CLINICAL STUDY PROTOCOL

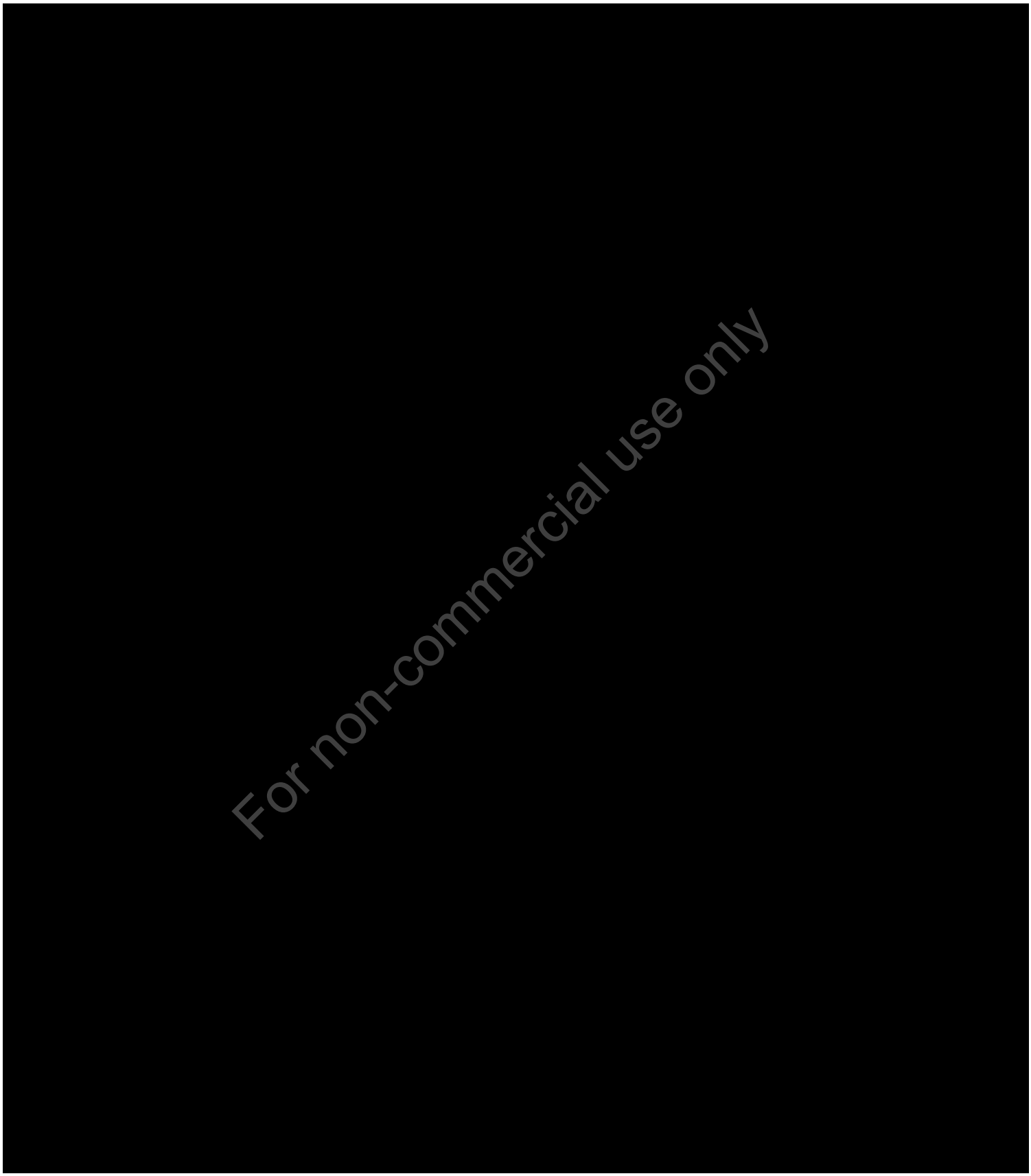
NCT Number: NCT04493242

Study Title: Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicles Infusion Treatment for COVID-19 Associated Acute Respiratory Distress Syndrome (ARDS): A Phase II Clinical Trial

Study Number: DB-EF-PHASEII-001

Protocol Version and Date:

Amendment 6: 08 Feb 2021



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Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicles Infusion Treatment for COVID-19 Associated Acute Respiratory Distress Syndrome (ARDS): A Phase II Clinical Trial

Protocol Number:	DB-EF-PHASEII-001
Investigational New Drug (IND) Number:	21669
Study Phase:	II
Study Type:	Double-Blinded, Placebo-controlled, Randomized Controlled Trial
Study Investigational Product:	Bone Marrow Mesenchymal Stem Cell (bmMSC)-Derived Extracellular Vesicles (via Intravenous Administration)
Administrative Amendment:	6
Date & Document Version:	8 February 2021 | 7.0
Administrative Amendment:	5
Date & Document Version:	31 December 2020 | 6.0
Administrative Amendment:	4
Date & Document Version:	2 November 2020 | 5.0
Administrative Amendment:	3
Date & Document Version:	7 October 2020 | 4.0
Administrative Amendment:	2
Date & Document Version	31 August 2020 | 3.0
Administrative Amendment:	1
Date & Document Version	7 August 2020 | 2.0
Original Protocol:	-
Date & Document Version	24 July 2020 | 1.0
Sponsor:	Direct Biologics, LLC 13492 Research Blvd, Ste 120-758 Austin, TX 78750

CONFIDENTIAL

This protocol is provided to you as an Investigator, potential Investigator, or consultant for review by you, your staff, and Ethics Committee/Institutional Review Board (EC/IRB). The information contained in this document is regarded as

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PROTOCOL AUTHORIZATION

INVESTIGATOR'S AGREEMENT

Title: Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicles Infusion Treatment for COVID-19 Associated ARDS: A Phase II Clinical Trial

Protocol Number: DB-EF-PHASEII-001

Signature of Investigator	dd/mm/yy
Printed Name of Investigator and Title	
Site Number(s):	

By my signature, I agree to supervise and oversee the conduct of this study and to ensure its conduct is in compliance with the protocol, informed consent, IRB/EC procedures, instructions from Direct Biologics representatives, the Declaration of Helsinki, International Conference on Harmonisation (ICH) Good Clinical Practices (GCP) guidelines, and the applicable parts of the United States (US) Code of Federal Regulations (CFR) and local regulations governing the conduct of clinical studies.

