3 studies, BID apremilast 30 mg resulted in statistically significant and clinically meaningful improvements in the signs and symptoms of PsA, PsO, and BD. The most commonly observed treatment-emergent adverse events (ie, those reported in ≥ 5% of subjects) have been diarrhea, nausea, headache (including tension headache), upper respiratory tract infections, and nasopharyngitis. The majority of treatment-emergent adverse events of diarrhea, nausea, and headache, occurred within the first 2 weeks of treatment and most resolved within 4 weeks. The majority of reported treatment-emergent adverse events were mild or moderate in severity and resolved while subjects continued apremilast treatment. The incidence of serious adverse events was low and comparable between apremilast and placebo treatment groups in the placebo-controlled periods and was not driven by any single preferred term or any specific individual organ toxicity. There was no evidence of an increased risk of serious or opportunistic infections in the phase 3 studies. The safety profile of apremilast is comparable across its approved indications in PsA, PsO, and BD.

As of 20 March 2020, the cumulative patient-years of exposure for apremilast since launch is 303,496 for the US. The worldwide exposure to commercial apremilast is approximately 488,415 unique patients. The safety profile of apremilast in the postmarketing setting remains similar to that observed in the registrational clinical program. The most frequently reported adverse events have been gastrointestinal (diarrhea, nausea, vomiting, abdominal discomfort) and nervous system disorders (headache). The benefit-risk balance of apremilast remains favorable for the approved indications.

Currently, apremilast requires titration during the first 5 days. During the initial phase 1 studies, more subjects reported gastrointestinal adverse events at the initiation of apremilast. In general, these events were mild to moderate in severity and did not lead to discontinuation. As a result, a titration regimen was implemented. For this study, as the potential effect of apremilast in the inflammatory cascade is expected to be during the first days of therapy, no titration regimen will be used. It is not expected that the initiation of apremilast without titration will change the risk-benefit. Subjects will be closely monitored during the 14-day treatment period.

Although risks of treatment with apremilast are known, the benefits for patients with COVID-19 are potential and the absence of effective treatments of COVID-19 complications during this pandemic warrant investigations such as this study.

The above benefit risk assessment supports the use of apremilast in this clinical trial. Reference should be made to the Investigator's Brochure for further data on apremilast.

Dose Justification for Apremilast

The apremilast dose to be evaluated in this study is 30 mg BID, the same dose approved for treatment of PsA, PsO, and BD. Inhibition of PDE4 results in the down-regulation of pro-inflammatory cytokines. In clinical studies of PsA and PsO, compared to placebo, treatment with BID apremilast 30 mg resulted in significant suppression on a range of serum cytokines, including IL-8, MCP-1, MIP-1β, TNFα, IL-6,

ferritin, and IL-2. Apremilast pharmacokinetics are not expected to differ between COVID-19 patients and other patient populations. It is expected that apremilast systemic levels attained with 30 mg BID in the approved indications will also be effective in modulating the increases in proinflammatory cytokines observed in COVID-19 infections and thus mitigate progression of the morbid clinical course.

In vitro studies have shown that the half-maximal concentration (IC_{50}) needed to inhibit PDE4 is 0.074 µM and inhibits cytokine production in peripheral blood mononuclear cells including TNF-α, IFN-γ and IL-12p70 with IC_{50} of 0.11 µM, 0.013µM and 0.12 µM, respectively (Schett et al, 2010). At the approved dose of 30 mg BID, the exposures in subjects with PsA is approximately 550 ng/mL (1 µM) at steady-state (Study CC-10004-PK-010). These exposures are well above the IC_{50} values required for inhibition of cytokine as well as chemokine production.

For the approved indications, apremilast is initially titrated from 10 mg once daily (QD) to 30 mg BID over the first 5 days of therapy, which is intended to reduce the gastrointestinal symptoms associated with initial therapy. In a phase 1 study in healthy subjects (Study CC-10004-PK-007), multiple doses of apremilast were evaluated as QD or BID dosing for 14 days (doses ranging from 40 mg QD to 80 mg QD and 40 mg BID). During the 14 days of dose administration, nausea occurred more frequently during the first week of dosing, and less frequently thereafter. In addition, this study evaluated the frequency of gastrointestinal related adverse events with and without dose titration for 40 mg QD for 14 days. The proportion of subjects who reported nausea was lower in the dose group with titration (44%) compared to the dose group without (78%). Overall, the dose titration group had fewer total number of adverse events reported (34 adverse events with dose titration versus 72 adverse events reported by the group without titration).

BID Apremilast 30 mg without titration has been evaluated in 3 phase 1 studies in healthy subjects and subjects with PsA (total n = 106 subjects) for a duration of 4 to 10 days (Studies CC-10004-PK-008, CC-10004-PK-010, and CC-10004-CP-020). The phase 1 Study PK-008 was a thorough QTc study in healthy subjects which evaluated apremilast 30 mg BID and 50 mg BID for 4 days. The most common adverse events observed in this study were headache (15/54, (28%), at 30 mg BID and 34/57, (60%), at 50 mg BID) followed by nausea (24% and 46% at 30 mg BID and 50 mg BID, respectively). In addition, two Phase 1 DDI studies evaluated apremilast 30 mg BID after multiple doses. Study CP-020 evaluated 30 mg BID for 10 days with an oral contraceptive. During the 10-day treatment period with 30 mg BID + oral contraceptive, the most frequently reported AEs were headache (27 subjects, 73%), nausea (22 subjects, 60%) and vomiting (10 subjects, 27%). No subject was discontinued from treatment. Study PK-010 evaluated APR 30 mg BID alone or in combination with methotrexate for 5 days in subjects with PsA. When apremilast 30 mg BID was administered alone, the AEs reported were mostly mild to moderate in nature (6/15, 40% reported 36 events). The regimen without titration was well-tolerated and there were no discontinuations reported due to gastrointestinal-related adverse events following start of therapy. Overall, the most common adverse event observed was headache, followed by nausea and vomiting, which were mostly mild in nature and did not require drug discontinuation.

For the treatment of COVID-19 infection, in which the development of organ dysfunction occurs rapidly, apremilast will not be initially titrated since rapid attainment of steady-state levels is expected to inhibit inflammatory cytokine production. This is necessary to establish clinical efficacy and is outweighs any expected benefit of initial titration for mitigating the development of generally mild gastrointestinal symptoms that were largely well tolerated in studies without titration.

Summary of Supportive Information for Apremilast Administration via Nasogastric Feeding Tube and Oral Syringe

Amgen conducted a study in the laboratory setting to evaluate the dose assurance and in-use stability of the apremilast 30-mg tablet when prepared for administration using polypropylene oral syringes and polyurethane nasogastric feeding tubes.

In this study, apremilast 30-mg tablet was disintegrated with water in an oral syringe. The resulting suspension was dispensed from the syringe into the nasogastric feeding tube. A dose accuracy experiment was performed in triplicate. The steps were the following:

1. Initially each nasogastric feeding tube was flushed with 10 mL water based on the manufacturer's instructions.
2. 20 mL water was drawn into a 60 mL clear oral polypropylene syringe containing a single apremilast 30-mg tablet. The tablet was disintegrated in the syringe with occasional shaking over 3 min. A pale pink cloudy solution with white precipitate resulted.
3. This solution was then pushed out of the syringe through the nasogastric feeding tube.
4. To ensure the total dose was administered, an additional 20 mL water was drawn into the oral syringe and flushed through the same nasogastric feeding tube.
5. The total volume flushed through the nasogastric tube was collected, and dose accuracy was evaluated.

Results from the dose accuracy assay demonstrated that acceptable recovery was achieved, namely recovery between 93.2% and 95.7%, within the acceptance criterion of 90.0 to 110.0%. These data support that the 30-mg apremilast tablet dose can be accurately delivered using disintegration in syringe in combination with flushing the suspension through a nasogastric feeding tube.

Second, an in-use stability study was conducted using the same procedure as the dose assurance study, except that the apremilast suspension was maintained in the oral syringe in a flat position for a total of two hours. This study was also performed in triplicate, and the percentage of label claim of apremilast and impurity profiles were determined by UHPLC/UV analysis. The results from this study demonstrated that the percentage label claim achieved was between 94.4% and 98.5%, which is within the acceptance criterion of 90.0 - 110.0%. Further, no significant changes in assay and impurities were observed. These data support that the 30-mg apremilast tablet dose can be suspended up to two hours in an oral syringe before dosing via nasogastric feeding tube.

The results from these studies support the following conclusions:
Apremilast 30-mg tablet dose assurance and in-use stability studies were conducted by using an oral syringe in combination with nasogastric feeding tubes to support alternate administration of apremilast.

- Apremilast 30-mg tablet suspension can be accurately delivered with commonly available nasogastric feeding tubes in combination with oral syringes.
- Apremilast 30-mg tablet suspension is stable at least 2 hours when disintegrated in a commercially available oral syringe at room temperature under ambient light exposure.
- Based on the study, these findings extend to apremilast 10-mg and 20-mg tablets.

1.1 Non-Clinical Studies

Please refer to section 5 of the Investigator's Brochure for details on the nonclinical program conducted in support of oral apremilast.

Apremilast is an orally active compound that modulates multiple inflammatory pathways through targeted PDE4 enzyme inhibition. Specifically, apremilast blocks the degradation of cAMP via potent inhibition of the PDE4 enzyme, resulting in an increase of cAMP in PDE4 expressing cells including monocytes, T cells, and neutrophils. The pharmacodynamic properties of apremilast include modulation of human peripheral blood mononuclear cell (HPBMC)- and T-cell-derived cytokines and anti-inflammatory, anti-nociceptive, and anti-angiogenic effects.

The biologic effects of apremilast have been assessed in numerous in vitro and in vivo models, and

nonclinical pharmacodynamic, pharmacokinetic, and toxicology studies have been conducted in mice, rats, rabbits, dogs, and cynomolgus monkeys. In vitro cellular assays demonstrated that apremilast is highly selective for PDE4 inhibition and showed no inhibition or binding to other receptors, kinases, or enzymes. In addition, results from various in vitro assays demonstrated that apremilast inhibits the production of numerous cytokines and chemokines by keratinocytes, monocytes, neutrophils, plasmacytoid dendritic cells (pDCs), and T cells. In vivo models of acute inflammation also demonstrated the anti-inflammatory activity of apremilast, including inhibition of systemic production of tumor necrosis factor alpha (TNF-α) and neutrophil infiltration.

Apremilast has been assessed in conventional studies of safety pharmacology, single- and repeated-dose toxicity, genotoxicity, and carcinogenic potential. The toxicological effects observed in animal studies were attributable to the pharmacological activity of apremilast.

General toxicity and mechanistic studies have been completed in mice, rats, and monkeys with longest durations extending to 6 months in mice and 12 months in monkeys. The systemic, comparative exposure of apremilast at the NOAELs in the 12-month monkey studies to humans receiving 30 mg apremilast BID was 7.2-fold in healthy subjects and 4.8 fold in psoriasis subjects.

Apremilast was neither genotoxic nor carcinogenic. Reproductive and developmental effects of apremilast included prolongation of estrous cycles in mice, prenatal embryo-fetal loss in mice and monkeys, and delayed fetal development (reduced ossification and fetal weight) in mice. Apremilast was not teratogenic.

In vitro, apremilast is not an inhibitor of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 and not an inducer of CYP1A2, CYP2B6, CYP2C9, CYP2C19, or CYP3A4. Apremilast is a substrate, but not an inhibitor of P-glycoprotein (P-gp) and is not a substrate or an inhibitor of organic anion transporter (OAT)1 and OAT3, organic cation transporter (OCT)2, organic anion transporting polypeptide (OATP)1B1 and OATP1B3, or breast cancer resistance protein (BCRP).

1.2 Clinical Studies

Please refer to the Investigator's Brochure for details on the clinical program conducted in support of oral apremilast. Clinical studies evaluating the efficacy and safety of apremilast have been performed in subjects with PsO, PsA, Behçet's disease (BD), rheumatoid arthritis (RA), atopic dermatitis (AD), ulcerative colitis (UC), asthma, and ankylosing spondylitis (AS) (study details and results summaries are provided in Section 6.2 of the Investigator's Brochure).

1.2.1 Efficacy

Apremilast is approved for the treatment of PsA, PsO, and oral ulcers associated with BD. Detailed information about the clinical effects of apremilast in these patient populations is provided in the prescribing information for apremilast.

https://www.pi.amgen.com/united_states/otezla/otezla_pi_english.pdf

1.2.2 Safety

The safety profile of apremilast has been well characterized in a broad and extensive clinical development program. In completed and ongoing phase 2 and phase 3 in subjects with PsA, PsO, and BD, the most

commonly observed (reported in ≥ 5% of subjects) treatment-emergent adverse events were diarrhea, nausea, headache (including tension headache), upper respiratory tract infections, and nasopharyngitis. The majority of treatment-emergent adverse events of diarrhea, nausea, and headache occurred within the first 2 weeks of treatment and most resolved within 4 weeks. The overall safety profiles of AS, BD, AD, RA, and UC were similar to the established safety profiles of PsO and PsA.

1.2.3 Pharmacokinetics and Pharmacodynamics

The pharmacokinetics of apremilast have been extensively characterized during the clinical development program in healthy subjects as well as subjects with moderate to severe PsO, PsA, and in subjects with oral ulcers associated with BD. Apremilast is rapidly absorbed following oral administration with a median time to maximum observed plasma concentration (t_{max}) of approximately 2.5 hours (ranging from 1 to 3 hours postdose) (Study CP-012 and Study PK-002). After reaching the maximum observed plasma concentration (C_{max}), apremilast plasma concentrations declined mono-exponentially with a terminal half-life of 6 to 9 hours. The absolute bioavailability of apremilast is approximately 73% (Study CP-012). Food has no clinically relevant effect on apremilast exposures and therefore, apremilast was administered without regards to food in all phase 2 and phase 3 studies.

The absorption, distribution, metabolism, and excretion (ADME) characteristics of radiolabeled [^{14}C]-apremilast were characterized in healthy male subjects following administration of a single oral dose of 20 mg suspension (Study PK-002). The study demonstrated that the mean total urinary and fecal radioactive recovery of apremilast and its metabolites was 97.1%, with mean contributions of 57.9% and 39.2% from urine and feces, respectively.

The major metabolic route of apremilast in humans was *O*-demethylation (50% of the dose metabolized via this pathway). Other minor metabolic routes included *O*-deethylation, *N*-deacetylation, hydroxylation (oxidative), hydrolysis of the imide ring, and a combination of these pathways. A pharmacologically inactive glucuronide conjugate of *O*-demethylated apremilast (metabolite M12) is the major circulating metabolite and its urinary excretion represents approximately 34% of the total administered dose.

In vitro studies have shown that apremilast metabolism is primarily mediated by CYP3A4, with minor contributions from CYP1A2 and CYP2A6. Results from clinical study with ketoconazole (strong CYP3A4 and P-gp inhibitor) demonstrated that increase in apremilast exposures (AUC and Cmax) were not considered clinically relevant and no dose adjustment is needed when apremilast is coadministered with CYP3A4 inhibitors.

The pharmacokinetics of apremilast is not affected by moderate or severe hepatic impairment. The pharmacokinetics of apremilast is not affected by mild or moderate renal impairment, however, dose reduction to 30 mg once daily (QD) is recommended in subjects with severe renal impairment. Apremilast pharmacokinetics is unaffected by age (young or elderly subjects) or gender (male or female).

The pharmacodynamics of apremilast have been studied extensively in multiple studies in subjects across the approved indications of PsO, PsA, and oral ulcers associated with BD. Biomarker analysis indicated that apremilast treatment was associated with a decrease in dendritic cells and T cells infiltrating the skin lesions within the epidermis and the dermis in the PsO setting. In the PsA indication, apremilast treatment was associated with a decrease in IL-1α, IL 6, IL-8, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 beta (MIP-1β), TNF-α, matrix metalloproteinase protein-3 (MMP-3), ferritin, and a small increase in von Willebrand factor (vWF) plasma protein levels.

2. INVESTIGATIONAL STUDY AGENT ADMINISTRATION IN THE I-SPY COVID-19 TRIAL

Intervention will be administered on an inpatient basis. Reported clinical SAEs and potential risks are described in §3.2.

2.1 Dose Regimen and Dose Groups

Study intervention within the apremilast arm is 30 mg BID for 14 days. Although the approved apremilast dosing requires a dose titration during the first 5 days, no titration will be used in this COVID-19 trial as the potential effect of apremilast in the inflammatory cascade is expected to be during the first days of therapy.

Patients who develop renal impairment during the course of study as natural progression of their disease will be identified and managed as per standard of care. Patients who develop worsening renal impairment with CrCl<30 ml/min will receive dose modification of apremilast from 30 mg twice a day to 30 mg once a day. Patients who require renal replacement therapy will need to have their apremilast dose held throughout the duration of renal replacement need. Once there is renal recovery and discontinuation of renal replacement therapy, as assessed by medical provider, patients may resume apremilast dosed as indicated per protocol guidance.

2.1.1 Apremilast Premedication Regimen and Prophylactic Medications

No specific premedication is required for apremilast routine treatment.

2.2 Eligibility

Eligibility criteria listed in the I-SPY COVID-19 TRIAL Protocol apply to all participants. In addition, the following criteria apply to those patients receiving apremilast.

Inclusion:

- No additional inclusion criteria

Exclusion:

- Current treatment with apremilast, or another agent of similar mechanism of action, for any indication within 1 week prior to first dose of investigational product.
- Concurrent use at screening or randomization or prior use of cytochrome P450 (CYP)3A inducers (e.g., rifampin, phenobarbital, carbamazepine) within 1 week prior to first dose of investigational product.

Note: Patients that meet these drug-specific exclusion criteria will be transferred to the backbone arm, but will be marked and excluded from the data analysis.

