Protocol Number: IRC-005

A Randomized Double-Blind, Phase 3 Study Comparing the Efficacy and Safety of High-Titer versus Low-Titer Anti-Influenza Immune Plasma for the Treatment of Severe Influenza A

Sponsored by:

Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
Division of Clinical Research (DCR)
National Institute of Allergy and Infectious Diseases (NIAID)
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Conducted by:

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
ALT	Alanine Aminotransferase
Anti-HBc	Hepatitis B Core Antibodies
APACHE	Acute Physiology And Chronic Health Evaluation (Score)
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate Aminotransferase
CAP	College of American Pathologists
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendment Of 1988
CLIP	Clinical Laboratory Improvement Program
COI	Conflict of Interest
CSO	Clinical Safety Office
DFA	Direct Fluorescent Antibody
DIN	Donation Identification Number
DSMB	Data and Safety Monitoring Board
ECMO	Extracorporeal Membrane Oxygenation
eCRF	Electronic Case Report Form
EUA	Emergency Use Authorization
FDA	Food and Drug Administration
FP24	Frozen Plasma-24
FFP	Fresh Frozen Plasma
GCP	Good Clinical Practice
HAI	Hemagglutination Inhibition
HBsAg	Hepatitis B Virus Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HTLV	Human T-Cell Lymphotropic Virus
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISBT	International Society of Blood Transfusion
IVIG	Intravenous Immune Globulin
IWRS	Interactive Web Response System
MAP	Mean Arterial Pressure
MOP	Manual of Operations
NAT	Nucleic Acid Testing
NA	Neuraminidase
NEWS (NEW score)	National Early Warning Score
NIAID	National Institute of Allergy And Infectious Diseases
NIRC	NIAID Influenza Research Collaboration
OCRPRO	Office of Clinical Research Policy And Regulatory Operations

OP	Oropharyngeal
OTC	Over the Counter
PCR	Polymerase Chain Reaction
PELOD	Pediatric Logistic Organ Dysfunction
PEWS (PEW score)	Pediatric Early Warning Score
PK	Pharmacokinetic
SAE	Serious Adverse Event
SERF	Safety Expedited Report Form
SOFA	Sequential Organ Failure Assessment
SSS	Social & Scientific Systems, Inc.
TACO	Transfusion-Associated Circulatory Overload
TRALI	Transfusion-Related Acute Lung Injury
UP	Unanticipated Problem
UPnonAE	Unanticipated Problem that is not an Adverse Event

PROTOCOL SUMMARY

Full Title: A Randomized Double-Blind, Phase 3 Study Comparing the Efficacy and Safety of High-Titer versus Low-Titer Anti-Influenza Immune Plasma for the Treatment of Severe Influenza A

Short Title: IRC-005

Clinical Phase: 3

IND Sponsor: OCRPRO, NIAID

Conducted by: NIAID Influenza Research Collaboration (NIRC)

Sample Size: 150
Accrual Ceiling: Up to 300 subjects will be screened to randomize 150 subjects

Study Population: Subjects aged two weeks or older, including children and pregnant women, hospitalized with severe influenza A infection

Study Duration: November 2015 – November 2018

Study Design: This randomized, double-blinded, multicenter Phase 3 trial will assess the efficacy and safety of anti-influenza immune plasma (also referred as "anti-influenza plasma") as an addition to standard of care antivirals in subjects hospitalized with severe influenza A infection (as defined in the inclusion criteria). Up to 40 sites in the United States will participate in this protocol. Adults and children may participate. There is no exclusion for pregnancy.

A total of 150 eligible subjects will be randomized in a 2:1 ratio to receive either high-titer anti-influenza plasma or control (low-titer) plasma. All subjects will receive standard care antivirals. Randomization will be stratified by:

- Severity: in the intensive care unit (ICU), non-ICU hospitalization requiring supplemental oxygen, or non-ICU hospitalization not requiring supplemental oxygen.
- Age category (child/adult)

Subjects will be assessed on Day 0 (baseline) and on Days 1, 2, 3, 7, 14, and 28. For participants who are not hospitalized on Days 2, 14, and 28, contact with the participant for the purpose of study data collection on those days may be performed by telephone.

All subjects will undergo a series of efficacy, safety, and hemagglutination inhibition (HAI) assessments during the study. Blood samples will be collected on Day 0, 1, 3, and 7. Oropharyngeal (OP) swabs for influenza PCR will be obtained on Days 0 and 3.

Study Agent: All plasma is from male donors to minimize the risk of transfusion-related acute lung injury (TRALI). The high-titer arm will receive human plasma with influenza A/H1N1 and A/H3N2 HAI titers of at least 1:80. The sponsor will try to supply units with as high a HAI as possible. The antigens for the HAI will be

for contemporary strains defined as strains contained within that year's seasonal trivalent influenza vaccine.

The control (low-titer) arm will receive human plasma with influenza A/H1N1 and A/H3N2 HAI titers of 1:10 or less.

Primary Objective: Evaluate the efficacy of treatment with high-titer versus low-titer anti-influenza immune plasma as an addition to standard care in subjects hospitalized with severe influenza A by clinical status at Day 7.

Primary Endpoint: Subject clinical status at Day 7 (6-point ordinal scale):

1. Death
2. In ICU
3. Non-ICU hospitalization, requiring supplemental oxygen
4. Non-ICU hospitalization, not requiring supplemental oxygen
5. Not hospitalized, but unable to resume normal activities
6. Not hospitalized with full resumption of normal activities

Secondary Objectives: 1. Evaluate the efficacy and safety of treatment with high-titer versus low-titer anti-influenza immune plasma plus standard care, using the following parameters:

- Subject clinical status (6-point ordinal scale) at Days 1, 2, 3, 14, and 28
- Duration of initial hospitalization
- 28-day mortality
- In-hospital mortality during initial hospitalization
- Composite of mortality and hospitalization at Days 7, 14, and 28
- Change from baseline to Day 3 and Day 7 in National Early Warning (NEW)/ Pediatric Early Warning (PEW) score
- Duration of supplemental oxygen use among those requiring oxygen at randomization
- Incidence of new oxygen use during the study
- Duration of ICU stay among those requiring ICU admission at randomization
- Incidence of new ICU admission during the study
- Duration of mechanical ventilation use among those requiring mechanical ventilation at randomization
- Incidence of new mechanical ventilation use during the study
- Duration and severity of acute respiratory distress syndrome (ARDS) among those meeting the definition of ARDS at randomization
- Incidence and severity of new ARDS during the study
- Duration of extra corporeal membrane oxygenation (ECMO) use among those requiring ECMO at randomization
- Incidence of new ECMO use during the study
- Change from baseline to Day 3 and Day 7 in sequential organ failure assessment (SOFA) score for age ≥ 18 years, and pediatric logistic organ dysfunction (PELOD) score for age < 18 years
- Disposition (home, rehabilitation, chronic nursing facility, initial hospitalization ongoing at 28 days, died in hospital) following the initial hospitalization

- Percent of subjects with influenza virus detectable in OP sample at Day 3

2. Compare the plasma and control groups for HAI titers at Days 1, 3, and 7.

3. Evaluate the safety of high titer anti-influenza plasma as compared to low titer plasma as assessed by:

- Cumulative incidence of grade 3 and grade 4 adverse events (AEs)
- Cumulative incidence of serious adverse events (SAEs)

PRÉCIS

Significant morbidity and mortality from influenza infections occur despite treatment with current antivirals. This randomized, double-blinded, multicenter phase 3 trial will assess the efficacy and safety of high-titer versus low-titer anti-influenza immune plasma in addition to standard care antivirals for the treatment of severe influenza A. Hospitalized subjects with severe influenza A will be eligible for study participation. Up to 40 sites in the United States will participate in this protocol. Adults and children may participate. There is no exclusion for pregnancy.