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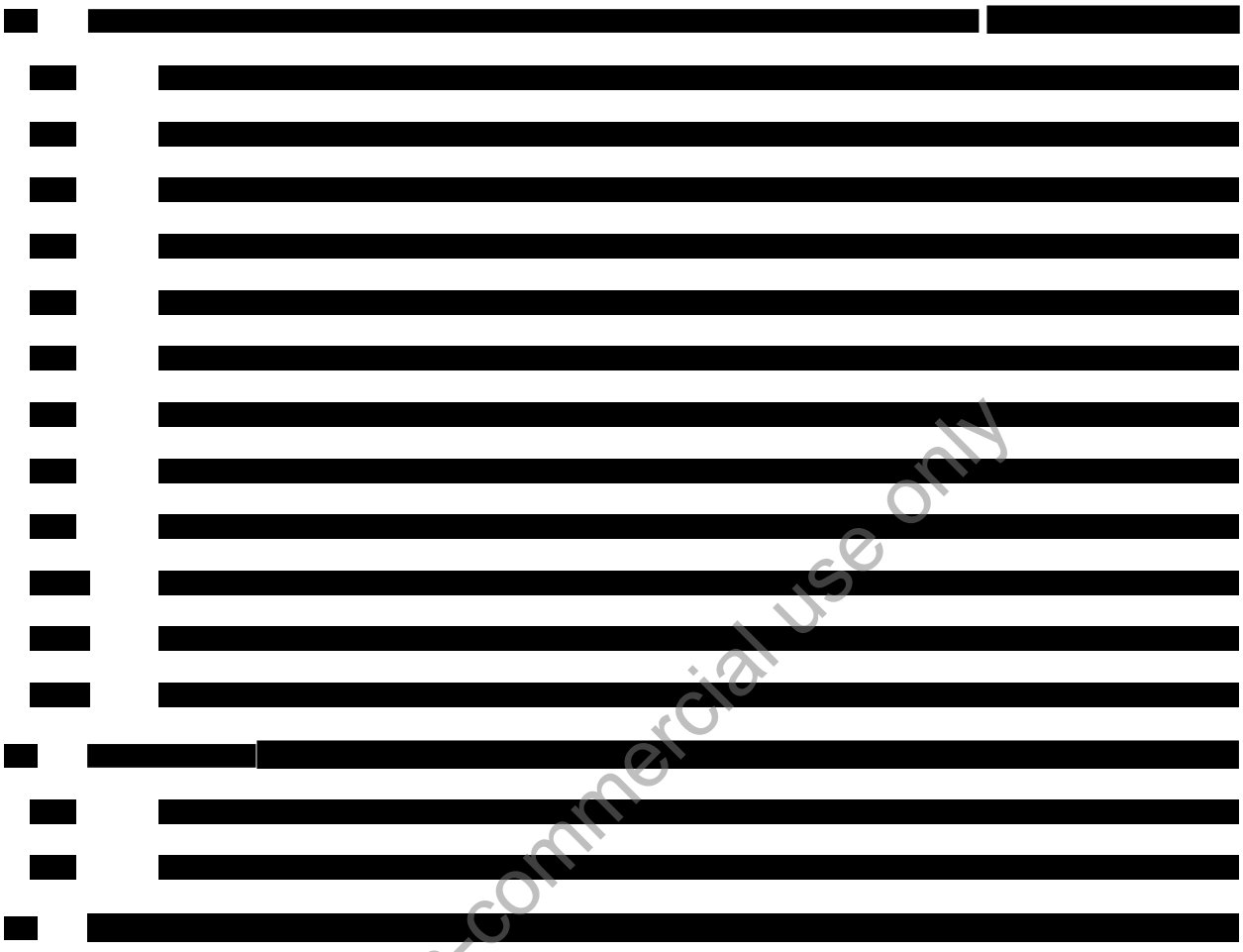

LIST OF IN-TEXT TABLES

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1. PROTOCOL SUMMARY

1.1 Synopsis

Title: Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicles Infusion Treatment for COVID-19 Associated Acute Respiratory Distress Syndrome (ARDS): A Phase II Clinical Trial

Study Description: ExoFlo™ Infusion Treatment for COVID-19 Associated ARDS (EXIT COVID-19), a multicenter, double-blinded, placebo-controlled, randomized control trial to evaluate the efficacy and safety in COVID-19 associated moderate to severe ARDS.

Objectives: To evaluate the safety and efficacy of intravenous (IV) administration of bone marrow mesenchymal stem cell derived extracellular vesicles (EVs), ExoFlo, versus placebo as treatment for COVID-19 associated moderate-to-severe Acute Respiratory Distress Syndrome (ARDS).

Endpoints: Primary Endpoint:

1) Improvement in partial pressure of arterial oxygen to fraction of inspired oxygen (PaO_2/FiO_2) ratio from pre-infusion baseline (Day 0) to Day 7. PaO_2 may be calculated from arterial blood gas (ABG) or imputed from the SpO_2 daily ([redacted]).

Note: Day 0 is designated as Day of Screening and Day 1 as Day of the First Study Intervention. Patients may be screened and treated within the same 24 hours—in this scenario, Day 1 will be synonymous with Day 0 & the pre-infusion value used will be from Day 1.

Secondary Endpoints:

2) Time to recovery as defined by the number of days from the first study treatment until return of oxygenation saturation (SpO2) ≥ 93% on room air (or PaO2/FiO2 ≥ 300 mmHg). If patient has chronic lung disease, recovery is defined as pre-COVID-19 SpO2 and O2 support.
3) Incidence of serious adverse events.

All-cause mortality.

Exploratory Endpoints:

5) Viremia: qualitative serum severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) ribonucleic acid (RNA) load on Days 0 or 1, 15, 29, and 61.
6) Acute phase reactants: C-reactive protein (CRP), D-dimer, Ferritin, interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) on Days 1, 4, 7, 10, 15, 29. CRP, D-dimer, & Ferritin also on Day 0.