2.3 Contraindications

Known hypersensitivity to apremilast or any excipients in formulation

2.4 Concomitant Medications

At Screening, all medications taken up to 30 days prior to the screening visit will be recorded.
In addition, supportive therapies given during the course of the study should be collected and recorded.

- Oxidative metabolism of apremilast is primarily mediated by CYP3A4. CYP3A inducers (i.e., rifampicin, phenobarbital, carbamazepine) are prohibited.
- Concurrent use of a PDE4 antagonist other than apremilast is prohibited
- Hydroxychloroquine in combination with azithromycin is prohibited

Caution should always be exercised when administering concomitant medications based on the individual medication profile and clinical risk-benefit assessment.

2.5 Clinical Evaluation and Procedures

Laboratory evaluations for general safety monitoring are described in master protocol §8.1─ 8.3.

2.6 Dose Modifications and Management of Toxicity

The reason for dose change of investigational product is to be recorded on the subject's eCRF.
The dose of apremilast investigational product is to be reduced as follows:

- Investigational product administration should be reduced to QD in subjects with creatinine clearance of < 30 mL/min (estimated by the Cockcroft-Gault equation).
- If gastrointestinal adverse events associated with the start of therapy are not mitigated with antiemetics and/or antidiarrheals, investigational product dosing may be reduced to QD for 1-3 days and then dose should be increased to 30 mg BID when symptoms resolve.

Investigational product is to be withheld if a subject requires dialysis. Treatment may be resumed if dialysis is no longer needed, if prior to day 14. If dosing is resumed, daily dose will be based on the subject's creatinine clearance.

Subjects will be withdrawn from the study if persistent or unacceptable treatment-related toxicity is observed.

2.6.1 Overdose

Apremilast was studied in healthy subjects at a maximum total daily dose of 100mg (given as 50mg twice daily) for 4.5 days without evidence of dose limiting toxicities. In case of an overdose, it is recommended that the patient is monitored for any signs or symptoms of adverse effects and appropriate symptomatic treatment is instituted.

An overdose of apremilast, regardless of the presence of an associated SAE, is considered an ECI and must be documented and reported.

Additionally, an SAE associated with an overdose of apremilast must be documented and reported according to the requirements for SAEs.

Please refer to the I-SPY COVID-19 MOP for instructions on reporting overdose.

3. INVESTIGATIONAL AGENT PHARMACEUTICAL INFORMATION

3.1 Investigational Study Agents (IND 150378, IND Sponsor: QLHC)

The apremilast drug product used for clinical trials is supplied as tablets for oral administration containing 30 mg of apremilast drug substance. The 30 mg tablets are beige-coated (Opadry II Beige), diamond shaped tablets with “APR” engraved on one side and “30” on the opposite side. The formulated tablet contains the following excipients: microcrystalline cellulose, lactose monohydrate, croscarmellose sodium, and magnesium stearate. Study drug will be provided in bottles.

All labels for apremilast oral tablets will meet all applicable requirements of the US FDA and Annex 13 of Good Manufacturing Practices and/or all local regulations, as applicable.

3.2 Reported Clinical AEs and Potential Risks of Apremilast

3.2.1 Potential Risks

Based on the nonclinical, clinical, and post marketing data available for apremilast, potential effects that could be encountered in subjects given apremilast based on planned population and duration of treatment are diarrhea, nausea, or vomiting, depression, and weight loss. (See “Company Core Safety Information for Apremilast” in Appendix A of the investigators brochure for additional details)

3.2.2 Previous Experience with Products of Same Class

GI related adverse effects are seen with other PDE inhibitors.

3.2.1 Observed AE’s in Human Trials

There are no adverse events of special interest for apremilast. Please refer to the investigator’s brochure, Appendix A, section 5 for details by individual clinical study of the observed adverse events conducted in support of apremilast. Across 3 multicenter, randomized, double-blind, placebo-controlled trials of apremilast, the majority of the most common adverse reactions occurred within the first two weeks of treatment and tended to resolve over time with continued dosing. Diarrhea, headache, and nausea were the most commonly reported adverse reactions. The most common adverse reactions leading to discontinuation for patients taking apremilast were nausea (1.8%), diarrhea (1.8%), and headache (1.2%). The proportion of patients with psoriatic arthritis who discontinued treatment due to any adverse reaction was 4.6% for patients taking apremilast 30 mg twice daily and 1.2% for placebo-treated patients.

3.3 Investigational Agent Availability

Apremilast is manufactured by Amgen. The investigational agent product is provided as tablets in bottles. Dose strengths of 30 mg are to be used in this trial. The investigator or designee will record the lot number, expiration date and the amount of study medication dispensed to each participant. The quantity of tablets, date/time of each dose, and box number of investigational product are to be recorded on the subject's eCRF

Apremilast is provided under a CTPA between Amgen and QLHC.

3.4 Investigational Agent Distribution

Shipment of investigational agents to a participating site will not be approved until documentation of IRB approval of the sponsor-approved protocol and consent is available, and the collection of all essential documents is complete.

Investigational agents may be requested by the investigator (or their authorized designees) at each organization. Investigational agents will be shipped directly to the institution or site where the agent will be prepared and administered. The transfer of agents between institutions is not permitted (unless prior approval from the sponsor is obtained). Agents are requested by completing the Investigational Agent Request Form (to include complete shipping contact information) and submitting the form to the sponsor-designated DCC, see I-SPY COVID MOP for additional details

Once QLHC or their designee establishes that the requesting site is authorized to receive investigational agents, the order will be forwarded to the manufacturer, who will ship the investigational agent directly to the study site. Instructions for ordering investigational agents are available in the I-SPY COVID MOP.

3.5 Investigational Agent Preparation and Handling

Apremilast tablets (30 mg) will be provided by Amgen. No preparation is required for apremilast tablets. Treatment will be administered on an inpatient basis. Only authorized site staff may supply or administer study intervention.

3.6 Investigational Agent Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all investigational agents. The investigator is required to maintain adequate records of receipt, dispensing, and final disposition of the study agent. On the receipt, record from whom study agent was received and to whom study agent was shipped, date, quantity, and batch or lot number. On the dispensing record, note quantities and dates study agents were dispensed to and returned by each participant.

3.7 Investigational Agent Packaging and Labeling

Apremilast is packaged and labeled by Amgen, according to GCP, FDA, and national requirements. Labels are printed and attached to the study agent vial or other packaging container prior to shipping to the site. Each is labeled with a single panel label that will include, but is not limited to, the following information:

- Blank spaces to write the study number and investigator name
- IND caution statement indicating that the agent is for clinical trial use only
- Agent identification
- Lot number
- Storage conditions
- Dosing instructions
- Blank spaces to write the participant's identification number, initials, and date dispensed

Each label must remain affixed to the blister pack and/or bottle. Additional information may be attached to a label on the product carton.

3.8 Investigational Agent Storage

Apremilast tablets should be stored below 30°C. Do not use drug product if tablets are broken, cracked, or otherwise not intact.

Detailed descriptions of the storage and handling instructions for apremilast tablets are provided in the MOP.

3.9 Investigational Agent Destruction/Disposal

Once agent accountability is performed, the participating sites should use local/institutional procedures for disposal of returned/unused study agent and bottles/containers. Copies of all certificates of destruction of any unused study agent must be provided to DCC. **Prior to destruction,** the pharmacist should contact the assigned study monitor.

Unused investigational agents shall be returned to the designated facility. Please contact DCC for instructions.

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Appendix H

Pulmozyme® (Dornase alfa) Inhalation Solution

I-SPY-COVID-19 Investigational Agent Information

INVESTIGATIONAL AGENT INFORMATION SUMMARY

Agent Chaperone: Jonathan Koff, Yale, jon.koff@yale.edu

Agent Co-Chaperone: Michelle Ng Gong, Montefiore, mgong@montefiore.org

General Information:

Agent Class: Mucolytic [recombinant human deoxyribonuclease I (rhDNase)]

Structural Class: Glycoprotein (260–amino acid with molecular mass of approximately 37 kd)

Manufacturer: Genentech

Phase of Development: Pulmozyme was approved by the US FDA in 1993 for treatment of cystic fibrosis (CF). It is presently approved in over 65 countries

Pharmaceutical Information:

Dosage Form: Single-use ampule for inhalation as an aerosol mist produced by a compressed air driven nebulizer system

Physical Description: Each Pulmozyme single-use ampule delivers 2.5 mL (2.5 mg) of the sterile solution to the nebulizer bowl. The aqueous solution contains 1.0 mg/mL dornase alfa, 0.15 mg/mL calcium chloride dihydrate, and 8.77 mg/mL sodium chloride. The formulation does not contain a preservative and is suitable for single-dose administration only. The nominal pH of the solution is 6.3

Strengths to be used in trial: 2.5 mg in 2.5 mL clear, colorless solution

Packaging Unit	14 unit cartons containing 14 single-use ampules
Storage Conditions:	Store ampules at 2°C to 8°C (36°F to 46°F) and protect from light. Refrigerate Pulmozyme during transport and do not expose to room temperatures for a total time of 24 hours.

Administration Information:

Route:	Inhalation with a nebulizer
Standard Regimen:	Non-intubated subjects will receive 2.5 mg BID until hospital discharge, improvement to room air (or baseline oxygen use prior to illness) for 24 hours, or total of 14 days of study drug, whichever comes first. If subjects are intubated, they will receive 5.0 mg BID in 10 mL normal saline until extubation or 14 days, whichever comes first. If intubated for less than 14 days, extubated subjects will receive 2.5 mg BID for a total Pulmozyme treatment of 14 days, or until hospital discharge, whichever comes first.
Agent Preparation:	Authorized site staff may supply and administer study intervention to inpatients following the instructions for use for the nebulizer system being used. See Section 3.5
Pre-medication:	Specific pre-medication is not required for routine treatment. See Section 2.1.1
Administration:	Treatment will be administered to subjects while hospitalized as inpatients via a nebulizer. Any nebulizer currently used in the hospital to deliver aerosolized medications will be acceptable for Pulmozyme. See Section 2.1 for further details.

Concomitant Medications: There are no known clinically important drug-drug interactions with dornase alfa.

Refer to §2.6 for side-effect management and dose reduction plans.

The above is intended as a summary only; please see the complete appendix for additional investigational agent information.

1. RATIONALE FOR TESTING

In December 2019, the Wuhan Municipal Health Committee (Wuhan, China) identified an outbreak of viral pneumonia cases of unknown cause. Coronavirus RNA was quickly identified in some of these patients. This novel coronavirus has been abbreviated as SARS-COV-2, and the disease caused by this virus has been designated COVID-19. Global efforts to evaluate novel antivirals and therapeutic strategies to treat COVID-19 have intensified. There is currently no vaccine to prevent SARS-CoV-2 infection or therapeutic agent to treat COVID-19. Therefore, there is an urgent public health need for rapid development of novel interventions.

Pulmozyme (dornase alfa) is recombinant human deoxyribonuclease I, the only drug in its class that acts as a mucolytic by cleaving extracellular chromosomal DNA from neutrophil extracellular traps (NET) and other cell-free DNA. The drug's on-label clinical use is to reduce the viscosity and quantity of airway mucus in individuals with cystic fibrosis, thereby improving mucociliary clearance (Yang and Montgomery, 2018) and reducing respiratory tract infections requiring parenteral antibiotics. Dornase alfa is commonly used in individuals with cystic fibrosis, including those with severe complications requiring mechanical ventilation in intensive care units, and is compatible with co-administration of other routine drugs. Off-label use of dornase alfa includes reports treating acute respiratory distress syndrome (ARDS), where the drug can lead to mucus plug clearance and accelerated recovery (Morris and Mullan, 2004; Riethmueller et al. 2006). A controlled clinical trial for treating ARDS with dornase alfa is currently underway (Pottecher et al. 2020). Dornase alfa has recently been proposed as a treatment for COVID-19 (Earhart et al. 2020)

The cellular and molecular mechanisms proposed for dornase alfa activity in severely distressed lungs of individuals with cystic fibrosis and many ARDS patients are as follows. Inflammation results in neutrophilia and neutrophil infiltration in the lungs, where these cells produce NETs, largely comprised of sticky, large chromosomal DNA that physically reinforces airway mucus viscosity and accumulation (Cheng and Palaniyar 2013; Martinez-Aleman et al. 2017). Thick mucus that clears poorly can lead to airway obstruction, bronchiectasis, lung injury, hypoxia and respiratory failure. Dornase alfa facilitates airway clearance by breaking up reinforcement of mucus by NETs, by far the greatest source of extracellular DNA in inflamed lungs (Cheng and Palaniyar 2013; Martinez-Aleman et al. 2017). Although the pathophysiology of SARS-CoV-2 is not fully investigated yet, current findings show that SARS-CoV-2 binds to cells of the respiratory tract via ACE-2 receptor and, after fusion of the virus with the cell membrane, replicates in these cells, causing apoptosis and cytopathic damage. Unlike mild COVID-19, which is often associated with fever and upper-airway symptoms, individuals with severe COVID-19 often progress to an ARDS condition: hypoxaemic respiratory failure associated with neutrophilia and neutrophil infiltration in the lungs, thick mucus in bronchi, and bronchiectasis (WHO 2020; Ye et al. 2020; Zhang et al. 2020; Barnes et al. 2020). Because lung neutrophilia in ARDS is generally known to involve high NET production it is rational to assume that NETs contribute to severe pathology in COVID-19. Lung neutrophilia and NET production have been shown to contribute to the development of ARDS in other severe viral respiratory infections, including H1N1 influenza (Narasaraju et al. 2011). By facilitating the clearance of NETs, dornase alfa not only facilitates sputum clearance in CF patients, but has additional anti-inflammatory activity. Dornase alfa has been shown to reduce NETs in the bronchoalveolar lavage (BAL) and sputum of participants with CF. In the Bronchoalveolar

Lavage for the Evaluation of Anti-inflammatory Treatment (BEAT) study, the percentage of neutrophils in bronchoalveolar lavage fluid significantly increased in untreated CF patients ($P<0.02$) while remaining constant in the dornase alfa-treated group. Levels of elastase and IL-8 also significantly increased from baseline in the untreated group ($P<0.007$ and $P<0.02$ for elastase and IL-8, respectively), but remained stable in patients receiving dornase alfa (Konstan and Ratjen, 2012).

Nebulized dornase alfa may effectively treat a deleterious effect of NETs in the airways by breaking down the DNA backbone of NETs in the COVID-19 lung and so promote recovery in individuals with COVID-19-related ARDS. Dornase alfa can be easily administered to mechanically ventilated patients and is well tolerated in intensive care unit settings. Dornase alfa is approved worldwide as a nebulized formulation, with an excellent safety profile and is well tolerated. The most common side effect is a hoarse voice. Moreover, dornase alfa could be administered in addition to other effective therapeutic treatments, including antiviral therapy for COVID-19 and selective immunosuppressive treatments.

Justification for Investigational Product Dose

Pulmozyme is an FDA-approved medication that is used in cystic fibrosis at doses currently approved for clinical use. For non-intubated subjects the FDA-approved dose of 2.5 mg will be used in this study. However, upon intubation for respiratory failure, mechanical ventilation and the ventilator circuit contribute to inefficient delivery of nebulized medications. Therefore, a dose of 5.0 mg in a volume of 10 mL normal saline will be used. In both non-intubated and intubated subjects, Pulmozyme will be administered twice daily (BID), which is consistent with FDA approval.

Duration of use is based upon the course of respiratory failure and mechanical ventilation for patients with COVID-19. To be effective, a drug needs to be used during the course of the respiratory failure. If the patient is still on mechanical ventilation, or requires high delivery of oxygen after 14 days, there may be other contributing factors that account for the continued dependence on oxygen or ventilator. Two weeks of Pulmozyme should be long enough to determine whether patients will have an acute clinical response to therapy.

1.1 Non-Clinical Studies

Please refer to section 4 of the Investigator's Brochure for details on the nonclinical program conducted in support of dornase alfa.

The rationale for studying Pulmozyme (rhDNase) as a mucolytic was based upon two factors: DNase's ability to cleave DNA and the knowledge that DNA is an extremely viscous polymerized polyanion. In lung diseases characterized by persistent airway infection, such as CF, DNA accounts for the increased viscosity of purulent airway secretions. In CF patients, the persistence of airway infection and inflammation with polymorphonuclear cells (PMNs) and the subsequent action of neutrophil proteases and oxidants are major factors contributing to destruction of lung tissue. DNA in sputum is thought to be derived from the large number of necrotic neutrophils that accumulate in the airways in response to infection. Concentrations of DNA in infected sputum range from 3−12 mg/mL. Solutions of pure DNA at these concentrations are highly viscous. In vitro testing with CF patients' sputa demonstrated that dornase alfa hydrolyzes the DNA in sputum of CF patients and reduces sputum viscoelasticity.

Single and multidose inhalation toxicology studies were conducted in rat and monkey for up to 26 weeks duration. The NOAEL for daily dosing in rats was considered to be 2232 µg/kg/day, and the NOAEL for daily dosing in monkeys was considered to be 2010 µg/kg/day.

rhDNase was administered by intratracheal instillation to rats at two consecutive

doses, 24 hours apart; the animals were then observed for 2 weeks. Doses ≥ 6 mg/kg were associated with the occurrence of transient, audible respiratory sounds, slight reductions in cumulative body weight gains, and an increased incidence of minimal to moderate pneumonitis on Day 3. Effects were reversible by Day 15 and the NOAEL was considered to be 2 mg/kg. In monkeys, rhDNase was administered by intratracheal instillation as two consecutive doses, 24 hours apart. Doses of 3 and 6 mg/day were well tolerated and 6 mg/day was considered to be the NOAEL.