A total of 150 eligible subjects will be randomized in a 2:1 ratio to receive either high-titer anti-influenza plasma or control (low-titer) plasma. All plasma is from male donors to minimize the risk of TRALI.

Subjects will be assessed on Study Day 0 (baseline) and on Study Days 1, 2, 3, 7, 14, and 28. For participants who are not hospitalized on Days 2, 14, and 28, contact with the participant for the purpose of limited study data collection for those days may be performed by telephone. Study visits on Days 1, 3, 7 must occur in person. All subjects will undergo a series of efficacy, safety, and HAI assessments during the study. Blood samples will be collected on Day 0, 1, 3, and 7. OP swabs for influenza PCR will be obtained on Days 0 and 3.

1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Influenza

Despite antivirals and vaccines, influenza is responsible for 226,000 excess hospitalizations and 30,000 - 50,000 deaths each year in the United States alone.[1] The number of deaths is estimated to be over 500,000 worldwide. Circulating H1N1 and H3N2 isolates are highly resistant to amantadine and rimantadine, and consequently, these drugs are not therapeutic. Currently, the circulating strains are susceptible to oseltamivir and zanamivir. Previous circulating seasonal H1N1 influenza viruses have high-level resistance to oseltamivir. Additionally, resistance to oseltamivir occurring on therapy has been described in seasonal and avian influenza. [2] Due to the morbidity and mortality occurring despite treatment with current antivirals as well as the limited therapeutic options for influenza, additional therapeutics for influenza are warranted. One potential therapeutic that is fairly rapidly available is the use of high-titer anti-influenza immune plasma.

1.2 Passive Immunotherapy in Influenza

1.2.1 Summary of Previous Pre-Clinical Studies

One small study in a BALB/c mouse model demonstrated the therapeutic efficacy of $F(ab)_2$ fragments against a lethal dose of H5N1 virus. [3] In this model, the mice received an intraperitoneal injection of 50, 100, or 200 µg $F(ab)_2$ fragments/mouse with normal horse antibody as a control, 24 hours after infection. Fifty micrograms of anti-H5N1 $F(ab)_2$ gave 70% protection, and 100 and 200 µg of anti-H5N1 $F(ab)_2$ gave 100% protection. In contrast, the antibody-negative control (200 µg of nonimmune equine antibody) did not provide protection, and all the mice in this group died (

Figure 1a).

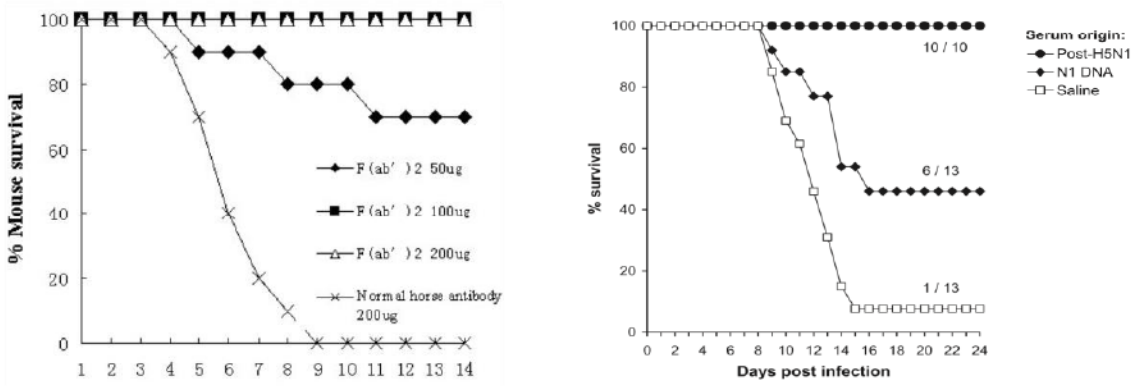

Figure 1: a) Therapeutic Efficacy of F(ab)2 Fragments Against H5N1; b) Efficacy of Passive Immunoprophylaxis Against H5N1

Researchers at St. Jude Children's Research Hospital tested the prophylactic efficacy of convalescent plasma. Eleven-week-old mice were intraperitoneally injected with 350 µL of serum collected from mice that survived challenge with A/Vietnam/1203/04 (H5N1) virus after 2 doses of an influenza neuraminidase (NA) encoding DNA vaccine. A negative control serum was pooled from saline-injected mice (Figure 1b). Recipient mice were challenged with 10 MLD_{50} of A/Vietnam/1203/04 18 hours after passive immunization. [4] The mice that received the immune plasma were 100% protected.

1.2.2 Summary of Previous Clinical Studies

1.2.2.1 1918 H1N1 Retrospective Review

Luke et al. conducted a meta-analysis of studies using convalescent blood products during the Spanish Influenza pandemic of 1918 and concluded that the approach may have been beneficial in the treatment of influenza pneumonia and ARDS. [5]

1.2.2.2 Hong Kong H1N1 2009 Plasma Cohort Study

A cohort study was conducted in Hong Kong by recruiting 93 patients aged > 18 years with severe H1N1 2009 infection requiring intensive care. [6] All subjects were offered treatment with 500 mL of convalescent plasma with a neutralizing H1N1 2009 antibody titer of > 1:160, collected from patients recovering from H1N1 2009 infection. Twenty subjects (21.5%) agreed to receive the plasma treatment, and 73 subjects declined. All subjects received standard antiviral treatment and other supportive medical care. Clinical outcome was compared in the subjects treated with plasma with those who declined plasma treatment as the "untreated" controls. Mortality in the treatment group was significantly lower than in the control group (20.0% vs. 54.8%; P = .01). The acute physiology and chronic health evaluation (APACHE) score in the control arm was 13. For similar severity of illness as measured by the APACHE score, mortality from H1N1 in other series is reported as 20-25%. [7-10] There were no adverse events (AEs) attributed to the convalescent plasma.

1.2.2.3 Hong Kong H1N1 2009 IVIG Randomized Controlled Trial

A multi-center, prospective, double-blind, randomized controlled trial of a hyperimmune intravenous immunoglobulin was also conducted in Hong Kong.[11] Convalescent plasma from patients who recovered from the 2009 pandemic influenza infection was made into an immunoglobulin (H-IVIG). Patients with severe A/H1N1 infection on standard antiviral treatment requiring intensive care and ventilatory support were randomized to receive H-IVIG or normal IVIG. Thirty-five patients were randomized to receive H-IVIG (17 patients) or IVIG (18 patients). H-IVIG treatment was associated with significantly lower day 5 and 7 post-treatment viral load when compared to the control (p=0.04 and p=0.02 respectively). Subgroup multivariate analysis of the 22 patients who received treatment within 5 days of symptom onset demonstrated that H-IVIG treatment was the only factor that independently reduced mortality [OR: 0.14, 95% CI, 0.02-0.92; p=0.04].