7) Immune cell counts: Absolute neutrophil count (ANC); CD3+, CD4+, CD8+ thymus cells also known as T lymphocytes (T cells); natural killer (NK) cells on Days 1, 4, 7, 10, 15, 29.
8) Sequential Organ Failure Assessment (SOFA) Score on Days 1, 15, 29 for patients who are still hospitalized.
9) Quality of life (QOL) assessment for patients who are discharged. EQ-5D-5L on Days 29 and 61. See Appendix 11.2 for sample EQ-5D-5L, which includes dimensions of mobility, self-care, usual activities, pain/discomfort, and anxiety/depression.

Study Population: Up to 120 adult male and female patients between 18 and 85 years of age hospitalized with COVID-19 associated ARDS.

Phase: Phase II

Site Number: 2-15

Description of Intervention: Patients will be randomized to one of the following:

Treatment Arm 1: PLACEBO Normal saline 100 mL

Treatment Arm 2: IP (Exoflo) 10ml dose in Normal saline 90 mL, which is approximately 800 billion EVs (Lot# P-441-1901-E5, P-441-2004-C5).

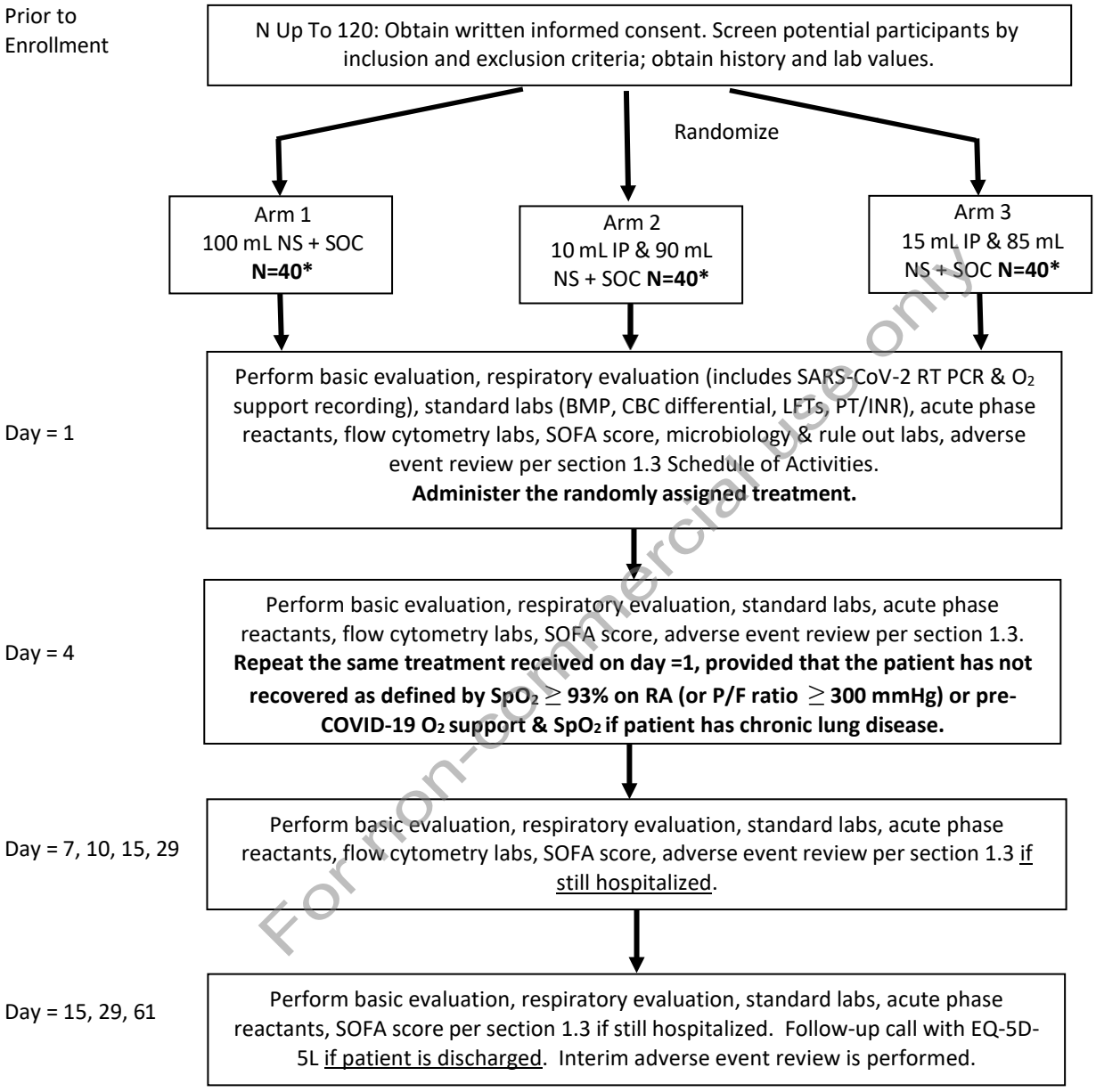
Treatment Arm 3: IP (Exoflo) 15ml dose in Normal saline 85 mL, which is approximately 1.2 trillion EVs (Lot# P-441-1901-E5, P-441-2004-C5).

The intervention will be infused over 60 minutes on Day 1 and repeated on Day 4 provided that the patient has not recovered as defined by SpO2 ≥ 93% on RA or PaO2/FiO2 ≥ 300 mmHg. If patient has chronic lung disease, recovery is defined as baseline SpO2 and O2 support.

Allocation of Intervention: Patients will be randomized 1:1:1 to each of the three treatment arms initially. The Interactive Response Technology will notify the DSMB such that a safety and interim analysis can be held following day 7 of the 60th patient randomized.

Duration: Each patient will be enrolled in the study for an estimated 60 days.