Acute (single-dose) IV toxicity studies in mice, rats, and monkeys revealed no evidence of overt toxicity at doses up to 10 mg/kg in all species. In multidose IV toxicity studies, rats and monkeys were given daily IV doses of rhDNase at 6 mg/kg for 5 days or up to 1.2 mg/kg for 2 weeks. The test article was well tolerated in all studies.

Pulmozyme produced no treatment-related increases in the incidence of tumors in a lifetime study in Sprague Dawley rats that were administered inhaled doses up to 0.246 mg/kg/day (approximately 30 times the MRHD in adults). There was no increase in the development of benign or malignant neoplasms and no occurrence of unusual tumor types in rats after lifetime exposure.

Pulmozyme tested negative in the following genotoxicity assays: the in vitro Ames assay, in vitro mouse lymphoma assay, and in vivo mouse bone marrow micronucleus assay. No evidence of impairment of fertility was observed in male and female rats that received intravenous doses up to 10 mg/kg/day (approximately 600 times the MRHD in adults).

1.2 Clinical Studies

Please refer to the Investigator's Brochure (section 5) for details on the clinical program conducted in support of dornase alfa for the treatment of CF.

1.2.1 Efficacy

Pulmozyme is approved for the treatment CF in adult and pediatric patients. Prescribing information can be found at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/103532s5175lbl.pdf

Two Phase III, multicenter, concurrent, randomized, placebo-controlled, 24-week studies (Z0342g and Z0343g) demonstrated the efficacy and safety of Pulmozyme in clinically stable CF patients with a forced vital capacity (FVC) > 40% of predicted (Fuchs et al. 1994). Administration of 2.5 mg of Pulmozyme once (QD) or twice daily (BID) reduced the risk of respiratory tract infection requiring parenteral antibiotics. Pulmozyme also improved forced expiratory volume in 1 second (FEV1) and FVC, reduced breathlessness, and improved general well being and CF-related symptoms. Administration of Pulmozyme did not induce drug allergy or anaphylaxis and did not increase the major complications of CF, such as hemoptysis, pneumothorax, or death.

Pulmozyme has demonstrated benefit for some patients on mechanical ventilation, although no large clinical trials have been conducted in this patient population. In a placebo-controlled, randomized, double-blind study in 100 infants ventilated after cardiac surgery, the instillation of rhDNase into the trachea BID resulted in a reduction in median ventilation time from 3.4 to 2.2 days ($p = 0.043$; Reithmueller et al. 2005). The incidence of atelectasis and time in the ICU were

also lower in the Pulmozyme-treated group. No adverse effects were reported.

1.2.2 Safety

Patients with CF have been exposed to Pulmozyme for almost 2 years (96 weeks) in clinical trials. In randomized, placebo-controlled clinical trials (Z0342g and Z0343g) of more than 600 patients (FVC ≥ 40% of predicted) treated with Pulmozyme 2.5 mg QD or BID for 6 months, most adverse events were observed with similar frequency in Pulmozyme and placebo patients and were judged to reflect the sequelae of the underlying lung disease. In most cases, events that were increased were mild, transient in nature, and did not require alterations in dosing. Few patients experienced adverse events resulting in permanent discontinuation from Pulmozyme, and the discontinuation rate was similar for those on placebo (2%) and Pulmozyme (3%). Events that were observed more frequently (≥ 3%) in Pulmozyme-treated patients than in placebo-treated patients are listed in Table 11 of the investigators brochure.

1.2.3 Pharmacokinetics and Pharmacodynamics

When 2.5 mg pulmozyme was administered by inhalation to eighteen CF patients, mean sputum concentrations of 3 μg/mL DNase were measurable within 15 minutes. Mean sputum concentrations declined to an average of 0.6 μg/mL two hours following inhalation. Inhalation of up to 10 mg TID of plumozyme by 4 CF patients for six consecutive days did not result in a significant elevation of serum concentrations of DNase above normal endogenous levels. After administration of up to 2.5 mg of pulmozyme twice daily for six months to 321 CF patients, no accumulation of serum DNase was noted. Pulmozyme is expected to be metabolized by proteases present in biological fluids. A human intravenous dose study suggested an elimination half-life of 3-4 hours for dornase alfa.

2. INVESTIGATIONAL STUDY AGENT ADMINISTRATION IN THE I-SPY COVID-19 TRIAL

Intervention will be administered on an inpatient basis. Reported clinical SAEs and potential risks are described in §3.2.

2.1 Dose Regimen and Dose Groups

Study intervention within the Pulmozyme arm is 2.5 mg BID by inhalation of an aerosol mist produced by a compressed air driven nebulizer system in non-intubated subjects. In intubated subjects, dose is 5.0 mg BID in 10 mL normal saline, by inhalation. Drug will be given for 14 days total, return to room air or baseline oxygen use for 24 hours, or until hospital discharge, whichever comes first.

2.1.1 Pulmozyme Premedication Regimen and Prophylactic Medications

No specific premedication is required for Pulmozyme routine treatment.

2.2 Eligibility

Eligibility criteria listed in the I-SPY COVID-19 TRIAL Protocol apply to all participants. In addition, the following criteria apply to those patients receiving Pulmozyme.

Inclusion:

- No additional inclusion criteria

Exclusion:

- Preexisting use of Pulmozyme

2.3 Contraindications

PULMOZYME is contraindicated in patients with known hypersensitivity to dornase alfa, Chinese Hamster Ovary cell products, or any component of the product.

2.4 Concomitant Medications

At Screening, all medications taken up to 30 days prior to the screening visit will be recorded.

In addition, supportive therapies given during the course of the study should be collected and recorded.

Clinical trials have indicated that Pulmozyme can be effectively and safely used

in conjunction with standard CF therapies including oral, inhaled and/or parenteral

antibiotics, bronchodilators, enzyme supplements, vitamins, oral or inhaled

corticosteroids, and analgesics. No formal drug interaction studies have been

performed. However, there are no known clinically important drug-drug interactions with Pulmozyme. Caution should always be exercised when administering concomitant medications based on the individual medication profile and clinical risk-benefit assessment.

2.5 Clinical Evaluation and Procedures

Laboratory evaluations for general safety monitoring are described in master protocol §8.1─ 8.3; additional evaluations/procedures necessary for this arm include:

DNAse I will be measured in plasma samples at baseline, 2d, 7d, and 14d of Pulmozyme administration

2.6 Dose Modifications and Management of Toxicity

Any potential dose modifications need to be discussed with the Sponsor.

As this is a drug that is already clinically used in patients with respiratory disease with minimal adverse effects, we anticipate that the drug or dose would not need to be delayed or reduced. Subjects will be withdrawn from the study if persistent or unacceptable treatment-related toxicity is observed.

2.6.1 Overdose

Single-dose inhalation studies in rats and monkeys at doses up to 180-times higher than doses routinely used in clinical studies are well tolerated. Cystic fibrosis patients have received up to 20 mg BID for up to 6 days and 10 mg BID intermittently (2 weeks on/2 weeks off drug) for 168 days. These doses were well tolerated.

An overdose of Pulmozyme, regardless of the presence of an associated SAE, is considered an ECI and must be documented and reported.

Additionally, an SAE associated with an overdose of Pulomzyme must be documented and reported according to the requirements for SAEs.

Please refer to the I-SPY COVID-19 MOP for instructions on reporting overdose.

3. INVESTIGATIONAL AGENT PHARMACEUTICAL INFORMATION

3.1 Investigational Study Agents (IND 150378, IND Sponsor: QLHC)

Pulmozyme drug product used for clinical trials is supplied as a single-use ampule. Each Pulmozyme single-use ampule delivers 2.5 mL (2.5 mg) of the sterile solution to the nebulizer bowl. The aqueous solution contains 1.0 mg/mL dornase alfa, 0.15 mg/mL calcium chloride dihydrate, and 8.77 mg/mL sodium chloride. The formulation does not contain a preservative and is suitable for single-dose administration only. The nominal pH of the solution is 6.3. For intubated patients, two 2.5 mL (2.5 mg) ampules plus an addition 5 mL of sterile normal saline will be used.

All labels for pulmozyme will meet all applicable requirements of the US FDA and Annex 13 of Good Manufacturing Practices and/or all local regulations, as applicable.

3.2 Reported Clinical AEs and Potential Risks of Pulmozyme

3.2.1 Potential Risks

Based on the nonclinical, clinical, and post marketing data available for Pulmozyme, dornase alfa is a generally safe drug. Potential effects that could be encountered in subjects given Pulmozyme based on planned population and duration of treatment are listed in section 3.2.1.

3.2.1 Observed AE's in Human Trials

Please refer to the investigator's brochure, section 5.3 for details by individual clinical study of the observed adverse events conducted in support of Pulmozyme.

Pulmozyme has been used for the treatment of CF for more than 15 years and medically significant toxicity is not common. Patients have been exposed to Pulmozyme for almost 2 years (96 weeks) in clinical trials. Common adverse events reported include voice alteration, pharyngitis, laryngitis, rash, chest pain, and conjunctivitis.

There have been no reports of anaphylaxis attributed to the administration of Pulmozyme. Urticaria, mild to moderate, and mild skin rash have been observed and have been transient. Within all of the studies, a small percentage (average of 2-4%) of patients treated with Pulmozyme developed serum antibodies to Pulmozyme. None of these patients developed anaphylaxis, and the clinical significance of serum antibodies to Pulmozyme is unknown.

3.3 Investigational Agent Availability

Pulmozyme is manufactured by Genentech. The investigational drug product is provided as clear, colorless solution in single-use ampules. A dose of 2.5 mg BID is to be used in this trial for non-intubated patients, and a dose of 5 mg BID will be used for intubated patients. The investigator or designee will record the lot number, expiration date and the amount of study medication dispensed to each participant.

Pulmozyme is provided under a CTPA between Genentech and QLHC.

3.4 Investigational Agent Preparation and Handling

Pulmozyme ampules (2.5 mg) will be provided by Genentech. Treatment will be administered on an inpatient basis. Only authorized site staff may supply or administer study intervention. Each Pulmozyme ampule should be squeezed prior to use in order to check for leaks. Discard ampules if the solution is cloudy or discolored. Once opened, the entire contents of the ampule must be used or discarded.

Pulmozyme should be administered via a nebulizer system or via a jet nebulizer connected to an air compressor with an adequate air flow and equipped with a mouthpiece or suitable face mask. Do not dilute or mix Pulmozyme with other drugs in the nebulizer. (SEE package insert [https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/103532s5175lbl.pdf] for additional details regarding recommended nebulizer systems). The nebulizer manufacturer's instructions on the use and maintenance of the equipment, including cleaning and disinfection procedures, should be followed. Filtered

nebulizers will be used to decrease aerosolization exposure to staff and caregivers. Any nebulizer currently used in the hospital to deliver aerosolized medications will be acceptable for Pulmozyme.

3.5 Investigational Agent Packaging and Labeling

Pulomyzme is packaged and labeled by Genentech, according to GCP, IMP, FDA, and national requirements. Labels are printed and attached the individual study ampule and to the outside carton prior to shipping to the site. Each carton is labeled with a single panel label that will include, but is not limited to, the following information:

- IND caution statement indicating that the agent is for clinical trial use only
- Agent identification
- Lot number
- Storage conditions
- Dosing instructions
- Blank spaces to write the study number, investigator's name, participant's identification number, initials, and date dispensed

Each label must remain affixed to the carton and the individual ampule.

3.6 Investigational Agent Storage

Store Pulmoyzme ampules under refrigeration at 2°C to 8°C (36°F to 46°F) and protected from light. Refrigerate ampules during transport and do not expose to room temperatures for a total time of 24 hours.

Detailed descriptions of the storage and handling instructions for dornase alfa are provided in the MOP.

3.7 Investigational Agent Destruction/Disposal

Once agent accountability is performed, the participating sites should use local/institutional procedures for disposal of returned/unused study agent and bottles/containers. Copies of all certificates of destruction of any unused study agent must be provided to DCC. **Prior to destruction,** the pharmacist should contact the assigned study monitor.

Unused investigational agents shall be returned to the designated facility. Please contact DCC for instructions.

3.8. AEs of Special Interest (AESIs)

Adverse events of special interest for this study include the following:

- Cases of potential drug-induced liver injury that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's law:
- Treatment-emergent ALT or AST > 3 ´ ULN in combination with total bilirubin > 2 ´ ULN
- Treatment-emergent ALT or AST > 3 ´ ULN in combination with clinical jaundice
- Data related to a suspected transmission of an infectious agent by the study drug (STIAMP), as defined below:

Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of the study drug is suspected

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Appendix I
Celecoxib plus High Dose Famotidine Combination Therapy

I-SPY-COVID-19 Investigational Agent Information

INVESTIGATIONAL AGENT INFORMATION SUMMARY

Agent Chaperone: Kashif Khan, MD, kashif.khan@med.usc.edu
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Albert Yen, MD, albert.yen@med.usc.edu
Se Fum Wong, MD, sefum.wong@med.usc.edu

General Information for Celecoxib:

Agent Class: Cyclooxygenase-2 (COX-2) selective enzyme inhibitor, nonsteroidal anti-inflammatory drug.

Structural Class: Small molecule (MW 381.4 g/mol)

Manufacturer: Pfizer

Phase of Development: Celecoxib was patented in 1993 and came into medical use in 1999 (1, 2). Label indications for celecoxib include osteoarthritis, rheumatoid arthritis, juvenile rheumatoid arthritis in patients 2 years and older, ankylosing spondylitis, acute pain, and primary dysmenorrhea (3). Off-label uses include reduction of the number of adenomatous colorectal polyps (colorectal adenomas) in adults and children with familial adenomatous polyposis (FAP) (4-6).

Pharmaceutical Information:

Dosage Form: Single dose, capsules for oral administration.

Physical Description: Celecoxib; CELEBREX® capsules: 400 mg white, with reverse printed white on green band with markings of 7767 on the cap and 400 on the body. The formulated capsules contain the following inactive ingredients: croscarmellose sodium, edible inks, gelatin, lactose monohydrate, magnesium stearate, povidone and sodium lauryl sulfate.

Strengths to be used in trial: 400 mg capsules.

Packaging Unit: The capsule formulation of celecoxib (Celebrex 400 mg) will be provided in bottles of 60 capsules (400 mg per capsule)

Storage Conditions:	Store at room temperature 20°C to 25°C (68°F to 77°F); excursions permitted between 15°C to 30°C (59°F to 86°F)

Administration Information:

Route:	Oral (subjects should take ***high dose*** celecoxib by swallowing or dissolved in water and administered through a feeding tube if intubated.
Standard Regimen:	***High dose oral*** 400 mg two time daily (BID) per os (PO) for a total course of ***7 days***.*
Agent Preparation:	No agent preparation is required. See Section 3.5
Pre-medication:	Specific pre-medication is not required for routine treatment. See Section 2.1.1
Administration:	Treatment will be administered to subjects while hospitalized as inpatients for up to ***7-days***. If subject is discharged before 7 days, oral high dose celecoxib should be stopped. Subjects should be administered ***high dose*** celecoxib 400 mg two times daily (BID) at approximately 12-hour intervals with food, and at approximately the same time each day (±2 hours). For intubated subjects, instructions for G-tube celecoxib administration are in the Pharmacy Manual. See Section 2.1 for further details.

*This study will test only a very short exposure, 400mg PO BID for 7 days. Given this short exposure, the regimen is likely very safe to test in the COVID inpatient setting. Celecoxib has been used in millions of people and is standardly administered short term pre and postoperatively for pain control in many ERAS (Enhanced Recovery After Surgery) pathways in hospitals across the country, especially for gynecologic and orthopedic procedures.

Concomitant Medications:
This is the language from the package insert, which is based upon long-term use (months-years):

- Celecoxib and anticoagulants such as warfarin have a synergistic effect on bleeding. The concomitant use of celecoxib and anticoagulants have an increased risk of serious bleeding compared to the use of either drug alone.
- Controlled clinical studies showed that the concomitant use of NSAIDs and analgesic doses of aspirin does not produce any greater therapeutic effect than the use of NSAIDs alone. In a clinical study, the concomitant use of an NSAID and aspirin was associated with a significantly increased incidence of GI adverse reactions as compared to use of the NSAID alone
- NSAIDs may diminish the antihypertensive effect of angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), or beta-blockers (including propranolol).
- In patients who are elderly, volume-depleted (including those on diuretic therapy), or have renal impairment, co-administration of an NSAID with ACE inhibitors or ARBs may result in deterioration of renal function, including possible acute renal failure.

- The concomitant use of celecoxib with digoxin has been reported to increase the serum concentration and prolong the half-life of digoxin.
- NSAIDs have produced elevations in plasma lithium levels and reductions in renal lithium clearance. The mean minimum lithium concentration increased 15%, and the renal clearance decreased by approximately 20%. This effect has been attributed to NSAID inhibition of renal prostaglandin synthesis.
- Concomitant use of NSAIDs and methotrexate may increase the risk for methotrexate toxicity (e.g., neutropenia, thrombocytopenia, renal dysfunction).
- Concomitant use of celecoxib and cyclosporine may increase cyclosporine's nephrotoxicity.
- Concomitant use of celecoxib with other NSAIDs or salicylates (e.g., diflunisal, salsalate) increases the risk of GI toxicity, with little or no increase in efficacy
- Concomitant use of celecoxib and pemetrexed may increase the risk of pemetrexed-associated myelosuppression, renal, and GI toxicity (see the pemetrexed prescribing information).
- Celecoxib metabolism is predominantly mediated via cytochrome P450 (CYP) 2C9 in the liver. Co-administration of celecoxib with drugs that are known to inhibit CYP2C9 (e.g. fluconazole) may enhance the exposure and toxicity of celecoxib whereas co-administration with CYP2C9 inducers (e.g. rifampin) may lead to compromised efficacy of celecoxib.
- *In vitro* studies indicate that celecoxib is an inhibitor of CYP2D6. Therefore, there is a potential for an *in vivo* drug interaction with drugs that are metabolized by CYP2D6 (e.g. atomoxetine), and celecoxib may enhance the exposure and toxicity of these drugs.
- Concomitant use of corticosteroids with celecoxib may increase the risk of GI ulceration or bleeding.