1.2.2.4 IRC002 – Severe Seasonal Influenza Plasma Randomized Trial

The NIAID Influenza Research Collaboration (NIRC) conducted a randomized study using high-titer anti-influenza plasma. Subjects of any age were eligible if they had confirmed influenza A or B and had evidence of severe influenza defined as oxygen saturation of < 93% on room air or tachypnea (respiratory rate > 20 for adults, with higher rates defined for children). Ninety-eight subjects were enrolled between January 2011 and March 2015, including 11 children and 2 pregnant women. Subjects were randomized to receive standard care (including antivirals) or standard care plus 2 units of high-titer anti-influenza plasma defined as influenza A/H1N1 and A/H3N2, or influenza B HAI titer of at least 1:40. The primary endpoint was proportion of participants with normalized respiratory status over time. The final study analysis is noted below.

At baseline (Day 0), 82% required oxygen, 43% were on mechanical ventilation, 58% were in the ICU, and 38% met criteria for acute respiratory distress syndrome (ARDS). Five subjects in the standard care arm and one subject in the plasma treatment arm died (ITT, logrank p=0.093). Fifty-three percent of subjects in the standard care arm and 67% of subjects in the plasma treatment arm normalized respiratory status by Day 28 (ITT logrank p=0.069; Figure 2a). More subjects in the plasma treatment arm normalized respiratory status in the 24-hour period between screening and baseline assessments. However, even when these were excluded there is still a trend towards efficacy in the plasma treatment

arm (ITT logrank p=0.26; Figure 2b). The treatment effect was primarily seen in subjects randomized within 4 days of symptoms.

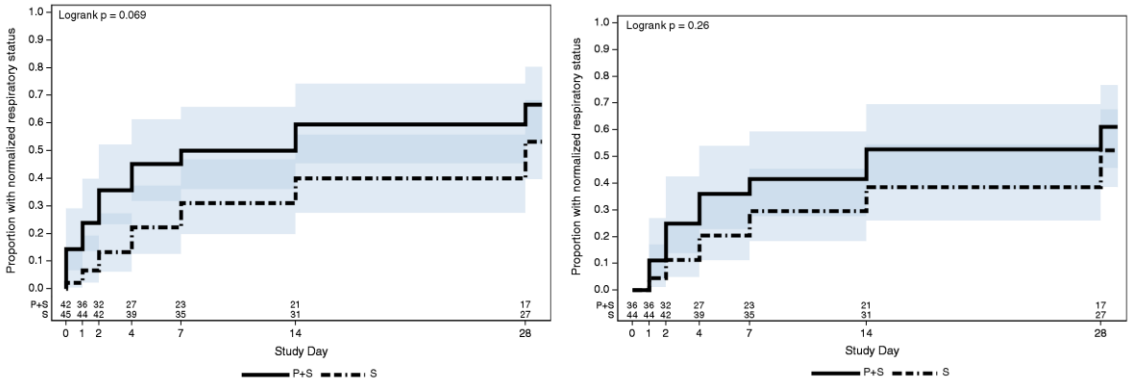

Figure 2: a) Proportion of participants with normalized respiratory status over time, by randomized treatment (ITT population); b) Proportion of participants with normalized respiratory status over time, by randomized treatment (modified ITT population - excluding participants with normalized respiratory status at baseline)

Similar trends of efficacy were seen in proportion of subjects without ARDS (Figure 3a) and improvement of SOFA score (Figure 3b).

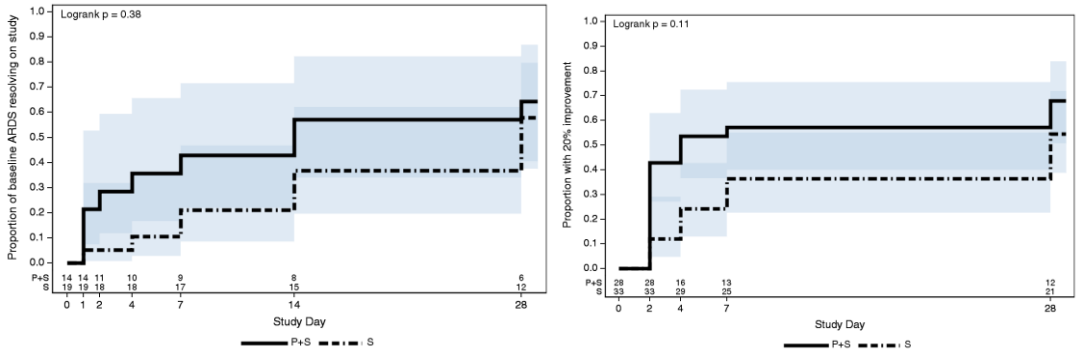

Figure 3: a) Proportion of participants without ARDS present over time, by randomized treatment (ITT population restricted to subjects with ARDS at baseline); b) Proportion of participants with 20% improvement in score, by randomized treatment (ITT population) – Adults

Similar trends in efficacy were seen in days of hospitalization, days in the ICU, and days on mechanical ventilation (Table 1). No benefit was seen in days on oxygen (Table 1), resolution of symptoms, fever, or viral shedding (data not shown).

Table 1: Summary of hospital/ICU stays and supplemental oxygen/mechanical ventilation during study follow-up, by randomized treatment

Table 2: Disposition and other secondary endpoints by randomized treatment (PEP)

	Total (N=87)	Randomized treatment: Anti-influenza Plasma Standard Care (N=42)	Randomized treatment: Standard Care Alone (N=45)	P-Value*
Days in hospital				
Median (Q1, Q3)	9 (4, 21)	6 (4, 16)	11 (5, 25)	0.13
Number of hospital admissions				
1	78 (90%)	40 (95%)	38 (84%)	0.096
2	7 (8%)	2 (5%)	5 (11%)	
4	2 (2%)	0 (0%)	2 (4%)	
ICU admission				
No	32 (37%)	18 (43%)	14 (31%)	0.097
Yes - 1 or more episodes	50 (57%)	24 (57%)	31 (69%)	
Days in ICU				
Median (Q1, Q3)	3 (0, 12)	2.5 (0.0, 9.0)	3 (0, 13)	0.37
Supplemental oxygen				
No	12 (14%)	8 (19%)	4 (9%)	0.61
Yes - 1 or more episodes	75 (86%)	34 (81%)	41 (91%)	
Days on supplemental oxygen				
Median (Q1, Q3)	8 (2, 28)	7 (1, 28)	8 (3, 28)	0.52
Mechanical ventilation				
No	43 (49%)	24 (57%)	19 (42%)	0.12
Yes - 1 or more episodes	44 (51%)	18 (43%)	26 (58%)	
Days on mechanical ventilation				
Median (Q1, Q3)	1 (0, 11)	0 (0, 6)	3 (0, 14)	0.14
*Wilcoxon rank sum				

While the IRC002 protocol required units to have an HAI of ≥ 1:40, the plasma units used for influenza A generally had an influenza A/H1N1 and A/H3N2 HAI titer of at least 1:80, and for influenza B the HAI titer was closer to 1:40. The HAI titers over time are the equivalent of pharmacokinetics (PK) for this intervention. The subjects' serum was tested to contemporary strains (i.e., not all subjects were tested to all strains). In the plasma arm, this intervention raised the HAI for influenza A/H1N1 from 1:29 to 1:63 at Day 2 and 1:81 at Day 4 (standard care: 1:32 on Day 0, 1:40 on Day 2, 1:41 on Day 4). A similar increase was seen in A/H3N2. However, in influenza B, the HAI in the active and standard care arms were similar and did not increase significantly by Day 2.