1.2 Schema



Abbreviations: AE=adverse event; BMP=basic metabolic profile; CBC=complete blood count; EQ-5D-5L=quality of life assessment; IP=investigational product; IV=intravenous; LFT-liver function test; NK cells=natural killer cells; NS=normal saline; P/F ratio_= partial pressure of arterial oxygen to fraction of inspired oxygen ratio; PT/INR= prothrombin time/international normalized ratio; RNA=ribonucleic acid; SOC=standard of care; SOFA=sequential organ failure assessment score; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

1.1 Schedule of Activities*

NOTE: If eligibility criteria are met, patients may receive the first study intervention on the same day as screening, such that Day 1 is synonymous with Day 0 and the pre-infusion value from Day 1 may be used in place of the value from Day 0. See table footnotes A-J for answers to other common site questions.

Table 1. Schedule of Activities.

	Screen	Day						
PROCEDURES	**0 or 1**	**1**	**4**	**7**	**10**	**15**	**29**	**61**
Visit	***0***	***1***	***2***	***3***	***4***	***5***	***6***	***7***
BASIC EVALUATION								
Informed consent	X							
Inclusion/Exclusion	X	X						
Demographics	X							
Medical history	X							
Days of Illness Before Admission	X[A]							
Concomitant Meds	X	X	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
Vital Signs[C]	X	X	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
Height & Weight[I]	X[I]							
Physical examination	X	X	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
Glasgow Coma Score	X	X	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
Pregnancy Test[D]	X[D]	X[D]						
EKG[I]	X[I]	PRN AS INDICATED; N/A AFTER D/C						
Randomization		X						
Administer IV Study Intervention		X	X[E]					
RESPIRATORY EVAL								
SARS-CoV-2 RT-PCR[F]	X	X[J]				X[B]	X[B]	X[B]
Record Prone Posn (Y/N, Freq)	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
PaO2/FiO2 ratio	X	CALCULATE DAILY UNTIL D/C; SEE *SECTION 11.1*						
Record O2 Support	X	DAILY UNTIL D/C; NOTE NC (LPM), FM (LPM), NRB, BiPAP (FiO2), HFNC O2 (FiO2), MV (FiO2, PEEP), HFOV (FiO2).						
CXR or CT chest	X	PRN AS INDICATED; N/A AFTER D/C						

STANDARD LABS								
BMP	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
CBC with differential	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
LFTS (including Bilirubin)	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
PT/INR	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
PTT	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	X[B]
ACUTE-PHASE REACTANTS								
CRP	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
D-dimer	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
Ferritin	X	X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
Il-6		X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
TNF-α		X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
FLOW CYTOMETRY								
T-lymphocyte panel (stain $CD3^+$, $CD4^+$, $CD8^+$)		X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
NK cells count ($CD3^-$ $CD56^+$ subset of lymphocyte gate)		X[J]	X[B]	X[B]	X[B]	X[B]	X[B]	
METRIC								
SOFA Score		X[J]				X[G]	X[G]	
EQ-5D-5L (Call Survey)							X[H]	X[H]
MICROBIOLOGY								
Urinalysis[I]	X[I]	PRN AS INDICATED; N/A AFTER D/C						
Urine culture[I]	X[I]	PRN AS INDICATED; N/A AFTER D/C						
Blood culture x 2[I]	X[I]	PRN AS INDICATED; N/A AFTER D/C						
Sputum culture[I]	X[I]	PRN AS INDICATED; N/A AFTER D/C						
RULE OUT TESTS								
Mycoplasma IgM[I]	X[I]							
QuantiFERON Gold[I]	X[I]							
Legionella Ag[I]	X[I]							
***Strep. Pneumoniae* Ag**[I]	X[I]							
Influenza A/B PCR[I]	X[I]							
Adverse Events Review	X	DAILY UNTIL D/C; CALL F/U ON DAY 15, 29, 61						

Abbreviations: Ag=antigen; ANC=absolute neutrophil count; BiPAP=bilevel positive airway pressure; BMP=basic metabolic profile; CRP=C-reactive protein; CBC=complete blood count; char=characteristics; CT=computed tomography; CXR=chest x-ray; D/C=discharge; EKG=electrocardiogram; EQ-5D-5L=5-dimentional quality of life assessment; FiO2=fraction of inspired oxygen; FM=face mask; Freq=frequency; HF NC O2=high flow nasal cannula oxygen support; HFOV=high frequency oscillatory ventilation; IgM=immunoglobulin M; IP=investigational product; IV=intravenous; LFT-liver function test; LPM=liters per minute; Meds=medications; MV=mechanical ventilation; N/A=not applicable; NC=nasal cannula; NK cells=natural killer cells; NRB=nonrebreather; PCR=polymerase chain reaction; PEEP=positive end expiratory pressure; POS=positioning; PT/INR= prothrombin time/international normalized ratio; PTT=partial prothrombin time; Quant=quantitative; RT-PCR=reverse transcriptase polymerase chain reaction; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; SOFA=sequential organ failure assessment score; T cells=thymus cells also known as T lymphocytes; TNF-α=tumor necrosis factor alpha.

A: The number of COVID-19 symptomatic days prior to the current admission is recorded along with the number of days from admission to first study administration.

B: Patients will be monitored for 72 hours as inpatients following each study intervention. Recovery will be defined in the study as SpO_2 reaching ≥ 93% on RA (or P/F ≥ 300 mm Hg) or pre-COVID-19 SpO_2 or O_2 support if patient has chronic lung disease.

- Following the first treatment on Day 1, the patient may be discharged on Day 4 or later.
- Following the second treatment on Day 4, the patient may be discharged on Day 7 or later.
- If a patient received the first but not the second treatment, he/she may be discharged prior to Day 7.
- Following patient's discharge, no labs will be drawn. Follow-up via phone-call only on Days 29 and 61.

C: During the infusion, measurements +/- 2 minutes of specified times for q5min vitals and +/- 5 minutes of specified times for q15min vitals are considered permissible; following the infusion, vital sign measurements are permissible +/-15 minutes of the specified times. Temperature is required only 5 minutes prior and q15min during the course of the infusion.

D: Serum pregnancy test (in women of childbearing potential) will be obtained on Day 0. Urine dipstick obtained on Day 1 at 4 hours prior to the first study treatment if >24 hours passed since the serum pregnancy test.