Refer to §2.6 for side-effect management and dose reduction plans.

General Information For Famotidine:

Agent Class:	Famotidine: Histamine blocker; a histamine-2 (H_2) receptor antagonist and inverse agonist.
Structural Class:	Famotidine: Small molecule (MW 337.4 g/mol)
Manufacturers:	J&J
Phase of Development:	Famotidine tablets for oral use were initially approved by the FDA in 1986. Low dose formulations received an OTC approval in 1995. Famotidine is presently approved in adult and pediatric patients 40 kg and above for the treatment of active duodenal ulcer (DU), active gastric ulcer, symptomatic non-erosive gastroesophageal reflux disease (GERD), and erosive esophagitis due to GERD, diagnosed by biopsy. It is also approved in prescription form adults for the treatment of pathological hypersecretory conditions (e.g., Zollinger-Ellison Syndrome, multiple endocrine neoplasias), and reduction of the risk of DU recurrence. Famotidine is currently available in 10 mg (OTC or

prescription), 20mg (OTC or prescription), and 40 mg (prescription) tablet forms. Famotidine for injection is marketed with and without preservatives in 10mg/ml and 0.4 mg/ml forms. Famotidine OTC tablets (10 mg, 20 mg) are marketed as branded PEPCID AC® as well as various generic versions.

Pharmaceutical Information:

Dosage Form:	Single dose, film-coated tablet for oral administration (10mg, 20mg, 40mg tabs). Liquid suspension for oral administration (40mg/5ml). Injectable liquid for intravenous administration (10mg/ml).
Physical Description:	PEPCID® or generic equivalent drug product used for this clinical trial is supplied as a tablet for oral administration containing 20 mg of famotidine drug substance. The 20 mg are white tablets coded PAC 20 on one side and the other side is plain. The formulated tablet contains the following inactive ingredients: carnauba wax, hydroxypropyl cellulose, hypromellose, iron oxides, magnesium stearate, microcrystalline cellulose, pregelatinized starch, talc, and titanium dioxide.
Strengths to be used in trial:	20 mg tablet.
Packaging Unit:	The tablet formulation of PEPCID® 20 mg will be provided in bottles.
Storage Conditions:	Famotidine; Store in well-closed, light-resistant containers and store at controlled room temperature (59°F to 86°F).

Administration Information:

Route:	Oral (patients should take ***high dose*** PEPCID® for by swallowing or dissolved in water and administered through a feeding tube if intubated)
Standard Regimen:	The course of treatment is a total of 21 days. ***High dose*** 80 mg PO four times daily (QID) for 7 days. Upon completion of the ***high dose***, subjects should receive a lower 40 mg PO twice daily (BID) for a course of 14 days.
Agent Preparation:	No agent preparation is required. See Section 3.5
Pre-medication:	Specific pre-medication is not required for routine treatment. See Section 2.1.1

Administration:	Treatment will be administered to subjects while hospitalized as inpatients. If a subject is discharged before 21 days, famotidine should be stopped. Subjects should be administered 80 mg (***high dose***) PEPCID® four times daily at approximately 6-hour intervals without restriction of food or drink, and at approximately the same time each day (±2 hour) for 7 days. Upon completion of the ***high dose***, subjects should receive a lower 40 mg PO twice daily (BID) at approximately 12-hour intervals without restriction of food or drink, and at approximately the same time each day (±2 hour) for an additional course of 14 days. For intubated subjects, instructions for G-tube PEPCID® administration are in the Pharmacy Manual.

Concomitant Medications:

Systemic exposure of concomitant drugs dependent on gastric pH for absorption may be significantly reduced by famotidine leading to loss of efficacy. Concomitant administration of famotidine with dasatinib, delavirdine mesylate, cefditoren, and fosamprenavir is not recommended.

Famotidine is considered a weak CYP1A2 inhibitor and may lead to substantial increases in blood concentrations of tizanidine, a CYP1A2 substrate. Avoid concomitant use with famotidine. If concomitant use is necessary, monitor for hypotension, bradycardia or excessive drowsiness. Refer to the full prescribing information for tizanidine.

Refer to §2.6 for side-effect management and dose reduction plans.

The above is intended as a summary only; please see the complete appendix for additional investigational agent information.

1. RATIONALE FOR TESTING

COVID-19

Since the outbreak of the SARS-COV2 infection (COVID-19) in December 2019, this rapidly evolving pandemic has infected at least 20 million people and has claimed 730,000 lives globally (John Hopkins COVID-19 data source, as of 10 August 2020). Compared to the typical, annual influenza epidemic, COVID-19 is associated with a mortality rate that is 10-fold higher, with a disproportionately higher rate of death among the elderly and individuals with underlying comorbidities (7). Although the vast majority of patients appear asymptomatic, or present with mild symptoms, experience from China has revealed that 14% of patients present with severe disease and 5% have a critical presentation associated with respiratory failure, shock, or multi-organ system dysfunction (7). The morbidity and mortality associated with this illness has been staggering; in the United States, 19% of COVID-19 patients with known disposition have required hospitalization, with 6% of all patients requiring ICU admission (8). The clinical course in severe cases of COVID-19 infection is characterized by a hyperinflammatory immune response and often a rapid progression to acute respiratory distress syndrome (ARDS), resulting in a high ICU mortality rate, ranging from 39% to 72% for those admitted to the ICU (9). These high ICU

mortality rates from China were also observed in Italy, where the ICU mortality was 26% with worse outcomes for older patients (≥ 64 years of age, 36%) compared to younger patients (≤ 63 years of age, 15%) (10).

Currently, treatment options for COVID-19 are limited. Up to the recent approval for the antiviral remdesivir, clinical management of the disease has been limited solely to supportive measures, with frequent off-label use of unproven or experimental therapies. Therefore, there is a significant high unmet need for an effective therapy for management of COVID-19. Additionally, recent data from COVID-19 infections have demonstrated an increase in proinflammatory cytokines, such as IL-6 and tumor necrosis factor (TNFα), with higher levels being observed in patients with more severe disease (11, 12). Characterization of the disease to date has identified this inappropriate hyperinflammatory immune response as a key driver leading to the clinical decompensation observed in COVID-19 patients; thus, a therapeutic regimen that mitigates this immune response might be able to alter the clinical disease course.

This study is being conducted to determine whether the combination of famotidine and celecoxib, concomitantly administered at a high dose regimen as adjuvant therapy with standard of care (SOC), can safely and effectively be used to mitigate, treat, or cure COVID-19 or limit the harm of the COVID-19 pandemic in accordance with the Secretary of the Department of Health and Human Services' (HHS's) Declaration under the Public Readiness and Emergency Preparedness Act for medical countermeasures against COVID-19 (COVID-19 Declaration) effective February 4, 2020. The purpose of this study is to test whether the combination of famotidine and celecoxib as an adjuvant therapy with standard of care results in clinical benefit in patients hospitalized with COVID-19. This study is authorized to proceed under an approved investigational new drug application (IND) in accordance with the public health and medical response of FDA, an Authority Having Jurisdiction as described under the PREP Act, to prescribe, administer, deliver, distribute or dispense this Covered Countermeasure as defined by and following the HHS's COVID-19 Declaration.

Background and Dose Justification the use of a Combination of Celecoxib/Famotidine for the Therapeutic Treatment of COVID-19 Patients

There are multiple different pathophysiologic patterns commonly associated with viral infections. One is the consequence of direct virus injury, and the other involves host immune-mediated pathology. While infection by the Betacoronavirus/Sarbecovirus SARS-CoV-2 is required for development of COVID-19 disease, it is estimated that up to 80% of SARS-CoV-2 positive patients are asymptomatic and do not progress to COVID-19 (13-18). Therefore, SARS-CoV-2 infection is necessary but not sufficient for development of clinical COVID-19 disease. A predominant pathophysiology in COVID-19 involves host immune-mediated processes that are most frequently observed in high risk patients; elderly and those with pre-existing medical conditions including (in descending order) hypertension, obesity, metabolic (diabetes), cardiovascular, neurologic, renal, chronic lung, and asthmatic diseases (8). Virtually all of these risk factors are associated with chronic pro-inflammatory states (19, 20).

Celecoxib and famotidine are potent systemic paracrine inflammatory signaling inhibitors, and prior reports indicate that each may independently act to reduce the morbidity and mortality associated with host responses to SARS-CoV-2 infection in those patients that develop COVID-19. The FDA market authorized indications for the prescription drug celecoxib

("CELEBREX®") are based on its activity in inhibiting synthesis of various prostaglandins primarily via inhibition of COX-2 enzyme activity (21). Celecoxib also inhibits 5-lipoxygenase (22), which plays a key role in controlling secondary inflammatory responses by regulating production of leukotrienes (23). FDA market authorized indications for famotidine ("PEPCID®") reference the activity of this over-the-counter drug as a histamine H_2 receptor antagonist (24). Tomera, Malone and Kittah (25) hypothesize that the mechanism of action of both agents is COX-2 inhibition (celecoxib) and histamine H2 receptor antagonism/inverse agonism (famotidine). Neither agent is purported to act via direct inhibition of SARS-CoV-2 viral replication.

Celecoxib as a potential therapeutic for COVID-19

Celecoxib is the only FDA-approved non-steroidal anti-inflammatory drug that is selective for (inducible) prostaglandin H synthase 2 (cyclooxygenase-2 or COX-2) enzyme inhibition (26). Prostaglandin E2 (PGE2) is one of the principal mediators of acute inflammation (27, 28), is synthesized by the COX-2 enzyme, and among many other activities can activate mast cell degranulation (29). Based on the observation of elevated PGE2 levels in both SARS (30) and SARS-CoV-2 (31), and informed by relevance of these host inflammatory pathway-related mechanisms of PGE2 activity, Hong et al reasoned that administration of a COX-2 specific inhibitor may have therapeutic benefit for treating COVID-19. In a prospective clinical trial, oral celecoxib (200mg PO twice daily (BID)) was administered to 25 hospitalized patients with COVID-19 (31). This study resulted in improved clinical outcomes, with 2 of 11 cases administered celecoxib 100 mg PO BID demonstrating COVID-19 disease progression after discontinuation, and 3 of 7 controls not treated with celecoxib also demonstrating progression. Elevated urinary PGE2 levels were markedly dampened in the celecoxib treated group, and chest CT images showed rapid improvement (31). A brief report showed more rapid recovery with increase to 400mg bid of celecoxib. A recent retrospective analysis supports the safety and potential efficacy of celecoxib when administered to patients with COVID-19 (32).

COX-2 is expressed upon induction in a variety of cell types, including lung fibroblasts (33), vascular endothelial cells (34-36), Type II alveolar pneumocytes (37), and alveolar macrophages (38). All of these cell types are found in compromised pulmonary gas exchange tissue during the prodromal period of COVID-19 (39). Vascular endothelial cells and Type II alveolar pneumocytes are primary targets for infection and replication of SARS-CoV-2, and alveolar macrophages are permissive to infection (40). SARS-CoV can transactivate the inducible COX-2 gene promoter in infected cells. SARS transcriptional upregulation of COX-2 is mediated by the nucleocapsid protein (N- directly via specific N2 protein sequences) and spike glycoprotein (S – via indirect transactivation cascade), driving overexpression of COX-2 in a dose dependent manner in infected cells (41, 42). The ability of SARS-CoV-2 to similarly transactivate COX-2 expression is untested, and homology analysis of the analogous N2 sequences of this and other Betacoronaviruses have not been reported. SARS-CoV-2 infection is associated with high levels of PGE_2 production based on urinary levels of PGE_2 , which in one study of hospitalized cases were 9x higher on average than levels observed in normal uninfected individuals (170±40ng/ml vs 18.1±3.8ng/ml, $p<0.01$) and in some cases were observed to spike to 3,000 ng/ml or 150X normal (31). These investigators postulated that excessive PGE_2 may play a key role in the pathophysiology of COVID-19 by binding to EP2, EP3 and EP4 receptors. EP2 receptor binding is associated with fever, pain, acute inflammation, and enhanced vascular permeability. EP3 receptor binding leads to edema, inflammatory mucus secretion, increased viscosity of alveolar exudate, and blockage of alveolar and respiratory bronchiole oxygen exchange via a mast cell activation-dependent pathway (29). EP4 receptor binding causes

bronchial contractions and spasms with resulting increased airway resistance (43), and is associated with respiratory and hemodynamic disorders, ARDS and multi- organ failure. Excessive PGE_2 binding to EP4 is also associated with inhibition of T lymphocyte functionality by promoting amplification, differentiation and proliferation of Th1 and Th17 subtypes (44). In addition, PGE_2 and thromboxane A2 (TXA2) activation can cause platelet aggregation and thrombosis (45). All of these signs and symptoms of excessive signaling by the products of arachidonic acid metabolism resulting from COX-2 activation are features of COVID-19 disease.

Celecoxib has previously demonstrated positive COVID-19 outcomes and moderation of urinary PGE-2 by its COX-2 antagonism in one prospective clinical study (31). A randomized double blind clinical trial (RCT) of severe influenza infection found that treatment with celecoxib reduced Il6, National Early Warning Scores (NEWS), and mortality from 27% to 12% (p 0.037) (46-48). Animal models of influenza also support celecoxib in combination with other agents. In one murine H5N1 model, triple therapy with celecoxib, mesalazine and zanamivir significantly improved survival rate and time to death while having no impact on viral load compared to zanamivir alone (49), and if zanamivir was added late, no mice survived, but adding celecoxib increased survival from 0% to 53% and was associated with significantly higher levels of CD4+ and CD8+ T lymphocytes (49). Celecoxib added to zanamivir in an H7N9 murine model increased survival from 18 to 70% while reducing the lung histopathology, IL6, and RANTES (50). In the H7N9 rat model, even if the viral replication had been suppressed with an antiviral, levels of cytokines and chemokines were still similar to the untreated mice. These findings may explain why remdesivir failed to reduce mortality in the NIAID study since once the SARS-CoV-2 virus has triggered a cytokine storm; even if viral replication is suppressed by antiviral therapy, the proinflammatory cytokines and chemokines will continue to drive the immunopathologic progression.

Prior rigorous, detailed molecular studies have conclusively demonstrated that both the SARS spike protein (via an indirect cascade) (42) and the SARS nucleocapsid protein (via direct transcriptional activation) (41) upregulate transcription from the COX-2 promoter. Mutation, gel shift and classical co-transfection studies were used to define two specific sequences within the SARS nucleocapsid protein N2 region which interact with the nuclear factor-kappa B-A and CCAAT/enhancer binding protein DNA sequences of the human COX-2 promoter. These findings suggest that SARS activates COX-2 expression in infected cells, and thereby upregulates production of PGE2 and other related prostaglandin metabolites from those cells. Clinical data indicate that SARS-CoV-2 infection is associated with substantially elevated urinary levels of PGE2 (31), and recent work has shown both in-vitro and in-vivo COX-2 upregulation. An initial assessment of the applicability of the SARS N2 findings to SARS-CoV-2 was performed by examining sequence conservation between these viruses (25). The findings demonstrate that a region of the nucleocapsid N2 domain spanning N2a and N2b, previously considered to be unstructured with no specific function (51), appears to be a highly conserved domain incorporating previously demonstrated COX-2 transactivator functionality. This domain includes a 49 aa segment defined in the SARS-CoV-2 N protein sequence accession P0DTC9.1 as 218-ALALLLLDRLNQLESKVSGKGQQQQGQTVTKKSAAEASKKPRQKRTATK-266 (Figure 7). This domain incorporates both the C-terminal and N-terminal regions previously defined by mutational analysis as critical for transactivation of COX-2 by SARS but extends both beyond these regions and spans the intervening sequences. This sequence is highly conserved in SARS, SARS-CoV-2, Pangolin coronaviruses, Horseshoe bat coronaviruses, and Civet cat coronaviruses, but this high degree of conservation does not extend to corresponding isolates of MERS or another animal and human Betacoronaviruses.

In addition to the conserved sequences associated with transactivation of the COX-2 promoter, both of the nuclear localization sequences (NLS) associated with N2 were also found to

be conserved between SARS and SARS-CoV-2 (as well as other SARS-like Sarbecoviruses obtained from Civet, Bat, and Pangolin samples) (25). NLS are required for nuclear transport of nucleocapsid protein from cytoplasmic polyribosome translation complexes if the Sarbecovirus nucleocapsid protein COX-2 transactivator domain is to exert an effect on infected cell transcription. Currently unknown is whether horseshoe bats, Pangolins and Civet cats express COX-2 and have similar transcriptional control elements, but it is possible that the minimal point mutations which have been identified in these two nuclear localization sequences may modulate the efficiency of transport and thereby subtly influence relative transcription transactivation activity in the different Sarbecoviruses. Considering the high degree of sequence conservation across both the COX-2 transactivation and associated NLS domains, it is reasonable to conclude that SARS-CoV-2 infection and N protein expression may result in direct transactivation of COX-2 promoter-mediated transcription and expression in infected cells. This mechanism may significantly contribute to the previously reported production of high levels of PGE2 and other related prostaglandins, and consequent dysregulated signaling to cells involved in innate and adaptive immune responses to SARS-CoV-2 (including mast cell activation as well as histamine production and release). While this signaling may explain aspects of the hyper-inflammatory host response to infection that characterizes COVID-19, it does not resolve why some SARS-CoV-2 infected patients develop the disease, while others do not.