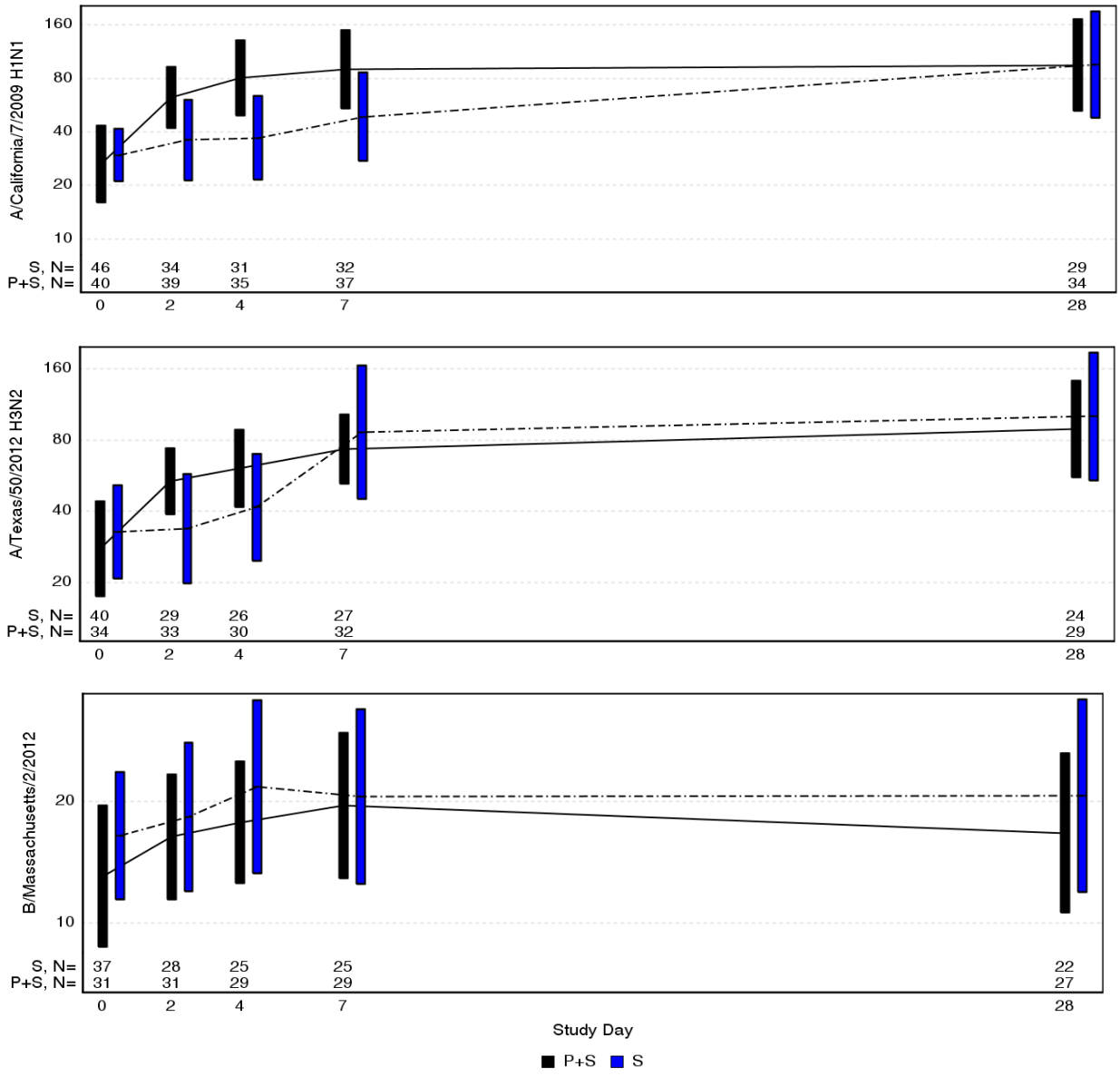

Figure 4: Influenza H1N1, H3N1 and B HAI titer over time, by treatment received

Lastly, there was a trend in higher level of disposition after hospital discharge, with more subjects in the plasma treatment arm returning home, and more subjects in the control arm being hospitalized at Day 28 (Table 2).

Table 2: Disposition following last hospital discharge, by randomized treatment (ITT population)

	Total (N=87)	Randomized treatment: Anti-influenza Plasma + Standard Care (N=42)	Standard Care Alone (N=45)	P-Value*
Disposition after last hospital discharge				
Released home - home health care not required	35 (41%)	21 (50%)	14 (33%)	0.029
Released home with home health care	13 (15%)	7 (17%)	6 (14%)	
Transferred to long term care facility	12 (14%)	6 (14%)	6 (14%)	
Hospitalization ongoing at Day 28	18 (21%)	7 (17%)	11 (26%)	
Discharged to hospice care - home or inpatient	1 (1%)	0 (0%)	1 (2%)	
Deceased	6 (7%)	1 (2%)	5 (12%)	

Another way to analyze treatment efficacy is to compare the clinical status of patients in the plasma and standard care groups at 7 days using an ordinal scale of 6 clinical states. This analysis is used in the INSIGHT anti-influenza IVIG study, and is proposed as the primary endpoint for the current study. When this ordinal scale is applied to the IRC002 data, there is good evidence of efficacy (Table 3).

Table 3: Proposed primary endpoint (clinical status measured on a 6-point ordinal scale) applied to IRC002 data (post-hoc analysis)

		Total (N=87)	Randomized treatment: P+S (N=42)	S (N=45)	P-Value*
Category at Day 7	N	87	42	45	0.020
	Not hospitalized with resumption of normal activities	25 (29%)	17 (40%)	8 (18%)	
	Not hospitalized, but unable to resume normal activities	6 (7%)	5 (12%)	1 (2%)	
	Non-ICU hospitalization, not requiring supplemental oxygen	5 (6%)	0 (0%)	5 (11%)	
	Non-ICU hospitalization, requiring supplemental oxygen	19 (22%)	7 (17%)	12 (27%)	
	In the intensive care unit (ICU)	27 (31%)	13 (31%)	14 (31%)	
	Death	5 (6%)	0 (0%)	5 (11%)	

*Wilcoxon Test

1.3 Study Rationale

Of subjects hospitalized with severe influenza (combining both IRC002 treatment groups together), 29% remained in the ICU and 60% had died or remained in the hospital on Day 7 (Table 3). There is need for better treatments in this population. The data from IRC002 strongly suggests that the addition of high-titer immune plasma to standard care can improve outcomes in this population. This present study aims to confirm these results in a continued search for better treatments for severe influenza.