E: Not all patients who recovered will receive a repeat study intervention. Patients who recovered by Day 4 do not receive a repeat study intervention.

F: Qualitative SARS-CoV-2 RT PCR will be used for Day 0 or 1 (prior to the first dose), 15, 29, 61. If there is a shortage of tests, documentation of positive test within 14 days prior to admission suffices for Day 0 or 1.

G: SOFA Score will be administered only if patient is still hospitalized.

H: ED-5D-5L score will be administered over the phone only if patient is discharged. Note: This is not a meaningful measure for patients who are still hospitalized due to common use of IV sedatives or PO antipsychotics for delirium. A baseline prior to study infusion is not obtained because the patient is hypoxic, and the hospital staff needs to prioritize diagnostics and treatment rather than time-consuming quality of life metric.

I: The Screen column refers to tests obtained prior to the first study treatment, usually obtained on Day 0, i.e., 24 hours prior to the first study treatment. However, height & weight, EKG, microbiology, and rule out tests do not have to be repeated on Day 0 if obtained once following the current hospital admission.

J: All lab tests including SARS-CoV-2 viremia, standard tests, acute phase reactants, flow cytometry will be obtained around 1-2 hours prior to the first study treatment on Day 1. On Day 1, SOFA score will be calculated on the morning values prior to the study treatment.

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5. STUDY POPULATION

5.1 Inclusion Criteria

Eligibility for study enrollment includes meeting all of the following criteria:

1. Provision of signed and dated informed consent form (either by the individual or by the individual's healthcare proxy).
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or female aged 18-85.
4. COVID-19 positive as defined by positive Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) SARS-CoV-2.
5. Moderate to severe ARDS as defined by modified Berlin definition, * which includes timing within 1 week of known clinical insult or new or worsening respiratory symptoms; bilateral opacities not fully explained by effusions, or lung collapse; respiratory failure not fully explained by cardiac failure or fluid overload; $PaO_2/FiO_2 \leq 200$ mm Hg.
6. Hypoxia requiring noninvasive oxygen support such as Nasal Cannula (NC), Nonrebreather (NRB), Bilevel Positive Airway Pressure (BIPAP), Continuous Positive Airway Pressure (CPAP), high flow nasal cannula oxygen (HFNC O2) or mechanical ventilation (MV) despite initiating standard of care.
7. If the candidate is either a male or female of reproductive potential, he or she must agree to use of double barrier method of highly effective birth control contraception such as condoms with oral contraceptive pill or choose to remain abstinent if already practicing abstinence during the screening period. The required duration of usage of double barrier method OR maintenance of abstinence must include the time from the beginning of the screening period until 90 days following the last dose of the study treatment.

*Modified Berlin definition used in this study is the full Berlin definition, albeit without the PEEP specification, which implies mechanical ventilation. [redacted]

**To ensure flexible adaptation of products approved by the FDA for the treatment of severe COVID-19, standard of care is defined as the NIH Current COVID-19 Treatment Guidelines [redacted]

5.2 Exclusion Criteria

Exclusion from study enrollment includes meeting one or more of the following criteria:

1. Vulnerable populations such as pregnant patients, children, individuals with severe physical or mental disabilities who cannot provide meaningful consent.
2. Active malignancy requiring treatment within the last five years.
3. Major physical trauma in the last 5 days, including motor vehicle accidents, assaults, mechanical falls with sequalae of significant bleeding or craniofacial bruising, and surgeries.
4. Active tuberculosis or cystic fibrosis.
5. Severe chronic respiratory disease including chronic obstructive pulmonary disease or pulmonary fibrosis requiring home oxygen > 5L/min.
6. Use of extracorporeal membrane oxygenation (ECMO) during the current hospitalization.
7. Pre-existing pulmonary hypertension.
8. Severe pre-existing hepatic impairment (presence of cirrhosis, liver function tests (LFTs) ≥ 6x baseline, INR ≥ 2.0).
9. Pre-existing Chronic Kidney Disease (CKD) stage IIIb or End Stage Renal Disease (ESRD) prior to onset of COVID-19 (stage I, II, and IIIa are acceptable)
10. Irreversible coagulopathy (e.g., frequently occluded vascular access despite anticoagulation, precipitous platelet drops concurrent with end-organ damage suggesting consumptive process) or irreversible bleeding disorder (e.g., frequent bleeding from vascular access, endotracheal tubes, and foley).
11. Pneumonia clearly attributable to a non-COVID-19 related process, including aspiration pneumonia or pneumonia that is exclusively bacterial, or originating from a diagnosed alternative virus (e.g., influenza).
12. Patients who are not full code.
13. Endotracheal intubation duration ≤ 24 hours.
14. Moribund—expected survival < 24 hours.
15. Severe metabolic disturbances on presentation (e.g., ketoacidosis, pH < 7.3)