Recent evidence has supported the use of dexamethasone for treatment of COVID-19 (52, 53). Dexamethasone is a very broad-spectrum glucocorticoid inhibitor of inflammatory and immune processes, and components of dexamethasone activity may overlap with the pharmacologic activity of celecoxib (COX-2 inhibition and 5-lipoxygenase activity). The combination of dexamethasone and celecoxib treatment may contribute to development of aortic stenosis and cardiac valve calcification, and so combining dexamethasone and celecoxib treatment for more extended treatment may be contraindicated (54). The leukotriene receptor antagonist montelukast has also been advocated for treatment of COVID-19, and since celecoxib-mediated 5-lipoxygenase inhibition will reduce production of leukotrienes, concurrent use of these two agents may or may not provide additional benefits over celecoxib alone.

Famotidine as a potential therapeutic for COVID-19

Histamine is one of the predominant signaling molecules released by activated mast cells (55), although production and release of this autocrine/paracrine signal is also associated with basophils, neutrophils and histaminergic neuronal cells (56, 57). Histamine acts via four different G-coupled protein receptors, H1 through H4. Famotidine is a highly specific antagonist/inverse agonist of the histamine H2 receptor (58), and is typically used for treatment of gastro-esophageal reflux disease (GERD). A retrospective cohort study of 1,602 hospitalized COVID-19 patients reported that propensity-matched patients who received famotidine (low dose- oral or iv 20-40mg daily) within 24 hours of admission and during hospitalization had a significantly reduced risk of death/intubation or death alone (59). In this study, serum ferritin was reported as an acute phase reaction indicator, and famotidine treatment also reduced maximum median ferritin levels to 708ng/ml (IQR 370-1152) versus 846ng/ml (IQR 406-1552) among non-user controls (rank-sum p=.03). In contrast, mortality reduction was not associated with those patients treated with proton pump inhibitors during hospitalization. These authors posited that the mechanism of action of famotidine in COVID-19 involved direct inhibition of viral replication via the SARS-CoV-2 main protease (3ClPro). More recent retrospective studies report a more mixed outcome from the standard over-the-counter famotidine dose (60, 61), and the small numbers of patients available for retrospective analysis in these series present considerable challenges when adjusting for confounding variables. A case series report involving low dose famotidine + cetirizine (20mg PO

BID) + cetirizine (10mg PO BID) reports a case fatality rate of 15.5% and a duration of hospitalization of 11 days, which is not different from the national average of 15.4% case fatality rate for hospitalized COVID-19 (62). When taken together with the various previously cited retrospective analyses, this finding suggests that famotidine dosing required to yield a significant reduction in clinical signs and symptoms must exceed the maximum dose required for the over-the-counter maximal labeled dose of 20mg PO BID. A limited outpatient case series has reported optimal reduction in patient reported COVID-19 symptoms with a dose of 80mg famotidine PO TID (63).

Studies from other laboratory groups have demonstrated that famotidine is not a direct inhibitor of SARS-CoV-2 replication and does not interact with or inhibit either the SARS-CoV-2 papain-like protease (Plpro) or main protease (64, 65). The most likely mechanism of action for this famotidine effect in improving hospitalized COVID-19 outcomes is via its on-target activity as a histamine receptor H2 antagonist (and inverse agonist) coupled to arrestin-biased activation (64). This finding has resulted in development of the hypothesis that SARS-CoV-2 infection-induced mast cell activation could account for some of the core pathologic cascade and much of the unusual symptomatology associated with COVID-19 (64, 66). Notable is that increased endothelial cell permeability has been attributed to histamine H2 activation and is blunted by famotidine pretreatment (67). High dose IV famotidine is currently being studied in a large randomized prospective study (NCT04370262) for treatment of hospitalized patients with moderately severe COVID-19.

SARS-CoV-2 infection-induced mast cell activation could account for some of the core pathologic cascade and much of the unusual symptomatology associated with COVID-19 (68). Many of the unique clinical symptoms observed during the early phase of COVID-19 are consistent with known effects of histamine release. Histamine may act as an autocrine regulator of mast cell cytokine and TNF-a release in a PGE2-dependent fashion and based on in vitro studies the autocrine feedback appears to be mediated by H_2 and H_3 (69) Histamine downregulates signals which drive monocyte (macrophage and dendritic cell) activation including antigen presentation to T cells . Of the various immune system cell types recently analyzed, the most profound defect observed in COVID-19 occur in monocytes and lymphocytes (70). Famotidine at the levels that are empirically clinically determined to overcome the effects of COVID-19 is able to overcome the effects of 10 micromolar histamine on monocytes (71). These famotidine levels are achieved with 60-80 mg QID famotidine dosing. Monocyte production of IL-12 and TNF-α is strongly inhibited by 100 nM to 10 μM histamine via H2 receptors (72, 73). Histamine is rapidly degraded but reaches levels as high as 100 nM in serum or nasal washing fluids (74, 75), therefore concentrations of histamine in tissue are likely to be significantly higher.

Tomera , Malone, and Kittah have recently reported a study of consecutive case series of 25 COVID-19 hospitalized patients treated with celecoxib and high dose famotidine together as adjuvant therapy (25). Patients were initially administered oral high dose famotidine 80 mg four times a day (QID) and oral celecoxib 200 mg twice daily (BID) following a 400 mg loading dose while hospitalized, with the most recently treated patients administered oral celecoxib 400 mg BID. In this series, famotidine dose selection was guided by the mast cell hypothesis and well described histamine H2 blockade pharmacokinetics (64). Although the pharmacokinetic calculations performed to support this dosing strategy did not account for local tissue levels of histamine (which will compete with famotidine for H2 receptor occupancy), 60mg PO TID was calculated to achieve 10-fold the half maximum inhibitory concentration (MIC50) for H2 blockade in the absence of histamine as a competitor. Given the abnormal tissue and tissue pharmacokinetics which may exist in this life-threatening condition and a drug known for a wide therapeutic index, 320mg famotidine PO daily (80mg PO QID) was selected for initial dose assessment (25). After hospital discharge, the aggressive high dose therapy was reduced to a

lower dosing regimen for continued outpatient therapy (7-24 days of 40 mg famotidine BID and 200 mg celecoxib BID). Summarized outcome measurements included time to discharge, changes in supplemental oxygen requirements, CT-chest findings, and laboratory changes in peripheral blood lactate dehydrogenase (LDH), C-reactive protein (CRP), ferritin, lymphocyte levels, and D-dimer. All 25 patients in the series survived hospitalized COVID-19 without mechanical ventilation or renal replacement therapy (RRT) and were discharged on room air within a median of 3 days (range 1-16 days). Statistically significant improvements from admission to discharge were observed for all aggregated outcome measures. In general, progressive improvement was noted in supplemental oxygen requirements and ground-glass CT findings. Acute kidney injury was either prevented or mitigated by treatment; HD famotidine contributed to this effect. This adjuvant combination therapy regimen was both safe and efficacious in this limited study.

Based upon the early clinical reports and mechanistic experiments, in the study proposed here we will be using a similar high dose celecoxib plus high dose famotidine combination dosing regimen as an adjuvant to SOC. Patients will receive high dose famotidine (80 mg PO QID) plus celecoxib (400 mg PO BID) for 7 days. Upon completion of this 7 day course of both high dose famotidine and high dose celecoxib, patients will continue to receive a lower dose of famotidine (40 mg PO BID) for 14 additional days. Celecoxib and famotidine will be stopped at hospital discharge. Relative to the Tomera and Kittah study (76), in this study the celecoxib dose will be administered at the higher 400 mg dose for the entire duration of the initial 7 day high dose regimen. This higher dose has been evaluated in previous clinical registration studies and has been deemed safe when administered for extended periods (6).

1.1 CELECOXIB

Detailed information regarding the efficacy and safety (including warnings and interventions for celecoxib administration can be found in the approved FDA label: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/020998s050lbl.pdf

1.1.1 Celecoxib Pharmacology and Non-Clinical Studies

Celecoxib is a nonsteroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgestic, and antipyretic properties. The anti-inflammatory and pain-relieving properties of celecoxib result from inhibition of prostaglandin (PG) synthesis by selective inhibition of PG G/H synthase-2 (encoded by gene PTGS2). The two PTGS isoforms, PTGS1 and PTGS2, are bisfunctional enzymes with both cyclooxygenase (COX) and hydroperoxidase activities, but they are commonly referred to as COX. Celecoxib was purposefully designed as COX-2-selective inhibitor. In vitro, celecoxib potently inhibits the transformation of arachidonic acid to prostaglandin precursors. Celecoxib concentrations reached during clinical therapy produce in vivo effects. Prostaglandins are mediators of inflammation, sensitize afferent nerves and potentiate the action of bradykinin in inducing pain in animal models. Celecoxib's analgesic and anti-inflammatory properties are believed to be due to a decrease of prostaglandins in peripheral tissues as a result of inhibition of prostaglandin synthesis. Nonselective NSAIDs inhibit both COX-1 and COX-2. Inhibition of COX-1 inhibits the production of prostaglandins and the production of the platelet activator, thromboxane A2. COX-1 is traditionally defined as a constitutively expressed enzyme and plays a role in the protection of the gastrointestinal mucosa, kidney hemodynamics, and platelet thrombogenesis. COX-2, on the contrary, is extensively

expressed in cells involved in inflammation and is upregulated by bacterial lipopolysaccharides, cytokines, growth factors, and tumor promoters. Celecoxib is approximately 30 times more selective for COX-2 inhibition over COX-1. In theory, this selectivity allows celecoxib and other selective COX-2 inhibitors to reduce inflammation (and pain) while minimizing gastrointestinal adverse drug reactions that are common with nonselective NSAIDs.

Carcinogenic potential of celecoxib was assessed in Sprague-Dawley rats given oral doses up to 200 mg/kg for males and 10 mg/kg for females (approximately 2-to 4-times the human exposure as measured by the AUC0-24 at 200 mg twice daily) or in mice given oral doses up to 25 mg/kg for males and 50 mg/kg for females (approximately equal to human exposure as measured by the AUC0-24 at 200 mg twice daily) for two years.

Celecoxib was not mutagenic in an Ames test and a mutation assay in Chinese hamster ovary (CHO) cells, nor clastogenic in a chromosome aberration assay in CHO cells and an *in vivo* micronucleus test in rat bone marrow.

Celecoxib had no effect on male or female fertility or male reproductive function in rats at oral doses up to 600 mg/kg/day (approximately 11times human exposure at 200 mg twice daily based on the AUC0-24). At ≥50 mg/kg/day (approximately 6-times human exposure based on the AUC0-24 at 200 mg twice daily) there was increased preimplantation loss.

Drug-Drug Interactions:

See section 7, Table 3 of the Celebrex label for a review of the clinically significant drug interactions with celecoxib.

1.1.2 Clinical Studies

1.1.2.1 Efficacy

Celecoxib is indicated for the treatment of pain and inflammation in osteoarthritis, acute pain in adults, rheumatoid arthritis, ankylosing spondylitis, primary dysmenorrhea and juvenile rheumatoid arthritis. The indications are as listed in the approved label.

1.1.2.2 Safety

The safety profile of celecoxib has been well characterized in a broad and extensive clinical development program and through post marketing experience.

Celecoxib was evaluated in controlled studies consisting of more than 8,500 patients that received a total daily dose of celecoxib of 200 mg (100 mg twice daily or 200 mg once daily) or more, including more than 400 treated at 800 mg (400 mg twice daily). Approximately 3,900 patients received celecoxib at these doses for 6 months or more; approximately 2,300 of these have received it for 1 year or more and 124 of these have received it for 2 years or more. The adverse events associated with these trials are presented in section 6.1 of the approved label for Celebrex.

Section 5 of the label list warnings and precautions for the following:

- Cardiovascular thrombotic events
- Gastrointestinal Bleeding, ulceration and perforation
- Hepatotoxicity
- Hypertension
- Heart failure and edema
- Renal toxicity and hyperkalemia
- Anaphylactic reactions
- Exacerbation of asthma related to aspirin sensitivity
- Serious skin reactions
- Premature closure of fetal ductus arteriosus
- Hematological toxicity
- Disseminated intravascular coagulation (in pediatric patients)

1.1.2.3 Pharmacokinetics and Pharmacodynamics

Celecoxib exhibits dose-proportional increase in exposure after oral administration up to 200 mg twice daily and less than proportional increase at higher doses. It has extensive distribution and high protein binding (~97% within the clinical dose range). Peak plasma levels of celecoxib occur approximately 3 hrs after an oral dose. Celecoxib has a half-life of approximately 11 hours. With multiple dosing, steady-state conditions are reached on or before Day 5.

Celecoxib metabolism is primarily mediated via CYP2C9. Three metabolites, a primary alcohol, the corresponding carboxylic acid and its glucuronide conjugate, have been identified in human plasma. These metabolites are inactive as COX-1 or COX-2 inhibitors. Celecoxib is eliminated predominantly by hepatic metabolism with little (<3%) unchanged drug recovered in the urine and feces. Following a single oral dose of radiolabeled drug, approximately 57% of the dose was excreted in the feces and 27% was excreted into the urine. The primary metabolite in both urine and feces was the carboxylic acid metabolite (73% of dose) with low amounts of the glucuronide also appearing in the urine.

Both renal and hepatic impairment affect the pharmacokinetics of celecoxib (see section 12.4 of the celecoxib label)

1.2 FAMOTIDINE

Detailed information regarding the efficacy and safety (including warnings and interventions for famotidine administration can be found in the approved FDA label: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/019462s039lbl.pdf

1.2.1 Famotidine Pharmacology and Non-Clinical Studies

Famotidine is specific competitive histamine type 2 receptor (H_2R) antagonist that inhibits production of gastric acid. It suppresses basal and stimulated secretion of gastric acid in a linear dose-dependent manner with oral doses of 5-40 mg, with evening administration of oral famotidine at doses of 10 mg and 20 mg resulting in a reduction of basal nocturnal acid output by 69% and 86%, respectively. Famotidine also decreases output of pepsin and may increase serum gastrin concentrations.

Famotidine is indicated for the treatment of conditions where a controlled reduction of gastric secretion is required, such as heartburn, hyperacidity, acid indigestion/dyspepsia, acid regurgitation, and gastroesophageal reflux. In addition, it aids in the prevention of these symptoms at night and when associated with the consumption of food and/or beverages. The indications are as listed in the approved label.

Famotidine has been assessed in conventional studies of safety pharmacology, single- and repeated-dose toxicity, genotoxicity, and carcinogenic potential.

Systemic effects of famotidine in the CNS, cardiovascular, respiratory or endocrine systems were not noted in pharmacology studies. Serum hormone levels, including prolactin, cortisol, thyroxine (T4), and testosterone, were not altered after treatment with famotidine.

Carcinogenic potential of famotidine was assessed in an oral carcinogenicity studies in rats and in mice. In the 106-week study in rats and the 92-week study in mice at oral doses of up to 2000 mg/kg/day (approximately 243 and 122 times, respectively, based on body surface area, the recommended human dose of 80 mg per day for the treatment of erosive esophagitis), there was no evidence of carcinogenic potential for famotidine.

Famotidine was negative in the microbial mutagen test (Ames test) using *Salmonella typhimurium* and *Escherichia coli* with or without rat liver enzyme activation at concentrations up to 10,000 mcg/plate. In *in vivo* studies in mice, with a micronucleus test and a chromosomal aberration test, no evidence of a mutagenic effect was observed.

Reproductive studies have been performed in rats and rabbits at oral doses of up to 2000 and 500 mg/kg/day, respectively, and in both species at intravenous doses of up to 200 mg/kg/day and have revealed no significant evidence of impaired fertility or harm to the fetus due to famotidine. While no direct fetotoxic effects have been observed, sporadic abortions occurring only in mothers displaying marked decreased food intake were seen in some rabbits at oral doses of 200 mg/kg/day (about 49 times the recommended human dose of 80 mg per day, based on body surface area) or higher. There are, however, no adequate or well-controlled studies in pregnant women.

Drug-Drug Iteractions:

CYP1A2: Famotidine is a weak CYP1A2 inhibitor.

Human Organic Anion Transporter (OAT) 1 and 3: In vitro studies indicate that famotidine is a substrate for OAT1 and OAT3. Following coadministration of probenecid (1500 mg), an inhibitor of OAT1 and OAT3, with a single oral 20 mg dose of famotidine in 8 healthy subjects, the serum AUC0-10h of famotidine increased from 424 to 768 ng●hr/mL and the maximum serum concentration (C_{max}) increased from 73 to 113 ng/mL. Renal clearance, urinary excretion rate and amount of famotine excreted unchanged in urine were decreased. The clinical relevance of this interaction is unknown.

Multidrug and Toxin Extrusion Protein 1 (MATE-1): An *in vitro* study showed that famotidine is an inhibitor of MATE-1. However, no clinically significant interaction with metformin, a substrate for MATE-1, was observed.

1.2.2 Clinical Studies

1.2.2.1 Efficacy

Famotidine is approved in adult and pediatric patients for the treatment of active duodenal ulcer (DU), active gastric ulcer, symptomatic non-erosive gastroesophageal reflux disease (GERD), and nerosive esophagitis due to GERD, diagnosed by biopsy. It is also approved in adults for the treatment of pathological hypersecretory conditions (e.g., Zollinger-Ellison Syndrome, multiple endocrine neoplasias), and reduction of the risk of DU recurrence. Detailed information about the clinical efficacy of famotidine in these patient populations is provided in the prescribing information for famotidine.

1.2.2.2 Safety

The safety profile of famotidine has been well characterized in a broad and extensive clinical development program and through post marketing experience.

Famotidine was studied in 7 US and international placebo- and active-controlled trials in approximately 2500 patients. The following adverse reactions occurred in greater than or equal to 1% of PEPCID-treated patients: headache, dizziness and constipation.