1.3.1 Discussion of Design and Controls

IRC002 was randomized, but not blinded and with no control intervention. While the primary endpoint chosen was objective (normalization of oxygenation and respiratory rate) and should not be influenced by the unblinded design, there remains the potential for bias influencing the study results. For example, the follow-up rate in IRC002 was less for those subjects randomized to receive standard care. Thus, assuring a completely blinded study was seen as critical for this study.

Several control arm interventions were considered for this study:

- Double Blind, with low-titer control plasma. This intervention would be easiest to blind. Additionally, it explicitly tests the hypothesis that the anti-influenza antibodies (as detected by HAI) are the component of plasma responsible for any treatment effect seen.
- Double Blind, with 5% albumin. The control would consist of adding 5% albumin to saline and putting it in a plasma bag with a standard ISBT label. It was thought that this may look like plasma from a distance, but by close inspection it would be easy to unblind. Blood Establishment personnel would be effectively unblinded. Due to the appearance, all units would have to be placed in an opaque bag, but hospital nurses would still need to inspect the bag and label per standard blood transfusion policies. Additionally, it was considered that the hospital nurse may question this plasma since it did not look like "normal" plasma. This would likely unblind the nurse administering the plasma. So additional staff would have to be used. However, even with these additional steps, it was considered unlikely that the blind could be maintained.
- Single Blind, with 5% albumin. This would blind subjects, but not blind the study team. This was considered more feasible due to some of the considerations above. However, single blind was not likely to add much scientific rigor above an unblinded study.
- No Blind, no control - similar to IRC002. The increased lost to follow-up rate in the control arm was attributed in part due to subjects not seeing value in participating in a study for which they were not "chosen" to receive the proposed treatment. Additionally, there is the concern that while the endpoints are objective the biases of investigators can never be completely excluded.

After careful considerations of all options, the use of low-titer plasma was seen as the best control, and would allow a fully blinded study to be performed.

Under IND 15771, the international study INSIGHT 006 (FLU-IVIG) is a multicenter, double-blind, randomized, placebo-controlled clinical trial comparing treatment with hyperimmune intravenous immunoglobulin (IVIG) versus saline placebo in hospitalized patients with locally confirmed influenza A or B who have a NEW score ≥ 2. By harmonizing study days, primary endpoint, and some secondary endpoint data between this plasma study and the FLU-IVIG study, additional study analysis can compare high-titer plasma vs. IVIG and high-titer plasma vs. saline to determine if treatment effect (if confirmed) is due to IgG or other components in human plasma. Therefore, this study was designed to have similar assessments, days of assessments, etc., to the INSIGHT 006 study.

A NEW score (or PEW score) of 3 is used in this IRC005 study. The use of this score along with the requirement for hospitalization is an attempt to define a population that is hospitalized due to clinical manifestations of severe influenza rather than other indications. The IRC002 study required only hypoxia or tachypnea to be enrolled. By the NEW score, either of these criteria would be a score of 2. Eight percent of the IRC002 adult population with influenza A would be excluded by using a cut off of NEW score ≥ 3, but the NEW score also allows enrollment of population with severe influenza that did not quite meet the IRC002 population so is unlikely to decrease enrollment.

Subjects with influenza B only are excluded from the IRC005 study. In IRC002, influenza B made up only 7% (7 subjects) of the study population. In general, it is hard to find units with high HAI titers to influenza B, so the cutoff of units used in the IRC002 study was 1:40. Analyzing the HAI titers for the 7 subjects with influenza B, it is not clear (though the numbers are very small) that the plasma-treated arm had higher HAI titers (Table 4). So for reasons of both low incidence and low HAI titers, this study will not enroll subjects with influenza B.

Table 4: B/Mass H3N2 HAI titer over time, by treatment received (as-treated population) among those with Influenza Type B

	Treatment Received									
	P+S					S				
Study Day:	0	2	4	7	28	0	2	4	7	28
N	3	3	2	3	3	4	2	2	3	3
Geometric Mean	25.2	80.0	56.6	100.8	127.0	16.8	80.0	80.0	63.5	80.0
95% CI	0.5, 1343.8	14.3, 447.6	0.7, 4624.3	13.8, 736.1	47.0, 343.2	2.6, 110.6	0.0, 534612	0.0, 3.573E9	0.3, 16091.2	0.8, 7613.5

2 STUDY OBJECTIVES

2.1 Primary Objective

Evaluate the efficacy of treatment with high-titer versus low-titer anti-influenza immune plasma as an addition to standard care in subjects hospitalized with severe influenza A.

The primary endpoint will be assessed by the subject's clinical status at Day 7 on a 6-point ordinal scale:

1. Death
2. In the ICU
3. Non-ICU hospitalization, requiring supplemental oxygen
4. Non-ICU hospitalization, not requiring supplemental oxygen
5. Not hospitalized, but unable to resume normal activities
6. Not hospitalized with full resumption of normal activities

2.2 Secondary Objectives

1. Evaluate the efficacy and safety of treatment with high-titer versus low-titer anti-influenza immune plasma as an addition to standard care, using the following parameters:

- The ordinal primary outcome assessed at Days 1, 2, 3, 14, and 28
- Duration of initial hospitalization
- 28-day mortality
- In-hospital mortality during initial hospitalization
- Composite of mortality and hospitalization at Days 7, 14, and 28
- Change from baseline to Day 3 and Day 7 in NEW/PEW score
- Duration of supplemental oxygen use among those requiring oxygen at randomization
- Incidence of new oxygen use during the study
- Duration of ICU stay among those requiring ICU admission at randomization
- Incidence of new ICU admission during the study
- Duration of mechanical ventilation use among those requiring mechanical ventilation at randomization
- Incidence of new mechanical ventilation use during the study
- Duration and severity of ARDS among those meeting the definition of ARDS at randomization
- Incidence and severity of new ARDS during the study
- Duration of ECMO use among those requiring ECMO at randomization
- Incidence of new ECMO use during the study
- Change from baseline to Day 3 and Day 7 SOFA score for age ≥ 18 years, and PELOD score for age < 18 years
- Disposition (home, rehabilitation, chronic nursing facility, initial hospitalization ongoing at 28 days, died in hospital) following the initial hospitalization

- Percent of subjects with influenza virus detectable in OP sample at Day 3

2. Compare the plasma and control groups for HAI titers at Days 1, 3, and 7.

3. Evaluate the safety of high titer anti-influenza plasma as compared to low titer plasma as assessed by:

- Cumulative incidence of grade 3 and grade 4 adverse events (AEs)
- Cumulative incidence of serious adverse events (SAEs)

3 INVESTIGATIONAL PLAN

3.1 General

Up to 300 subjects are planned for enrollment in order that 150 subjects may be randomized. Randomized adult subjects will receive 2 units of plasma. Randomized pediatric subjects will receive a weight-based infusion of plasma which, depending on the calculated plasma volume, may be one or two infusions. See Section 5.7 for specifics of the plasma dosing. All subjects will be followed through Study Day 28. The study population will consist of males and females hospitalized with severe influenza A.

3.2 Definitions

Definitions for the purpose of this study:

- **Enrolled:** For the purpose of collecting data and samples, and reporting AEs, a subject will be considered enrolled beginning from when the informed consent form is signed until the subject is considered "screen failure", "discontinued", or "completed".

- **Randomized:** For the purpose of study reporting and data analysis, subjects are considered randomized when a randomization number is assigned.