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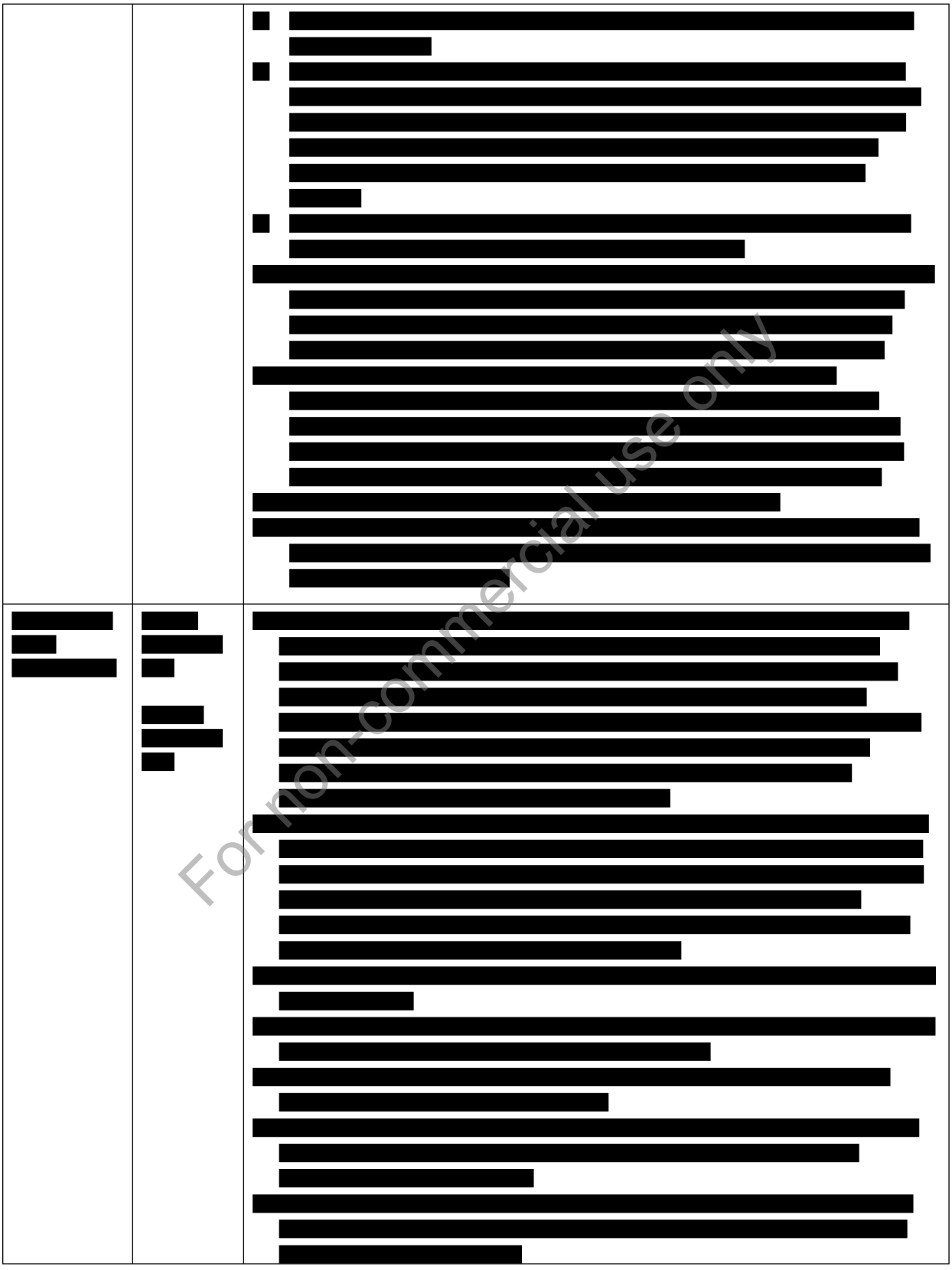