1.2.2.3 Pharmacokinetics and Pharmacodynamics

Famotidine is rapidly absorbed following oral administration with a median time to maximum observed plasma concentration (t_{max}) of approximately 1-3 hours. Plasma levels after multiple dosages are similar to those after single doses. The elimination half-life of famotidine is 2.5-3.5 hours. Famotidine is eliminated by renal (65 to 70%) and metabolic (30 to 35%) routes. Renal clearance is 250 to 450 mL/minute, indicating some tubular excretion. In adult patients with severe renal impairment (creatinine clearance less than 30 mL/minute), the systemic exposure (AUC) of famotidine increased at least 5-fold. In patients with moderate renal impairment (creatinine clearance between 30 to 60 mL/minute), the AUC of famotidine increased at least 2-fold.

The bioavailability of oral doses of famotidine is poor (40 to 45%) due to low gastroretention time. Bioavailability may be slightly increased by food, or slightly decreased by antacids; however, these effects are of no clinical consequence. Fifteen to 20% of famotidine in plasma is protein bound.

Famotidine undergoes minimal first-pass metabolism. Twenty-five to 30% of an oral dose was recovered in the urine as unchanged compound. The only metabolite identified in humans is the S-oxide.

2. INVESTIGATIONAL STUDY AGENT ADMINISTRATION IN THE I-SPY COVID-19 TRIAL

Intervention will be administered on an inpatient basis. Reported clinical SAEs and potential risks are described in §3.2.

2.1 Dose Regimen and Dose Groups

Famotidine
Famotidine will be administered concomitantly with celecoxib in this arm during the first 7 days, and then as a single agent for the next 14 days. Study intervention with famotidine within this dual drug arm is 80 mg (high dose) PEPCID® four times daily for the first 7 days by swallowing or through a feeding tube at approximately 6-hour intervals without restriction of food or drink, and at approximately the same time each day (±2 hour). For the next 14 days, famotidine should be administered at 40 mg twice daily by swallowing or through a feeding tube at approximately 12-hour intervals without restriction of food or drink, and at approximately the same time each day (±2 hour). For participants requiring mechanical ventilation, investigators will need to determine the most appropriate means for providing the drug product (ie, crushed tablet within feeding tube with adequate flush); preparation instructions for administration of famotidine using a G-tube or other enteral feeding tube can be found in the study Pharmacy Manual. If intolerance of the study drug intervention is observed, the participant will remain in the study for endpoints and safety observations (Master Protocol Section 9). Missed doses are not made up.

Celecoxib
Celecoxib will be administered concomitantly with famotidine in this arm for the first 7 days of this 21-day treatment regimen. Study intervention with celecoxib within this dual drug arm is 400 mg twice daily (BID) by swallowing or through a feeding tube at approximately 12-hour intervals with food or drink, and at approximately the same time each day (±2 hour) for a total of 7-days. For participants requiring mechanical ventilation, investigators will need to determine the most appropriate means for providing the drug product (ie, transfer contents of capsule within feeding tube with adequate flush); preparation instructions for administration of celecoxib using a G-tube or other enteral feeding tube can be found in the study Pharmacy Manual. If a feeding tube is not available for >24 hours or intolerance of drug is noted, the participant will discontinue study drug intervention, but will remain in the study for endpoints and safety observations (Master Protocol Section 9). Missed doses are not made up.

2.1.1 Premedication Regimen and Prophylactic Medications

No specific premedication is required for famotidine routine treatment.
No specific premedication is required for celecoxib routine treatment.

Prophylactic treatment for coagulopathies is managed as clinically needed. Critically ill patients with COVID are typically receiving either ICU prophylaxis with heparin, enoxaparin or a DOAC or therapeutic anticoagulation if they have known pulmonary embolism or deep venous thrombosis.

2.2 Eligibility
Eligibility criteria listed in the I-SPY COVID-19 TRIAL Protocol apply to all participants. In addition, the following criteria apply to those patients receiving famotidine/celecoxib.

Inclusion:

- No additional inclusion criteria

Exclusion:

- Dialysis or renal failure (eGFR<15ml/min), or reduced kidney function (either acute kidney injury or chronic kidney disease with a serum creatinine corresponding to an eGFR <30ml/min/1.73 m^2
- Patients that had an MI during their current hospitalization prior to enrollment
- Childs Pugh score of 10 or above

Note: Patients that meet these drug-specific exclusion criteria will be transferred to the backbone arm, but will be marked and excluded from the data analysis.

2.3 Contraindications

Famotidine

- Known serious hypersensitivity (e.g., anaphylaxis) to famotidine or other H_2 receptor antagonists or any excipients in formulation

Celecoxib

- Known hypersensitivity (e.g., anaphylactic reactions and serious skin reactions) to celecoxib, any components of the drug product.
- History of asthma, urticaria, or other allergic-type reactions after taking aspirin or other NSAIDs.

2.4 Concomitant Medications

At Screening, all medications taken up to 30 days prior to the screening visit will be recorded. In addition, supportive therapies given during the course of the study should be collected and recorded.

Famotidine

- Famotidine can reduce the absorption of other drugs, due to its effect on reducing intragastric acidity, leading to loss of efficacy of the concomitant drug. Concomitant administration of famotidine with dasatinib, delavirdine mesylate, cefditoren, and fosamprenavir is not recommended. See the prescribing information for other drugs dependent on gastric pH for absorption for administration instructions, including atazanavir, erlotinib, ketoconazole, itraconazole, ledipasvir/sofosbuvir, nilotinib, rilpivirine and posaconazole.
- Although not studied clinically, famotidine is considered a weak CYP1A2 inhibitor and may lead to substantial increases in blood concentrations of tizanidine, a CYP1A2 substrate. Avoid concomitant use with famotidine. If concomitant use is necessary, monitor for hypotension, bradycardia or excessive drowsiness. Refer to the full prescribing information for tizanidine.
- HD Famotidine will prolong warfarin.

Celecoxib NOTE: The language below is from the package insert, which is based upon long-term use of celecoxib (months-years)

- Celecoxib and anticoagulants such as warfarin have a synergistic effect on bleeding. The concomitant use of celecoxib and anticoagulants have an increased risk of serious

bleeding compared to the use of either drug alone.

- Controlled clinical studies showed that the concomitant use of NSAIDs and analgesic doses of aspirin does not produce any greater therapeutic effect than the use of NSAIDs alone. In a clinical study, the concomitant use of an NSAID and aspirin was associated with a significantly increased incidence of GI adverse reactions as compared to use of the NSAID alone
- NSAIDs may diminish the antihypertensive effect of angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), or beta-blockers (including propranolol).
- In patients who are elderly, volume-depleted (including those on diuretic therapy), or have renal impairment, co-administration of an NSAID with ACE inhibitors or ARBs may result in deterioration of renal function, including possible acute renal failure. These effects are usually reversible.
- The concomitant use of celecoxib with digoxin has been reported to increase the serum concentration and prolong the half-life of digoxin.
- NSAIDs have produced elevations in plasma lithium levels and reductions in renal lithium clearance. The mean minimum lithium concentration increased 15%, and the renal clearance decreased by approximately 20%. This effect has been attributed to NSAID inhibition of renal prostaglandin synthesis.
- Concomitant use of NSAIDs and methotrexate may increase the risk for methotrexate toxicity (e.g., neutropenia, thrombocytopenia, renal dysfunction).
- Concomitant use of celecoxib and cyclosporine may increase cyclosporine's nephrotoxicity.
- Concomitant use of celecoxib with other NSAIDs or salicylates (e.g., diflunisal, salsalate) increases the risk of GI toxicity, with little or no increase in efficacy.
- Concomitant use of celecoxib and pemetrexed may increase the risk of pemetrexed-associated myelosuppression, renal, and GI toxicity (see the pemetrexed prescribing information).
- Celecoxib metabolism is predominantly mediated via cytochrome P450 (CYP) 2C9 in the liver. Co-administration of celecoxib with drugs that are known to inhibit CYP2C9 (e.g. fluconazole) may enhance the exposure and toxicity of celecoxib whereas co-administration with CYP2C9 inducers (e.g. rifampin) may lead to compromised efficacy of celecoxib.
- *In vitro* studies indicate that celecoxib is an inhibitor of CYP2D6. Therefore, there is a potential for an *in vivo* drug interaction with drugs that are metabolized by CYP2D6 (e.g. atomoxetine), and celecoxib may enhance the exposure and toxicity of these drugs.
- Concomitant use of corticosteroids with celecoxib may increase the risk of GI ulceration or bleeding.

Caution should always be exercised when administering concomitant medications based on the individual medication profile and clinical risk-benefit assessment.

2.5 Clinical Evaluation and Procedures

Laboratory evaluations for general safety monitoring are described in master protocol §8.1─ 8.3.

2.6 Dose Modifications and Management of Toxicity

The reason for dose change of investigational products is to be recorded on the subject's eCRF.

Patients who develop hepatic impairment during the course of study as natural progression of their disease will be identified and managed as per standard of care. For hepatic impairment, a Child's Pugh class is calculated using the definitions and table below:

	1 Point	2 points	3 points
Bilirubin	<2mg/dL	2-3 mg/dL	>3mg/dL
Albumin	>3.5 g/dL	2.8-3/5 g/dL	<2.8
INR	<1.7	1.7-2.2	>2.2
Ascites	absent	Slight	Moderate
Encephalopathy	None	Grade 1-2	Grade 3-4

Child's Pugh class A: 5-6 , Child's Pugh class B: 7-9 , Child's Pugh class C: 10 or greater . Note that patients with a Child's Pugh score of 11 and above at entry are excluded from the trial per the main protocol exclusion criteria

Celecoxib and famotidine are to be discontinued permanently if:

- A subject develops worsening kidney function defined as a 2x increase in SCr over pre-enrollment baseline and/or a serum creatinine corresponding to an eGFR < 30 mL/min.1.73m^2.
- Myocardial infarction (MI), defined as either new ischemic EKG changes/abnormal wall motion by imaging/ischemic thrombus on angiography) and troponin elevation with at least 1 value above 99% UNL.
- Radiographic (CT or MRI) or clinical concern for stroke caused by ischemia or hemorrhage, persisting ≥24 hours or until death.
- Severe/worsening heart failure, defined as an increase in or institution of IV inotropic therapy, or mechanical circulatory support.

Celecoxib is to be dose reduced to:
- 400 mg QD if Childs Pugh Class B at entry
- 400 mg QD if LFT (AST/ALT) abnormalities elevated to Grade 3
- 400 mg QD if bilirubin abnormality elevated to Grade 3

Celecoxib is to be discontinued if:
- Grade 4 LFT abnormalities occur
- Grade 4 Bilirubin abnormalities occur

Famotidine is to be dose reduced to:

- 40 mg PO four times daily for 7 days if serum creatinine corresponds to an eGFR 30-59 mL/min/1.73m^2. Upon completion of the high dose, subjects should receive a lower 20 mg PO twice daily (BID) for a course of 14 days.

Famotidine is to be discontinued if:

- Thrombocytopenia, defined as a platelet count of < 50,000 mm^3 develops
- Prolonged QTc develops on ECG obtained as part of routine clinical care

Famotidine and Celecoxib will be discontinued permanently if any persistent or unacceptable treatment-related toxicity is observed. As for all patients whose drug is discontinued, the reason will be recorded.

2.6.1 Overdose

Famotidine
The types of adverse reactions in overdosage of famotidine are similar to the adverse reactions encountered with use of label approved recommended dosage. Oral doses of up to 640 mg/day have been given to adult patients with pathological hypersecretory conditions with no serious adverse effects. In the event of overdosage, treatment should be symptomatic and supportive. Unabsorbed material should be removed from the gastrointestinal tract, the patient should be monitored, and supportive therapy should be employed. Due to low binding to plasma proteins, famotidine is eliminated by hemodialysis. There is limited experience on the usefulness of hemodialysis as a treatment for famotidine overdosage.

Celecoxib
Symptoms following acute NSAID overdosages have been typically limited to lethargy, drowsiness, nausea, vomiting, and epigastric pain, which have been generally reversible with supportive care. Gastrointestinal bleeding has occurred. Hypertension, acute renal failure, respiratory depression, and coma have occurred, but were rare.

No overdoses of celecoxib were reported during clinical trials. Doses up to 2400 mg/day for up to 10 days in 12 patients did not result in serious toxicity. No information is available regarding the removal of celecoxib by hemodialysis but based on its high degree of plasma protein binding (>97%) dialysis is unlikely to be useful in overdose.

Manage patients with symptomatic and supportive care following an NSAID overdosage. There are no specific antidotes. Consider emesis and/or activated charcoal (60 to 100 grams in adults, 1 to 2 grams per kg of body weight in pediatric patients) and/or osmotic cathartic in symptomatic patients seen within four hours of ingestion or in patients with a large overdosage (5 to 10 times the recommended dosage). Forced diuresis, alkalinization of urine, hemodialysis, or hemoperfusion may not be useful due to high protein binding.

An overdose of famotidine or celecoxib, regardless of the presence of an associated SAE, is considered an ECI and must be documented and reported.

Additionally, an SAE associated with an overdose of famotidine or celecoxib must be documented and reported according to the requirements for SAEs.

Please refer to the I-SPY COVID-19 MOP for instructions on reporting overdose.

3. INVESTIGATIONAL AGENT PHARMACEUTICAL INFORMATION

3.1 Investigational Study Agents (IND 150378, IND Sponsor: QLHC)

PEPCID used for clinical trials is supplied as tablets for oral administration containing 20 mg of famotidine drug substance. The 20 mg are white tablets coded PAC 20 on one side and the other side plain. The formulated tablet contains the following inactive ingredients: carnauba wax, hydroxypropyl cellulose, hypromellose, iron oxides, magnesium stearate, microcrystalline cellulose, pregelatinized starch, talc, and titanium dioxide. Study drug will be provided in bottles.

All labels for PEPCID oral tablets will meet all applicable requirements of the US FDA and Annex 13 of Good Manufacturing Practices and/or all local regulations, as applicable.

CELEBREX used for clinical trials is supplied as capsules for oral administration containing 400 mg of celecoxib drug substance. The 400 mg capsules are white, with reverse printed white on green band with markings of 7767 on the cap and 400 on the body. The formulated tablet contains the following inactive ingredients: croscarmellose sodium, edible inks, gelatin, lactose monohydrate, magnesium stearate, povidone and sodium lauryl sulfate. Study drug will be provided in blister packs.

All labels for Celebrex capsules will meet all applicable requirements of the US FDA and Annex 13 of Good Manufacturing Practices and/or all local regulations, as applicable.

3.2 Reported Clinical AEs and Potential Risks of Famotidine

3.2.1 Potential Risks

Famotidine

Based on the nonclinical, clinical, and post marketing data available for famotidine the most common potential effects that could be encountered in subjects given famotidine based on planned population and duration of treatment are headache, dizziness and constipation.

Central nervous system (CNS) adverse reactions, including confusion, delirium, hallucinations, disorientation, agitation, seizures, and lethargy, have been reported in elderly patients and patients with moderate and severe renal impairment treated with famotidine. Since famotidine blood levels are higher in patients with renal impairment than in patients with normal renal function, dosage adjustments are recommended in patients with renal impairment.

Celecoxib

Based on the nonclinical, clinical, and post marketing data available for celecoxib the most common potential effects that could be encountered in subjects given celecoxib based on planned population and duration of treatment are: abdominal pain, diarrhea, dyspepsia, flatulence, peripheral edema, accidental injury, dizziness, pharyngitis, rhinitis, sinusitis, upper respiratory tract infection, rash.

3.2.2 Previous Experience with Products of Same Class

Famotidine: Headache, dizziness and constipation related adverse effects are seen with other H_2 antagonists.

Celecoxib: Celecoxib is a selective COX-2 inhibitor. Compared to other NSAIDS that are non-selective COX-1 and COX-2 inhibitors, it has less cardiac and nephrotoxicity.

3.2.3 AE's of Special Interest for this trial

Famotidine
The adverse events of special interest for famotidine in this trial include:

- Thrombocytopenia, defined as a platelet count of < 50,000 mm^3
- Sustained ventricular arrhythmia or torsades de pointes

Celecoxib
The adverse events of special interest for celecoxib in this trial include:

- Cardiovascular thrombotic events (stroke or myocardial infarction)
- Gastrointestinal Bleeding, ulceration and perforation requiring blood transfusion or surgical intervention
- Severe/worsening heart failure, defined as an increase in or institution of IV inotropic therapy, or mechanical circulatory support
- Acute kidney injury, defined as the need for dialysis or a serum creatinine corresponding to an eGFR of <30 ml/min/1.73 m2
- Anaphylaxis
- Stevens Johnson Syndrome

3.3 Investigational Agent Availability

PEPCID® is manufactured by J&J. The investigational agent product is provided as tablets in bottles (50 tablets per bottle). Dose strengths of 20 mg are to be used in this trial. The investigator or designee will record the lot number, expiration date and the amount of study medication dispensed to each participant. The quantity of tablets, date/time of each dose, and box number of investigational product are to be recorded on the subject's eCRF

PEPCID® is provided under an agreement between J&J and QLHC.

CELEBREX is manufactured by Pfizer. The investigational agent product is provided as capsules. A dose strength of 400 mg are to be used in this trial. The investigator or designee will record the lot number, expiration date and the amount of study medication dispensed to each participant. The quantity of capsules, date/time of each dose, and box number of investigational product are to be recorded on the subject's eCRF

CELEBREX is provided under an agreement between Pfizer and QLHC.

3.4 Investigational Agent Distribution

Shipment of investigational agents to a participating site will not be approved until documentation of IRB approval of the sponsor-approved protocol and consent is available, and the collection of all essential documents is complete.

Investigational agents may be requested by the investigator (or their authorized designees) at each organization. Investigational agents will be shipped directly to the institution or site where the agent will be prepared and administered. The transfer of agents between institutions is not permitted (unless prior approval from the sponsor is obtained). Agents are requested by completing the Investigational Agent Request Form (to include complete shipping contact information) and submitting the form to the sponsor-designated DCC, see I-SPY COVID MOP for additional details

Once QLHC or their designee establishes that the requesting site is authorized to receive investigational agents, the order will be forwarded to the manufacturer, who will ship the investigational agent directly to the study site. Instructions for ordering investigational agents are available in the I-SPY COVID MOP.