- **Screen Failures:** Subjects are considered screen failures when they meet one of the following criteria after signing consent:
  ◦ Screening tests reveal that the subject is ineligible.
  ◦ Subject withdraws consent before being randomized.

- **Discontinued:** Subjects are considered discontinued when they meet one or both of the following criteria:
  ◦ Subject withdraws consent after being randomized (refer to Section 4.4).
  ◦ Subject is withdrawn by investigator after being randomized (refer to Section 4.5).

- **Completed:** Subjects are considered completed when they are followed through Study Day 28 or followed through to death at or prior to 28 days.

3.3 Overview of Study Design

This randomized, double-blinded, multicenter Phase 3 trial will assess the efficacy and safety of high-titer versus low-titer anti-influenza immune plasma for the treatment of severe influenza A. Hospitalized subjects with severe influenza A (as defined in the inclusion criteria) will be eligible for study participation. Up to 40 sites in the United States will participate in this protocol. Adults and children may participate. There is no exclusion for pregnancy.

A total of 150 eligible subjects will be randomized in a 2:1 ratio to receive either high-titer or low titer (control) anti-influenza plasma on Study Day 0 (refer to Section 5.7– Plasma Dose). All subjects will receive standard care antivirals (generally this would be oseltamivir).

Subjects will be assessed on Study Day 0 (baseline) and on Study Days 1, 2, 3, 7, 14, and 28. For participants who are not hospitalized on Days 2, 14, and 28, contact with the participant for the purpose of study data collection on those days may be performed by telephone.

All subjects will undergo a series of efficacy, safety, and PK (HAI) assessments during the study. Blood samples will be collected on Day 0, 1, 3, and 7. OP swabs for influenza PCR will be obtained on Day 0 and 3.

4 STUDY POPULATION

4.1 Inclusion Criteria for Enrollment (Screening)

1. Subjects must be aged 2 weeks or older.
2. Hospitalization due to signs and symptoms of influenza
   Note: The decision for hospitalization will be made by the treating clinician. To be considered eligible, the hospitalization may either be an initial hospitalization, or a prolongation of a hospitalization due to a respiratory illness that was found to be from influenza. Influenza could be a component of a larger respiratory syndrome (i.e. COPD exacerbation thought to be triggered by influenza). However, respiratory syndromes that are not likely due to the virus should not be included (i.e. a subject that had mild influenza then developed pulmonary embolism and respiratory distress from the embolism).
3. Study plasma available on-site or available within 24 hours after randomization.
4. Not previously screened nor randomized in this study
5. Willingness to have blood and respiratory samples obtained and stored.
6. Willingness to return for all required study visits and participate in study follow up.

4.2 Inclusion Criteria for Randomization

1. Locally determined positive test for influenza A (by PCR, other nucleic acid testing, or by rapid Ag) from a specimen obtained ≤ 48 hours prior to randomization.
2. Onset of illness ≤ 6 days before randomization, defined as when the subject first experienced at least one respiratory symptom or fever.
   Note: For subjects with chronic respiratory symptoms (chronic cough, or COPD with baseline dyspnea), the onset of symptoms is defined as the point when the symptoms changed during this illness).
3. Hospitalized due to influenza, with anticipated hospitalization for more than 24 hours after randomization. Criteria for hospitalization will be up to the individual treating clinician.
4. NEW or PEW score ≥ 3 within 12 hours prior to randomization.
5. ABO-compatible plasma available on-site or available within 24 hours after randomization.

4.3 Exclusion Criteria for Randomization

Subjects who meet any of the following criteria will be excluded from study participation:

1. Strong clinical evidence in the judgment of the site investigator that the etiology of illness is primarily bacterial super-infection in origin. Co-infection would be allowed, as there may be benefit to resolving influenza illness faster. Super-infection, where influenza illness occurred and is resolving, and new bacterial illness causing deterioration should be excluded. (e.g., if the subject's respiratory infection is thought unlikely to benefit from additional antiviral therapy, this exclusion criteria would be met).
2. Prior treatment with any anti-influenza investigational drug, anti-influenza investigational IVIG, or anti-influenza investigational plasma therapy within 30 days prior to screening. Other investigational drug therapies (non-influenza) and administration of plasma and/or IVIG for non-influenza reasons are allowed.
3. History of allergic reaction to blood or plasma products (as judged by the site investigator).

4. A pre-existing condition or use of a medication that, in the opinion of the site investigator, may place the individual at a substantially increased risk of thrombosis (e.g., cryoglobulinemia, severe refractory hypertriglyceridemia, or clinically significant monoclonal gammopathy). Prior IVIG use alone would not meet exclusion criteria, but the investigator should consider the potential for a hyper-coagulable state.
5. Subjects who, in the judgment of the site investigator, will be unlikely to comply with the requirements of this protocol, including being uncontactable following discharge from hospital.
6. Medical conditions for which receipt of 450-700 mL (or pediatric equivalent) of intravenous fluid may be dangerous to the subject (e.g., decompensated congestive heart failure).

4.4 Subject Withdrawal

Subjects (or their legal surrogates if subjects are or become unable to make informed decisions) can terminate study participation at any time without prejudice. Subjects who indicate interest in withdrawing from the study should be asked about possible limited further study data collection and/or data usage, rather than full withdrawal. The most important data to obtain is safety data through Day 28 and primary endpoint data (clinical status at Day 7). This would require, at a minimum, a Day 28 by telephone contact for vital status (date of discharge, disposition, and any adverse events that occurred during the study) and a review of the available subject information for clinical status at Day 7. This is not considered full withdrawal of consent. The modified consent for limited follow-up should be noted in the source documentation.

Subjects who fully withdraw consent will not be contacted further. The reason for withdrawal from the study is to be recorded in the source records and eCRF.

Randomized subjects who withdraw from the study will not be replaced.

4.5 Discontinuation of Subject by Investigator

The investigator also has the right to withdraw subjects for either of the following reasons:

- The subject is lost to follow-up.
- The subject experiences an AE, including complications incurred during plasma infusion, and the investigator believes that continuation in the study would be detrimental to the subject. In such cases, it is recommended that the subject remain in the study with safety follow-up through Day 28. The option of continued participation with limited intervention or even just observational data collection should be considered prior to investigator initiated discontinuation due to AE. Considerations for limited follow up are described in Section 4.4.
- Non-compliance with study procedures to the extent that it is potentially harmful to the subject or to the integrity of the study data.

Lost to follow-up is defined as unsuccessful contact after at least two documented telephone calls.

Any randomized subject withdrawn from the study will not be replaced.

4.6 Discontinuation of Study

The Office of Clinical Research Policy and Regulatory Operations (OCRPRO), as the study sponsor, and the Food and Drug Administration (FDA), have the right to terminate this study at one or all sites at any time. The reviewing IRB/IEC has the right to terminate the study at the sites it is responsible for at any time.

5 TREATMENT

Subjects will be randomized on Study Day 0 in a 2:1 ratio to receive standard care plus either high-titer or low-titer anti-influenza plasma. Subjects will receive plasma directly compatible with their ABO blood type.

5.1 Materials, Supplies, and Study Blinding

The study plasma is obtained under IND 14125. The investigational product anti-influenza immune plasma is collected from FDA-licensed, registered blood establishments in accordance to standard criteria.