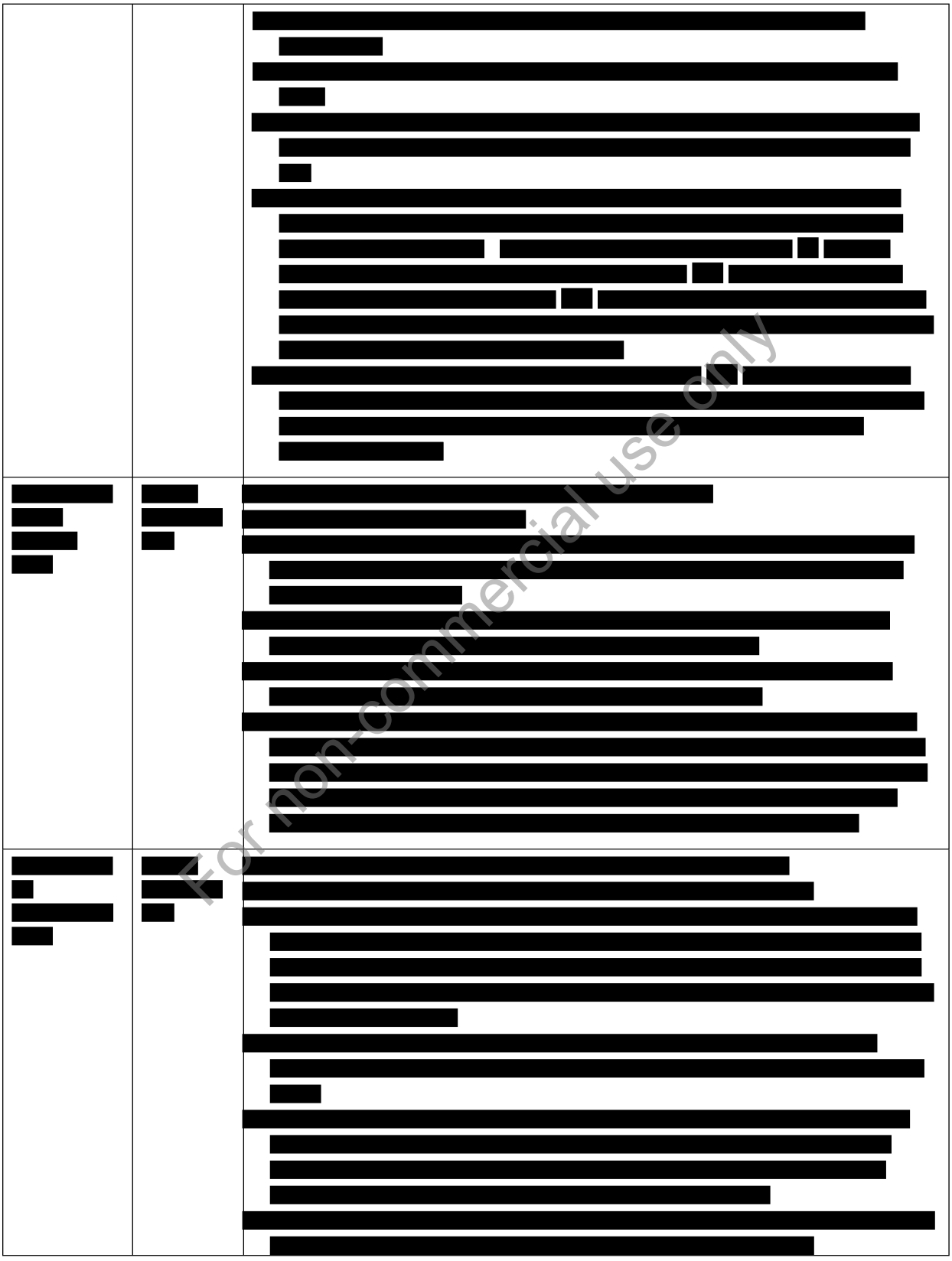

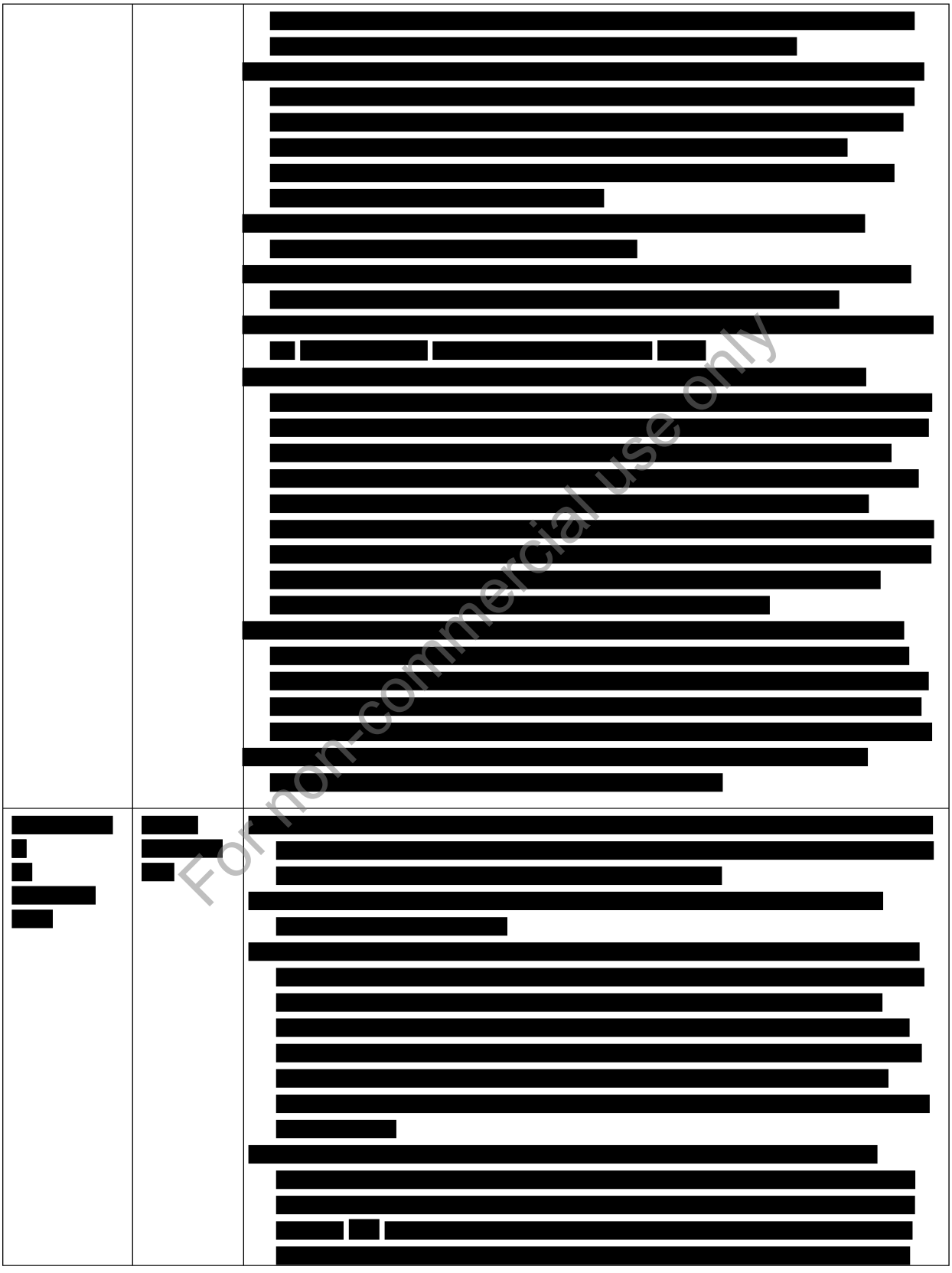

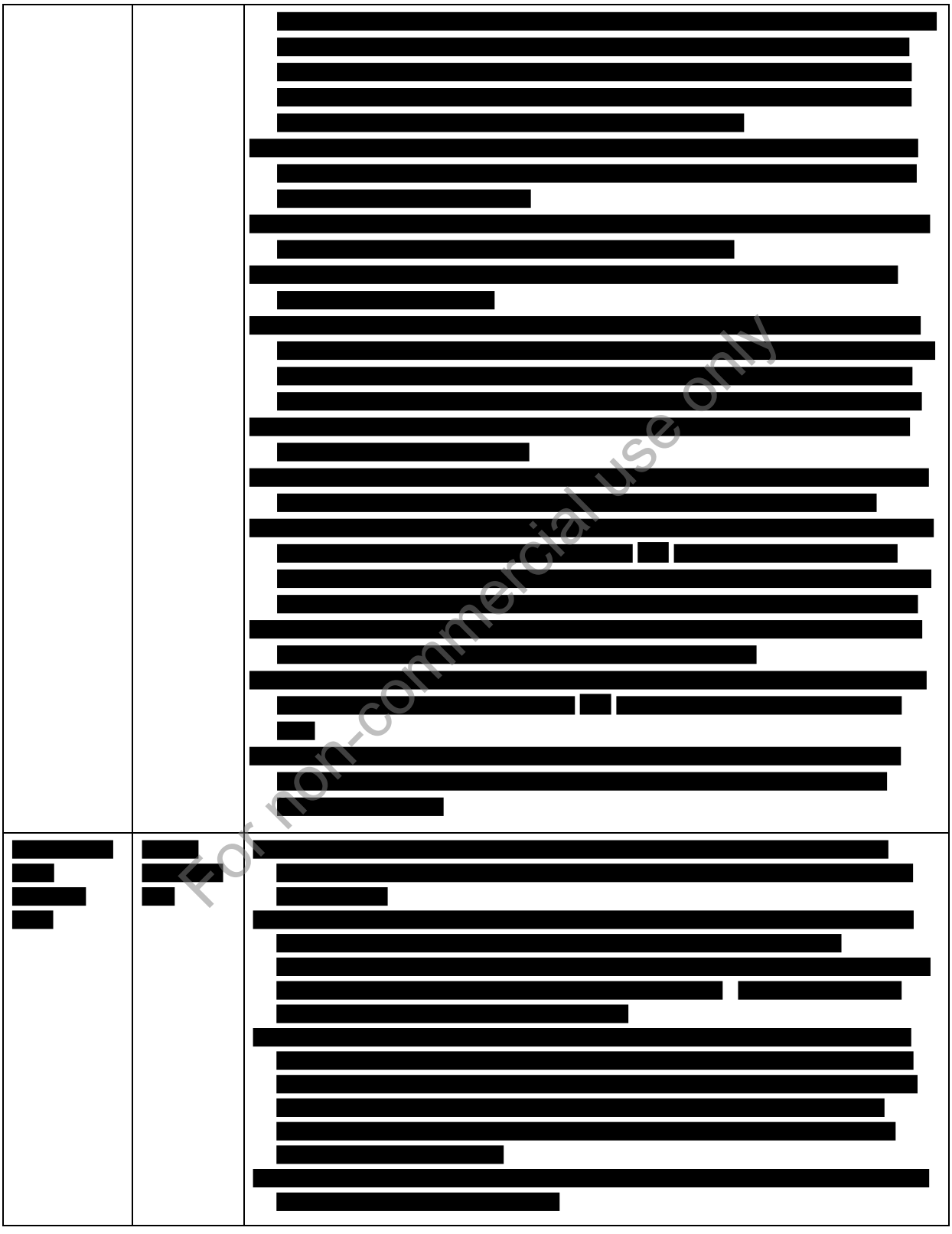

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