3.5 Investigational Agent Preparation and Handling

Famotidine
PEPCID® tablets (20 mg) will be provided by J&J. Treatment will be administered on an inpatient basis only. Only authorized site staff may supply or administer study intervention while patient is hospitalized. No preparation is required for PEPCID® tablets, unless patients are on a feeding tube, in which case refer to the Pharmacy Manual.

Briefly, the procedure for administration through a feeding tube is:

1. Study drug should be administered with or without food or feeds.
2. After administering tube feeding, flush the tube with water prior to study drug administration.
3. Transfer tablets to the desired dosage (four tablets for 80 mg or two tablets 40 mg) to a clean pill crusher and grind it to a powder. Transfer the powder to a clean medicine cup.
4. Add 30 ml of room temperature water to the medicine cup and stir vigorously to suspend the material.
5. Draw the suspension into a large syringe (eg, 60 mL syringe) and administer via the feeding tube.
6. After this first administration, flush the tube with room temperature water.
7. To ensure all study drug has been delivered, add 30 ml of room temperature water to medicine cup and stir to include any potentially remaining material.
8. Draw the fluid into the same large syringe (eg, 60 mL syringe) and transfer to the patient via the feeding tube.
9. After this final administration of the study drug, flush the tube with room temperature water.
10. Document the date and time, the patient's response (if any response occurred), and your assessment of study drug delivery.

11. Document all liquids administered, including feed and flushes, on the patient's intake and output record.

Celecoxib
Celebrex capsules (400 mg) will be provided by Pfizer. Treatment will be administered on an inpatient basis only. Only authorized site staff may supply or administer study intervention while patient is hospitalized. No preparation is required for Celebrex capsules, unless patients are on a feeding tube, in which case refer to the Pharmacy Manual.

Briefly, the procedure for administration through a feeding tube is:

1. Study drug should be administered with food or feeds. Administer the study drug immediately after feeding the patient.
2. After administering tube feeding, flush the tube with water prior to study drug administration.
3. Transfer the contents of one 400 mg capsule (400 mg total) to a clean medicine cup.
4. Add 30 ml of room temperature water to the medicine cup and stir vigorously to suspend the material.
5. Draw the suspension into a large syringe (eg, 60 mL syringe) and administer via the feeding tube.
6. After this first administration, flush the tube with room temperature water.
7. To ensure all study drug has been delivered, add 30 ml of room temperature water to medicine cup and stir to include any potentially remaining material.
8. Draw the fluid into the same large syringe (eg, 60 mL syringe) and transfer to the patient via the feeding tube.
9. After this final administration of the study drug, flush the tube with room temperature water.
10. Document the date and time, the patient's response (if any response occurred), and your assessment of study drug delivery.
11. Document all liquids administered, including feed and flushes, on the patient's intake and output record.

3.6 Investigational Agent Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all investigational agents. The investigator is required to maintain adequate records of receipt, dispensing, and final disposition of the study agent. On the receipt, record from whom study agent was received and to whom study agent was shipped, date, quantity, and batch or lot number. On the dispensing record, note quantities and dates study agents were dispensed to and returned by each participant

3.7 Investigational Agent Packaging and Labeling

PEPCID® is packaged and labeled by J&J, according to GCP, FDA, and national requirements. Celebrex is packaged and labeled by Pfizer, according to GCP, FDA, and national requirements.

Labels are printed and attached to the study agent vial or other packaging container prior to shipping to the site. Each is labeled with a single panel label that will include, but is not limited to, the following information:

- Blank spaces to write the study number and investigator name
- IND caution statement indicating that the agent is for clinical trial use only
- Agent identification
- Lot number
- Storage conditions
- Dosing instructions
- Blank spaces to write the participant's identification number, initials, and date dispensed

Each label must remain affixed to the bottle/box (primary container). Additional information may be attached to a label on the product carton.

3.8 Investigational Agent Storage

PEPCID® tablets should be stored in well-closed, light-resistant containers and stored at controlled room temperature (59°F to 86°F). Do not use drug product if tablets are broken, cracked, or otherwise not intact.

Celebrex capsules should be stored in well-closed, light-resistant containers and stored at controlled room temperature (59°F to 86°F). Do not use drug product if capsules are broken, cracked, or otherwise not intact.

Detailed descriptions of the storage and handling instructions for PEPCID® tablets are provided in the MOP.

3.9 Investigational Agent Destruction/Disposal

Once agent accountability is performed, the participating sites should use local/institutional procedures for disposal of returned/unused study agent and bottles/containers. Copies of all certificates of destruction of any unused study agent must be provided to DCC. **Prior to destruction,** the pharmacist should contact the assigned study monitor.

Unused investigational agents shall be returned to the designated facility. Please contact DCC for instructions.

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Appendix J
IC14

I-SPY-COVID-19 Investigational Agent Information

INVESTIGATIONAL AGENT INFORMATION SUMMARY

Agent Chaperone:	Thomas Martin, MD - U of Washington, trmartin@uw.edu
Agent Co-Chaperones:	Mark Wurfel, MD, PhD – U of Washington, mwurfel@uw.edu Ellen Burnham MD – U. of Colorado, ellen.burnham@cuanschutz.edu

General Information:

Agent Class:	Competitive antagonist
Structural Class:	Chimeric monoclonal antibody
Manufacturer:	Implicit Bioscience Ltd.
Phase of Development:	Phase II/III

Pharmaceutical Information:

Dosage Form:	A sterile, single-use 30 mL vial containing 25 mL of IC14 at 5 mg/mL (125 mg total)
Physical Description:	Each vial contains IC14 in solution at 5 mg/mL
Strengths to be used in trial:	5 mg/mL stock solution to be administered in a dose of 4 mg/kg iv on Day 1, then 2 mg/kg iv on Days 2, 3, 4
Packaging Unit	Single-dose vials
Storage Conditions:	Store between 2-8° C

Administration Information:

Route:	IV
Regimen:	Dosing regimen is 4 mg/kg on Day 1, followed by 2 mg/kg on Days 2, 3, 4
Agent Preparation:	No agent preparation is required.
Pre-medication:	No premedication is required.
Administration:	Treatment will be administered as an iv infusion to subjects while hospitalized as inpatients

Concomitant Medications: No warnings or precautions are required for concomitant medications.

The above is intended as a summary only; please see the complete appendix for additional investigational agent information

1. RATIONALE FOR TESTING

1.1 CD14 Pattern Recognition Receptor in lung injury and COVID infection

A range of clinical and experimental evidence supports the hypothesis that targeting CD14 is an appropriate strategy to modulate the host innate immune response that causes serious illness and organ damage in COVID-19 illness (Martin et al, 2020). The primary pathway by which the innate immune system is alerted to the presence of noxious stimuli is through Pattern Recognition Receptors (PRRs). PRRs are activated by exogenous PAMPs as well as DAMPs. This innate immune response driven by the recognition of PAMPs is critical for the host response to microbial pathogens such as viruses, bacteria, and fungi. PAMPs are recognized by a family of membrane toll-like receptors (TLRs) that activate macrophages and other innate immune cells. Multiple experimental studies have shown that both the membrane-bound and soluble forms of CD14 bind a diverse group of PAMPs and DAMPs. The membrane-bound form of CD14 is required for maximal innate immune responses through TLRs (Wright 1995; Aderem et al. 2000; Ulevitch 1999) and other intracellular pathways that comprise the innate immune system. Given that CD14 is a proximal component of the recognition of PAMPs and DAMPs by a variety of TLRs, it represents an attractive pharmacologic target. Blockade of CD14 may reduce recognition of viral and bacterial products and the initiation of events that lead to the production of pro-inflammatory cytokines and recruitment of inflammatory cells.

CD14 exists in membrane-bound and soluble forms and may be thought of as a master regulator of immune responses (Di Gioia et al. 2015). PAMPs and DAMPs drive and amplify innate immune responses that damage the lungs and other organs. CD14 may also play a role in inflammasome activation driven by NLRP3 and other closely related proteins. Membrane CD14 (mCD14) on the cell surface is critical to immune responses in the lung epithelium and is present on alveolar macrophages (Lin et al. 2004). Membrane CD14 and TLR4 mediate responses to respiratory syncytial virus (Kurt-Jones et al. 2000) and mCD14 is required for influenza A-driven cytokine production in murine macrophages (Pauligk et al. 2004). Activation of TLR3 by double-stranded RNA released by respiratory viruses is mediated by CD14 (Lee et al. 2006). Coronavirus lung pathology involves TLRs (Li et al. 2020) that require or utilize CD14 either as a co-receptor or for transport of immunogenic ligands to endosomes. CD14 mediates pathogenic oxidative stress in acute lung injury driven by oxidized phospholipids (Di Gioia et al. 2020) that are present in samples from patients with ARDS caused by infections with H5N1 avian influenza A virus (IAV) or SARS-CoV1 (Imai et al. 2008). In patients with COVID-19 infection, there is a clear increase in the number of circulating CD14+ monocytes, suggesting an enhanced inflammatory phenotype that is related to adverse clinical outcomes (Zhang et al. 2020). RNA profiling has shown that innate immunity pathways are activated in bronchoalveolar lavage cells of patients with COVID-19 (Zhou et al. 2020) and that the elevated $CD14^{hi}$ monocyte population expressing IL-1β persists into the recovery phase (Wen et al. 2020). Thus, CD14+ monocytes, macrophages, and dendritic cells central to innate immunity pathways have emerged as fundamental aspects of the immunopathogenesis of COVID-19 infection (Merad et al. 2020).

Soluble CD14 (sCD14) is produced in the liver and spleen and shed from the surface of inflammatory cells. Soluble CD14 is abundant in serum of sepsis patients and correlates with clinical severity (Landmann et al. 1995). In addition to conferring innate immune reactivity on cells that do not constitutively express mCD14 (e.g., endothelial cells) (Pugin et al. 1993), sCD14 may directly stimulate pathogenic pro-inflammatory cytokine/chemokine production in a manner independent of microbial products (Lévêque et al. 2017). We have found that soluble CD14 in the lungs is strongly associated with deleterious inflammatory responses in the lungs of patients with ARDS (Martin et al. 1997). Furthermore, a recent proteomics study showed high levels of sCD14 in plasma samples that increased dramatically with increasing severity of disease, leading the authors to suggest that CD14 could be an appropriate target for intervention in these patients (Messner et al. 2020).

In summary, CD14-dependent signaling is a key step in the initial pathways that amplify acute inflammatory reactions in the lungs and worsen the clinical severity of lung injury. Taken together, the available data provide strong support for CD14 as a key regulator of innate immune responses in the lungs and suggest that targeting both membrane-bound and soluble CD14 could be a highly effective strategy to limit the severity of inflammatory responses in the lungs and systemic circulation of patients with SARS-CoV-2 induced lung and organ injury, particularly when added to a direct antiviral drug such as remdesivir (Beigel et al. 2020; Spinner et al. 2020). Clinical experience with an anti-CD14 antibody suggests that CD14 can be inhibited in humans safely, without causing broad immunosuppression.

1.2 Non-Clinical Studies

The effect of anti-CD14 has been observed in experimental animal and human models. In non-human primates, LPS infusion causes a response that models septic shock. Treatment with two different anti-CD14 mAbs abrogated the systemic effects of LPS infusion with preservation of mean arterial pressure (MAP), reduced circulating cytokine levels, and a relatively preserved lung epithelial permeability barrier (Leturcq et al. 1996). In rabbits, treatment with an anti-CD14 mAb protected against the lethal effects of repeated LPS exposure (Schimke et al. 1998). In a model of Gram-negative pneumonia in rabbits, University of Washington investigators found that treatment with an anti-CD14 mAb protected rabbits from the systemic effects of a localized pulmonary infection (Frevert, et al. 2000). Treated animals had improved MAP and required less intravenous fluid. The treated animals had slower clearance of the bacteria instilled in the lungs, which was not seen in a separate study of bacterial clearance in antibiotic treated rabbits (Axtelle et al. 2003). These data show that anti-CD14 treatment can attenuate the adverse systemic effects of Gram-negative infections. More recently, studies in porcine and primate models of *E. coli* bacteremic sepsis have shown that pre-treatment with a different anti-CD14 monoclonal antibody reduced circulating levels of IL-1β, IL-6, and TNFα, as well as tissue levels of IL-6 in the lungs, liver, spleen, and kidneys (Thorgersen et al. 2010; 2013) and significantly improved the associated coagulopathy and myocardial dysfunction (Keshari et al. 2020). Notably, anti-CD14 did not significantly alter the load of *E. coli* bacteria or LPS measured in lung tissue. LPS levels detected in liver and spleen were higher in the anti-CD14-treated animals suggesting augmented clearance of circulating LPS.

Taken together these preclinical data support the utility of the CD14-blocking antibody, IC14, as a therapeutic intervention in lung injury. Dampening inflammation via transient blockade of CD14 can protect the host from the extreme pro-inflammatory environment present early in the development of COVID-19 illness, leading to less severe lung injury and better clinical outcomes.

1.2 Clinical Studies

1.2.1 Efficacy

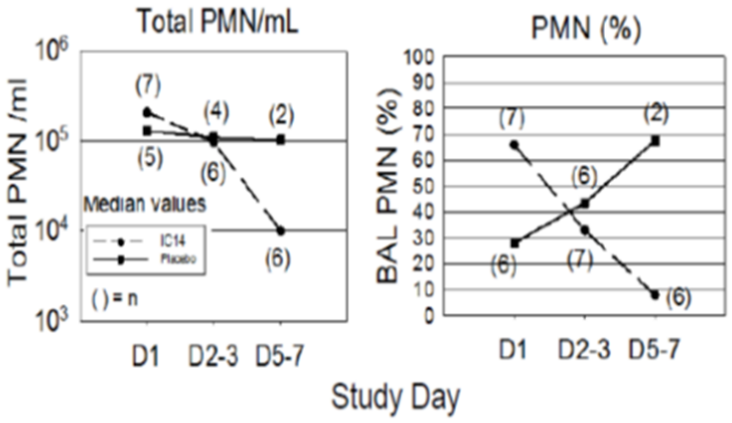
Human LPS-Challenge Study. In a human model of LPS infusion, researchers showed that IC14 treatment mitigated the inflammatory effects of intravenous LPS (Verbon et al. 2001). This phase 1 study showed that an IC14 is well tolerated and has the expected anti-inflammatory effects in humans.

Community acquired pneumonia. A phase 2, randomized double-blind placebo-controlled study of IC14 in 180 patients with community-acquired pneumonia (84 treated with IC14) did not show a significant decrease in incidence of therapeutic failure, but the treatment was well tolerated with no significant increase in TEAEs, SAEs, or secondary infections (ICOS Abbreviated Clinical Study Report 2004).

Severe Sepsis. In a randomized, double-blind, placebo-controlled, dose-ranging, multiple-center, phase 1 trial in patients with severe sepsis, IC14 was administered intravenously to four different groups (n=8/group): 1) single dose of 1 mg/kg, 2) single dose of 4 mg/kg, 3) 4 doses of 4 mg/kg/day, 4) 1 dose of

4 mg/kg on Day 0 followed by 3 doses of 2 mg/kg/day (Reinhart et al. 2004)(Axtelle et al. 2003). A placebo arm was included for each group (n=2/group). Single and multiple doses of IC14 were generally well tolerated and did not induce anti-drug antibody formation or increase the incidence of secondary bacterial infection. Patients in the multi-day IC14 groups showed a trend towards reduced multi-organ dysfunction score (MODS) (p=0.06). No significant differences in mortality or inflammatory cytokine production were noted in this small study. One SAE occurred (hypotension and rash) in a treated patient, which resolved with ranitidine, dimetindene, and prednisolone.

Adult Respiratory Distress Syndrome (ARDS). An NIH-sponsored double-blind randomized placebo-controlled phase 2 study of 13 critically ill patients with ARDS supports the use IC14 to treat lung injury resulting from increased epithelial and endothelial permeability. This study administered IC14 or placebo to critically ill patients with ARDS at the University of Washington (Seattle, WA). Patients with ARDS were randomized to receive either IC14 4 mg/kg/day x 1 day and 2 mg/kg/day x 3 days (n = 7) or placebo for 4 days (n = 6). Bronchoalveolar lavage (BAL) was performed at baseline and again on Days 2-3 and 5-7 to measure leukocyte counts and differentials, total protein, and pro-inflammatory cytokines and chemokines in the BAL fluid. This trial was stopped early because patients were being enrolled in competing trials; nevertheless, the data showed trends for reductions in total and percentage of neutrophils in lung lavage fluid, as well as plasma IL-6 and IL-8, consistent with an improvement in lung and systemic inflammation (Figure 1). Notably, the pre-treatment BAL markers of lung inflammation were higher in the IC14-treated patients, suggesting worse lung inflammation at baseline. No IC14-related safety concerns were noted. Taken together, this trial suggests that administration of IC14 is feasible and safe in critically ill patients with ARDS and may reduce markers of inflammation indicative of breakdown of the endothelial and epithelial permeability barrier.