All plasma is from male donors to minimize the risk of TRALI.

All plasma used in this study will have the following characteristics:

- Negative Anti-HIV-1/2
- Negative Anti-HTLV-I/II
- Negative Anti-HCV
- Negative HBsAg
- Negative for anti-HBc
- Negative serologic test for syphilis
- Negative West Nile Virus (WNV) NAT
- Negative HCV NAT
- Negative HIV NAT
- Negative for Zika NAT

The blood establishments collecting the plasma follow all current FDA guidance.

All plasma units used in this study have anti-influenza antibodies to influenza A (A/H1N1 or A/H3N2) as needed for this study.

- Active arm will receive human plasma (FFP or FP24) with both an influenza A/H1N1 and A/H3N2 HAI titer of at least 1:80. The sponsor will try to supply units with as high a HAI as possible. The antigens for the HAI will be for contemporary strains defined as strains contained within that year's seasonal trivalent influenza vaccine.
- Control arm will receive human plasma (FFP or FP24) with both an influenza A/H1N1 and A/H3N2 HAI titer of 1:10 or less.
- Refer to Section 5.7 Plasma Dose, for dosing information.

Both active and control plasma will be in standard plasma unit bags, with a study-specific ISBT label. High-titer units and low-titer units will have the same label. Blood establishment personnel and study team will remain blinded until after analysis.

5.2 Randomization

Subjects who meet all criteria for enrollment will be randomized using an interactive web response system (IWRS) to receive high-titer anti-influenza plasma or low-titer plasma in a 2:1 ratio. A central randomization scheme has been prepared by the sponsor. Randomization is stratified by:

- Severity: in the ICU, non-ICU hospitalization requiring supplemental oxygen, or non-ICU hospitalization not requiring supplemental oxygen
- Age category (child/adult)

5.3 Rationale for Selection of Doses in the Study

Study plasma dosing is detailed in Section 5.7. The total volume of plasma in adults can be estimated by multiplying actual body weight in kilograms by 40 mL/kg. [12] For a 70-kg person, the plasma volume would be estimated to be 2800 mL (40 mL/kg x70 kg). For nonimmune subjects assume this plasma volume has a HAI = 0.

The volume of a unit of study plasma is approximately 250 mL (range 225-350 mL). Therefore, the adult study dose of 2 units of plasma would be equal to approximately 450-700 mL. A plasma volume of 500 mL at a HAI titer of 1:160 would increase HAI in plasma to approximately 1:25. A plasma volume of 500 mL at a HAI titer of 1:80 would increase HAI in plasma to approximately 1:13.

In IRC002, in general, this model worked. Subjects receiving plasma had a geometric mean influenza A/H1N1 HAI titer of 1:26 on Day 0, 1:63 on Day 2, and 1:81 on Day 4, while the standard care alone group had a HAI titer of 1:30 on Day 0, 1:36 on Day 2, and 1:37 on Day 4. As expected, the titers on Day 28 were essentially the same, (1:95 and 1:96 respectively) meaning any HAI increase from the plasma infusions is gone by Day 28.

For children and neonates, the total volume of plasma is estimated between 37.3 and 54.8 mL/kg. [13, 14] Using a mean of 45 mL/kg, an anti-influenza plasma dose of 4 mL/kg would provide the same increase in HAI as would be expected in a 70-kg subject receiving 1 unit of plasma. The total dose of 2 units of plasma in adults is equivalent to 8 mL/kg in children (either as one infusion or two infusions, as noted in Section 5.7).

5.4 Study Plasma Supply, Packaging, and Labeling

Sites have been given an initial supply of study plasma after IRB approval. The sites are resupplied with study plasma, as it is needed.

Study plasma must be shipped with enough dry ice to ensure that the plasma remains frozen throughout the shipping process. All investigational products will be labeled with a standard ISBT label containing a study-specific plasma product code.

5.5 Site Receipt and Verification

The site blood bank will acknowledge receipt of the supply of study plasma via the IWRS, verifying that the material remained frozen during transit, was received undamaged, and that the proper number of units were received.

5.6 Study Plasma Storage

Study plasma must be maintained at -20°C or colder. Any excursion warmer than -18°C should follow the site SOP regarding suitability for infusion. If the plasma is not useable this should be documented, then a deviation should be filed and the sponsor should be notified.

Any temperature excursion warmer than -20°C should be recorded and communicated to the sponsor. While this plasma may be useable for infusion, the temperature excursion may impact the plasma's suitability for manufacturing (if units with high titer antibody are unused at the end of the season or at expiration). By communicating temperature excursions to the sponsor, the ultimate disposition of these units can be determined.

5.7 Plasma Dose

Specific study plasma unit(s), identified by donation identification number (DIN), will be assigned to subjects in the IWRS.

- Adult subjects will receive 2 units of assigned study plasma.
    - If an adult subject is of very small weight and/or stature, and the investigator thinks it is not safe to administer 2 units, then less than two full units can be administered and the reason for not administering the full two units should be described in the source documentation and entered in the eCRF.
- Pediatric subjects ≥ 56 kg, will receive 8 mL/kg not to exceed 2 units of assigned study plasma divided into 2 infusions (approximately 4 mL/kg of study plasma per infusion) (i.e. in larger children, this may be less than 8 mL/kg). Specify the total dose (volume) to be administered in the plasma order to ensure accurate dosing.
- Pediatric subjects ≥ 28 kg but < 56 kg will receive 8 mL/kg of assigned study plasma divided into 2 infusions (approximately 4 mL/kg of study plasma per infusion). Specify the total dose (volume) to be administered in the plasma order to ensure accurate dosing.
- Pediatric subjects < 28 kg will receive 8 mL/kg of assigned study plasma given in one infusion. The total dose of 8 mL/kg can be accomplished with one unit of plasma, thereby avoiding exposing the children to more than one donor. Specify the total dose (volume) to be administered in the plasma order to ensure accurate dosing.

Subject Type	Dosage	Units of Plasma
Adult	450 -700 mL	2
Pediatric ≥ 56 kg	4 mL/kg + 4mL/kg= 8 mL/kg total (448-700 mL)	2
Pediatric ≥ 28 kg but < 56 kg	4 mL/kg + 4mL/kg =8 mL/kg total (< 448 mL)	2
Pediatric < 28 kg	8 mL/kg (< 224 mL)	1

5.8 Study Plasma Preparation

After receipt of the IWRS randomization notification, the appropriate site staff will order/ request the plasma by specifying the plasma ABO type and the DIN(s) included in the randomization notification.

Site blood bank/transfusion services should verify the plasma to be infused and that the DIN(s) on the plasma unit(s) match the DINs specified in the plasma order, and should prepare the study plasma according to standard departmental procedures.

The sponsor must be informed promptly of any study plasma units that are thawed but not administered to the assigned subject. If study plasma is thawed but not administered to the assigned subject, it must be destroyed in accordance with standard blood bank procedures. When dosing pediatric subjects, any allocated unused plasma must also be destroyed in accordance with standard blood bank procedures.

5.9 Study Plasma Administration

Study plasma should be administered as soon as possible. The plasma should be started no later than 12 hours after randomization for units that are available at a site, and no later than 24 hours for units that are shipped from the repository for that subject. The plasma infusion rate will be determined by the individual sites based on institutional policy.