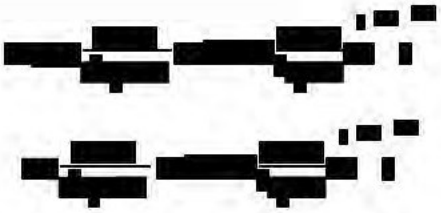

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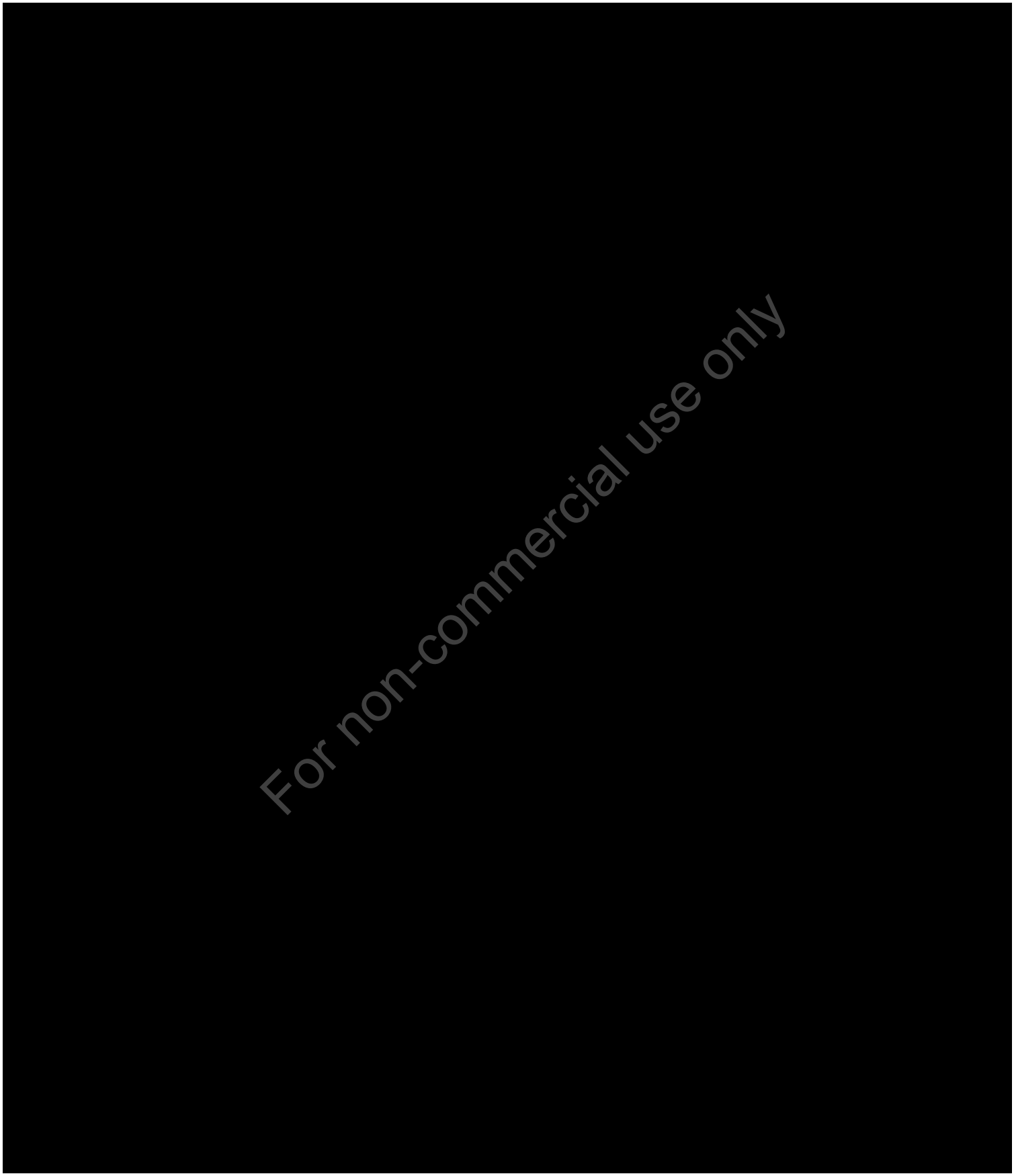

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