		Day 1 (baseline)	Day 3	Day 7
Plasma IL-6 pg/ml Median (IQR)	IC14	449.8 (42.4-4867.2)	109.5 (42.4-267.3)	90.2 (4.8-108.9)
	Placebo	180.8 (92.8-371.3)	75.3 (42.4-586.0)	319.1 (121.8-516.4)
BALF IL-6 pg/ml Median (IQR)	IC14	1047.6 (227.9-1396.2)	41.6 (25.6-738.3)	23.8 (4.8-45.2)
	Placebo	79.6 (7.0-979.7)	200.4 (13.3-4147.4)	173.6 (21.1-326.1)

Figure 1. IC14 Pilot Trial in ARDS

1.2.2 Safety

Previous Phase 1-2 studies with IC14 have documented a favorable safety profile and it has been generally well tolerated. Mild side effects reported in healthy subjects included headaches, rashes and tingling lasting a few hours, runny nose, fever and chills, sleepiness, and light headedness. One serious treatment related adverse event has been reported in a patient with sepsis. That patient experienced hypotension and diffuse exanthema during the IC14 infusion, which resolved with administration of ranitidine, prednisolone and dimetindene. None of the 161 patients who have received IC14 in clinical trials and 9 patients tested in expanded-access protocols have detectable anti-IC14 antibodies. Signs of asymptomatic pleocytosis in the eyes were noted in preclinical toxicology studies of nonhuman primates treated with IC14 antibody; however, there were no eye findings in 26 week long-term tox studies in primates treated with weekly doses

of 20 or 50 mg/kg. IC14 has not been associated with an increase in ophthalmologic infiltrates in 63 humans who have had eye examinations after treatment with IC14 (20 normal volunteers and 43 patients). In a phase 2 trial of IC14 for treatment of acute lung injury (ALI), there were no serious adverse events attributable to IC14.

1.2.3 Pharmacodynamics and Pharmacokinetics

The IC14-treatment regimen chosen for this study is based on results obtained from a study with IC14 in 40 septic patients, showing that a dose of 4 mg/kg, followed by 2 mg/kg daily for 3 days resulted in 99% saturation of mCD14 on circulating monocytes (Reinhart et al. 2004). This regimen resulted in >80% saturation of monocyte CD14 receptors for as long as 4 days, 60-70% saturation at 7 days, and 50% saturation for more than 8 days (Reinhart et al. 2004). Since the risk of developing organ dysfunction is greatest within the first several days, this dose was selected for this patient population. This dose has been well tolerated in previous phase 1/2 studies in patients with severe sepsis, severe community-acquired pneumonia and sepsis, and patients with acute lung injury (ICOS Abbreviated Clinical Study Report 2004; Investigator's Brochure).

In a prior study in 10 patients with ALS (ALS01), a single course of IC14 given over 4-5 days, at cumulative doses of 5-10 mg/kg, was safe and well tolerated. IC14 demonstrated nonlinear kinetics, consistent with target-mediated pharmacokinetics. Monocyte mCD14 receptor occupancy achieved the desired saturation quickly and persisted for 7 days, demonstrating that these IC14 exposure levels were clinically relevant, achievable, and durable. There was a fall of sCD14 in urine during treatment which reflects the decrease in circulating levels of free sCD14.

In an open-label, expanded-access study in ALS patients treated with IC14 8 mg/kg given every 2 weeks for up to 39 weeks, the monocyte mCD14 receptor occupancy rapidly achieved saturation that persisted at 80% two weeks after dosing.

2. INVESTIGATIONAL STUDY AGENT ADMINISTRATION IN THE I-SPY COVID-19 TRIAL

The IC14 intervention will be administered to inpatients via iv infusion.

2.1 Dose Regimen and Dose Groups

Patients hospitalized with pulmonary complications of SARS-CoV-2 infection will be randomized to IC14 at a dose of 4 mg/kg i.v. on Day 1, followed by 2 mg/kg i.v. once daily on Days 2, 3, and 4.

2.1.1 Premedication Regimen and Prophylactic Medications

Premedication is not needed based on clinical experience with IC14 in human subjects. There has been only one significant infusion reaction in 161 subjects treated in clinical trials and 9 patients treated in expanded-access protocols, and this was managed successfully with ranitidine, prednisolone, and dimetindene.

2.2 Eligibility

Eligibility criteria are listed in the I-SPY COVID-19 MASTER Protocol apply to all participants. In addition, the following criteria apply to those patients receiving IC14.

Inclusion (In addition to those defined in MASTER PROTOCOL): None.

Exclusions (in addition to those defined in MASTER PROTOCOL):

- Baseline platelet count <50,000/mm^3
- Presence of serious co-existing infection prior to onset of COVID-19 illness

a. HIV infection not virally suppressed and with pre-hospitalization CD4 counts ≤ 500 cell/mm3
b. Active tuberculosis or a history of inadequately treated tuberculosis
c. Active hepatitis B or hepatitis C viral infection

2.3 Contraindications

- See above Exclusion Criteria
- There are no known drug-drug interactions that contraindicate administration of IC14.

2.4 Concomitant Medications

At Screening, all medications taken up to 30 days prior to the screening visit will be recorded.
In addition, supportive therapies given during the course of the study should be collected and recorded.

Caution should always be exercised when administering concomitant medications based on the individual medication profile and clinical risk-benefit assessment.

2.5 Clinical Evaluation and Procedures

Laboratory evaluations for general safety monitoring are described in the Master Protocol §8.1─ 8.3

If an infusion reaction, including anaphylaxis, occurs during administration of the IC14 study drug, the subject should be treated according to accepted guidelines for anaphylaxis (Sampson 2006) and a blood sample should be collected within two hours for measurement of anti-drug antibodies and serum tryptase.

A serum specimen for anti-drug antibodies should be collected at the time of hospital discharge, or 28 days after the first dose of the IC14 study drug if the subject is still hospitalized.

All study subjects should be monitored for new eye findings, including light sensitivity, redness, pain or change in vision. New eye findings should be recorded as AEs. Dosing of the IC14 study drug should be discontinued if new eye findings develop during dosing.

All subjects should be scheduled for full eye examinations when they are clinically stable, free of COVID-19 symptoms and able to comply with the ocular examination requirements. The eye examination can be scheduled after the subject has been discharged from the hospital.

2.6 Dose Modifications and Management of Toxicity

All study subjects should be monitored for infusion reactions for at least 1 hour after administration of the IC14 study drug.

Dosing will be stopped if a subject shows evidence of an infusion reaction, including anaphylaxis, or develops a significant new infection during treatment, or develops thrombocytopenia (platelet count < 50,000/mm^3). If an infusion reaction occurs, a blood sample for measurement of tryptase should be drawn within two hours of the infusion reaction. Dosing also will be stopped if a subject develops a serious adverse event that is definitely or probably related to the IC14 study drug. No other dose

modifications are planned during the study. All subjects who have dosing stopped for any reason should be continued in the post-dosing observation period of the trial.

New infections will be treated with antibiotics as clinically appropriate. Infusion reactions will be managed with steroids and antihistamines. Thrombocytopenia will be managed with daily monitoring and platelet transfusions if clinically indicated for bleeding.

Dose Justification. Justification for the dosing regimen is provided in Section 1.2.3. above.

2.6.2 Overdose

IC14 is a monoclonal antibody with a favorable safety profile. In preclinical studies in primates, doses of up to 50 mg/kg weekly for 26 weeks were well tolerated. A lead-in period for Safety Review is not needed based on the lack of serious safety events in 161 human subjects treated in clinical trials and 9 subjects treated in expanded-access protocols to date.

An overdose of IC14 (defined as administration of study drug that is greater than the specified regimen dose and frequency), regardless of the presence of an associated SAE, is considered an adverse event of special interest (AESI) and must be documented and reported. Additionally, an SAE associated with an overdose of IC14 must be documented and reported according to the requirements for SAEs.

Please refer to the I-SPY COVID-19 MOP for instructions on reporting overdose.

2.6.4. Definition of Unacceptable Toxicity of IC14

The occurrence of any of the following toxicities after beginning of treatment will be considered unacceptable toxicity if assessed by the investigator to be definitely related or probably related related to study treatment. Occurrence of an unacceptable toxicity will result in a pause in randomization to the IC14 arm while the I-SPY COVID Safety Working Group further evaluates the event and determines whether it is safe to proceed.

1. Any life-threatening event occurring during dosing with IC14 or following the final dose, which is judged to be definitely related or probably related to IC14 (Note: the term life-threatening refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).

2. Important IC14-related medical events that may not result in death, be life-threatening, or require hospitalization, but may be considered serious when, based upon the appropriate medical judgement, they may jeopardize the participant and may require medical or surgical intervention to prevent a life-threatening event as defined above.

The I-SPY COVID Safety Working Group and DMC can decide to halt accrual and randomization into the IC14 arm at any time based on definite or probable toxicity observed during treatment.

3. INVESTIGATIONAL AGENT PHARMACEUTICAL INFORMATION

3.1 Investigational Study Agents (IND 150378, IND Sponsor: QLHC)

The IC14 drug product used for clinical trials is supplied as a 5 mg/mL solution packaged at a concentration of 25 mL in 30 mL glass vials (125 mg/vial). More than one vial might be required to prepare each dose. The IC14 study drug is colorless and will be diluted in a final volume of 250 mL of

sterile 0.9% w/v NaCl and administered as an iv infusion over 2 hours. The properties of the IC14 study drug solution are shown in Table 1.

Study drug will be provided as a pack size of 10 vials/box. All labels for IC14 will meet all applicable requirements of the US FDA and Annex 13 of Good Manufacturing Practices and all local regulations, as applicable.

Table 1. Properties of IC14 Study Drug

Component	Grade	Function	Concentration (mg/mL)	Amount per Vial
IC14 Drug Substance	Not Applicable	Drug Substance	5	125 mg
Polysorbate 20	NF	Stabilizing Agent	0.2 mL/L	0.005 mL
Sodium Acetate Trihydrate	USP	Buffer	3.71	18.55 mg
Sodium Chloride	USP	Tonicity Agent	7.01	35.05
Glacial Acetic Acid	USP	pH adjustment	as required	as required
Water for Injection	USP/EP	Solvent	QS to 25.0 mL	QS to 25 mL

3.2 Reported Clinical AEs and Potential Risks of IC14

3.2.1 Potential Risks

Potential risks include the following:

- Drug allergy. One episode of an anaphylactic infusion reaction likely due to drug allergy has occurred in 170 subjects who have been treated with IC14 (161 in clinical studies and 9 in an expanded access protocol). This episode occurred in a study subject with sepsis who developed hypotension and a rash during the IC14 drug infusion. The drug was stopped and the subject improved following treatment with steroids, famotidine, and dimetindene. Subjects who develop an infusion reaction, including anaphylaxis, during or following IC14 infusion should have the study drug stopped immediately and be treated according to accepted guidelines, beginning with injectable epinephrine (Sampson et al. 2006).
- Thrombocytopenia. Thrombocytopenia is the only adverse event that has occurred at a frequency of more than 5% above placebo. So far no bleeding events have been associated with IC14 treatment. Patients with baseline platelet count <50,000/mm^3 will be excluded from the study. Platelet counts will be monitored during the study.
- New infections. As with any biological response modifier, the possibility exists that treatment will be associated with the emergence of new infections in the lungs or elsewhere in the body. To date in 161 patients and normal volunteers treated with IC14, there has not been an increase in the number of IC14-treated subjects with clinically documented infections as compared with placebo. Significant new infections will be recorded as AEs of special interest (AESI) and will be defined and tracked using standard clinical diagnostic practices as directed by the participant's personal physicians.

- Eye inflammation. In preclinical toxicology studies, some primates treated in single and multiple dose studies had mild cellular infiltrates in the vitreous humor of the eyes, but no eye findings were noted in animals that had stopped dosing according to protocol and underwent postmortem examinations at 28 days. These findings were considered to be of minor toxicological significance. Notably, in a 26-week chronic toxicology study, none of the primates treated with weekly doses of 20 mg/kg or 50 mg/kg had eye findings by slit lamp examination during the study or at necropsy at the end of 26 weeks. There have been no findings in ophthalmological examinations in 63 subjects who have been treated with IC14, including patients (n=43) and normal subjects (n=20). The long-term toxicology study in primates and the experience in patients treated with IC14 indicate that IC14 does not pose a significant risk to the eyes. Refer to latest IB for more details.

3.2.2 Previous Experience with Products of the Same Class

The risks of monoclonal antibodies include infusion reactions, which can be treated with i.v. steroids and antihistamines. Risks of specific monoclonal antibodies depend on the target. Other antibodies to IC14 have not been used in patients. Monoclonal antibodies that modify steps in innate immunity can be associated with secondary infections, but an increase in the number of patients with infections has not been observed in the clinical experience with IC14 in patients with community-acquired pneumonia, sepsis, or ARDS.

3.2.1 Observed AEs in Human Trials

The most frequently reported adverse reactions (occurring in greater than 1% of patients and at least 5% higher frequency with IC14 versus placebo) included only thrombocytopenia (8.4% in IC14 vs. 2.7% in placebo). There have been no associated bleeding events. Changes in liver function and renal function also have occurred, but these did not occur with a frequency of $\geq$5% higher in IC14 than placebo and none of these events were reported as SAEs.

3.2.2 Adverse Events of Special Interest

Adverse events of special interest include:

- New infections
- Thrombocytopenia (platelet count <50,000/mm^3)
- Eye abnormalities (e.g. change in vision, redness, pain, light sensitivity)
- Liver function abnormalities (bilirubin, AST, ALT)
- Reduction in renal function

3.3 Investigational Agent Availability

IC14 is provided under a CTPA between Implicit Bioscience, Inc. and QLHC.

3.4 Investigational Agent Distribution

Shipment of investigational agents to a participating site will not be approved until documentation of IRB approval of the sponsor-approved protocol and consent is available, and the collection of all essential documents is complete.

Investigational agents may be requested by the investigator (or their authorized designees) at each organization. Investigational agents will be shipped directly to the institution or site where the agent will be prepared and administered. The transfer of agents between institutions is not permitted (unless prior approval from the sponsor is obtained). Agents are requested by completing the Investigational Agent

Request Form (to include complete shipping contact information) and submitting the form to the sponsor-designated DCC, see I-SPY COVID MOP for additional details.

Once QLHC or their designee establishes that the requesting site is authorized to receive investigational agents, the order will be forwarded to the manufacturer, who will ship the investigational agent directly to the study site. Instructions for ordering investigational agents are available in the I-SPY COVID MOP.

3.5 Investigational Agent Preparation and Handling

The IC14 study drug will be provided by Implicit Bioscience, Ltd. The calculated volume of IC14 solution at 5 mg/mL will be diluted to a total volume of 250 mL in a 250-mL bag of 0.9% NaCl and the entire bag of 250-mL dose solution will be infused over 2 hours. The IC14 should be inspected visually for particulate matter and discoloration prior to administration. The drug solution should be clear and colorless. The IC14 drug product should not be administered if it contains particulates or is discolored.

Only authorized site staff may supply or administer the IC14 study intervention.

3.6 Investigational Agent Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all investigational agents. The investigator is required to maintain adequate records of receipt, dispensing, and final disposition of the study agent. On the receipt, record from whom study agent was received and to whom study agent was shipped, date, quantity, and batch or lot number. On the dispensing record, note quantities and dates study agents were dispensed to and returned by each participant.

3.7 Investigational Agent Packaging and Labeling

The IC14 study drug is packaged and labeled by Implicit's GMP storage facility maintained by Caligor Coghlan Pharma Services (Bastrop, TX) according to GCP and FDA requirements. Labels will be printed and attached to the outer carton prior to shipping to the site. Each will be labeled with a single panel label that will include the name "I-SPY COVID". Additional information pre-printed on the carton will include, but is not limited to, the following:

- Blank spaces to write the study number and investigator name
- Agent identification
- Lot number
- Dosing instructions. Detailed dosing instructions and preparation of infusion materials are described in the Pharmacy Manual.
- Blank spaces to write the participant's identification number, initials, and date dispensed

Additional information may be attached to a label on the product carton.

3.8 Investigational Agent Storage

Glass vials (30 mL volume) of IC14 containing 125 mg of IC14 at 5 mg/mL are stored in tamper-resistant cardboard cartons that contain 10 vials per carton and are secured by a foam insert. IC14 must be stored and transported at 2-8^{o}C (36-46^{o}F) in an upright position and protected from light. The temperature of all drug shipments will be monitored and recorded by TempTale devices added to pre-conditioned and qualified shipping containers.

Detailed descriptions of the storage and handling instructions for IC14 are provided in the MOP.

3.9 Investigational Agent Destruction/Disposal

Once agent accountability is performed, the participating sites should use local/institutional procedures for disposal of returned/unused study agent and bottles/containers. Copies of all certificates of destruction of any unused study agent must be provided to DCC. **Prior to destruction,** the pharmacist should contact the assigned study monitor.

Unused investigational agents shall be returned to the designated facility. Please contact DCC for instructions.

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Quantum Leap Healthcare Collaborative

I-SPY COVID-19 TRIAL

MEMORANDUM FOR RECORD

To: I-SPY COVID-19 Site Investigators
From: I-SPY COVID-19 Clinical Trials OperationsWorking Group Quantum Leap Healthcare Collaborative
Subject: I-SPY COVID Trial Revision 4 Memo 1: Changes to Enrollment Eligibility
CC: FDA, Wake Forest central Institutional Review Board

Date: May 8, 2021

Dear Investigators:

The purpose of this Memorandum is to inform investigational sites and the Institutional Review Boards (IRBs)/Ethics Committee (EC) about upcoming changes to the section titled, **4.3 Exclusion Criteria, sub-section G** of the I-SPY COVID TRIAL Master Protocol, Amendment 4, version 4/1/2021.

The change reflects a decision to modify exclusion criteria for the time since requirement for high flow oxygen or ventilation from greater than 72 hours to greater than 120 hours (5 days).

Section 4.3 sub-section G will change from:

G. Time since requirement for high flow oxygen or ventilation greater than 72 hours.

To:

G. Time since requirement for high flow oxygen or ventilation greater than 120 hours (5 days).

This memo should be filed in your study folder. These changes take effect for new enrollments upon IRB approval. In addition, the changes will be implemented in the next protocol amendment.

If you have any questions, please do not hesitate to contact the I-SPY COVID-19 Project Management Office (PMO).

Sincerely,

I-SPY 2 COVID-19 Study Team
Quantum Leap Healthcare Collaborative

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