Before administration of study plasma, the investigator (or designated study staff) must verify the plasma label(s) to confirm that the DIN(s) on the plasma unit(s) match the IWRS randomization notification email for the subject, as well as to confirm the appropriate dose to be administered (refer to Section 5.7). Plasma DIN(s) and plasma product code(s) should be recorded in source documents at the time of infusion along with the volume administered.

The calendar date and 24-hour clock time for study plasma administration should be recorded for the start and end of each infusion. The intervals between units may occur per a site's blood establishment SOPs and/or normal practice. Pretreatment to minimize transfusion reactions (e.g. acetaminophen, diphenhydramine, etc.) according to local institutional policies are permitted in this study, but any such medications should be captured as concomitant medications.

If an AE develops during infusion, the infusion may be slowed temporarily or permanently discontinued, as deemed appropriate by the investigator. As a guide, local side effects (e.g., infusion site burning) or non-allergic systemic effects (e.g., chills) can generally be alleviated by slowing the rate of infusion, whereas allergic side effects (e.g., rash, wheezing, hypotension) should result in the cessation of study plasma infusion. All grade 3 or grade 4 AEs, and all SAEs occurring during study plasma administration should be captured as noted in Section 10.2. Additionally, any AEs resulting in plasma discontinuation (regardless of AE grade) should be recorded.

The investigator is ultimately responsible for making the decision to slow or stop study plasma infusion. If the infusion is discontinued, the subject should be treated according to best available local practices and procedures, and the Medical Monitor should be notified. Reason(s) for premature discontinuation of any plasma infusion must be documented in the medical record (source document) and on the eCRF.

5.10 Plasma Accountability

The investigator or his/her designee is required to maintain accurate study plasma accountability records. A binder containing instructions and the required accountability documentation will be provided to the investigator or his/her designee. When the study is completed, copies of the plasma accountability records will be maintained at the study site and all originals returned to the sponsor. Copies of the study plasma accountability records must be maintained with the rest of the documentation for the study. All unused study plasma must be returned to the repository or disposed of upon authorization by NIAID or its designee. All records regarding the disposition of study plasma must be available for inspection by the study monitors and regulatory authorities.

5.11 Concomitant Medications

Subjects will be monitored throughout the study for use of concomitant medications. Any prescription medications including IVIG, blood products, over-the-counter (OTC) preparations, herbal remedies, and/or nutritional supplements taken during the study period must be recorded on the eCRF.

All subjects with confirmed influenza infection will receive an anti-influenza antiviral (e.g., oseltamivir, zanamivir).

It is also anticipated that subjects may be treated with antibacterial agents, either for concomitant infection, or bacterial superinfection after influenza. The final decision for antibiotics (need, drug[s], and dose) and other medications will be made by the treating physician (except as noted below under 5.12 Prohibited Medications).

5.12 Prohibited Medications

Subjects may not receive investigational medications for influenza at any time during the study. Their use would not be considered standard care, and therefore withholding these compounds would still be consistent with best medical practice. This does not include licensed drugs available under an emergency use authorization (EUA) or approved medications at unapproved doses.

5.13 Treatment Compliance

Deviation(s) from the prescribed dosage regimen should be recorded in the eCRF and the subject's source document.

6 STUDY PROCEDURES

6.1 Schedule of Evaluations

A schedule of evaluations is presented as Table 5. A detailed presentation of assessments immediately follows the table. To ensure similar assessments of efficacy after plasma administration, the day of randomization will be noted to be Study Day 0, the first day after randomization is Study Day 1, etc. If the plasma is not administered until the day after randomization, the day of randomization should still be noted to be Study Day 0.

In an attempt to standardize the data, the follow up study visits should preferably occur in the morning (i.e. prior to noon). However, any visit within the window (as defined on the schedule of evaluations is acceptable).

Table 5: Schedule of Evaluations

	Screen	*Base line*	*Randomization*	*Study Plasma Administration*			*Follow-up*					
Day +/- Window	**−1 to 0**	**0**	**0**	**0**			**1**	**2***	**3**	**7 ± 1**	**14 ± 2***	**28 + 4***
Time				**Pre-infusion**	**1st unit plasma**	**2nd unit plasma)[5]**						
Evaluation/Procedure												
ELIGIBILTY												
Informed consent	X											
Demographics & Medical History	X											
Influenza A testing (PCR or rapid Ag)[1]	X											
ABO typing[2]	X											
STUDY DRUG ADMINISTRATION												
Randomize subject			X									
Plasma infusion					X	X						
STUDY PROCEDURES												
Vital signs including SpO_2	X	X		X	X	X	X	X	X	X	X	X
Clinical Data[3]	X	X					X	X	X	X	X	X
Concomitant medications		X		X			X	X	X	X	X	X
Adverse events		X			X	X	X	X	X	X	X	X
SAFETY LABORATORY												
CBC, Chemistry Panel, and PT/INR[4]		X					X		X	X		
Pregnancy test (for females of childbearing potential)	X											
RESEARCH LABORATORY PROCEDURES												
Blood for Serum		X					X		X	X		
Oropharyngeal Swab for virus isolation		X							X			

Notes:

1. Influenza test results from a specimen obtained ≤ 48 hours prior to randomization may be used.

2. Documented ABO typing may be utilized for screening and randomization; however, re-typing should be conducted prior to release of study plasma if required by institutional policies.

3. Refer to Section 6.3 of the protocol for details of clinical data to be collected.

4. CBC includes white cell count with differential (including neutrophil, and lymphocyte), hemoglobin, hematocrit, and platelets. Chemistry panel includes creatinine, glucose, total protein, total bilirubin, ALT/SGPT, AST/SGOT).

5. For Pediatrics < 28 kg, there is no second unit plasma infusion.

** This visit may be conducted via telephone. If conducted via telephone, the assessments should be restricted to the ordinal scale, oxygen requirements, new medical conditions, concomitant medication, and evaluation for AEs.*

6.2 Location and Personnel for Study Procedures

Hospitalization is a requirement for enrollment into this study. The decision regarding duration of hospitalization will be made by the treating physician. Day 0 assessments and study plasma administration will occur in a hospital.

Subsequent visits may occur in a health care facility or at home according to site practices and local law. Any sites performing home visits should have:

- IRB/IEC approval for home visits.
- Written study-specific procedures including obtaining samples with the storage condition and timeframe of this protocol that are approved by the study team.
- Documentation of institutional permission or policies permitting home visits including liability concerns.
- Adequate staff and resources to perform home visits (e.g., portable pulse oximeter for SpO_2 measurements).

Data collected for clinical indications may be used (i.e. review of clinical chart) if obtained on the given day /within the specified window. Otherwise assessments need to be performed by the study team. All study assessments should be performed by members of the investigative team that are specifically designated to perform such activities (according to site practices, local law, and as designated on the appropriate study documents).

6.3 Detailed Description of Assessments

6.3.1 Study Day –1 to 0: Screening

Subjects may be screened in the emergency room or hospital. Screening evaluations must be completed before randomization.

6.3.1.1 Informed Consent

One informed consent form will be used for both screening and enrollment into this protocol. Informed consent must be obtained prior to performing any study related activities.

The site investigator will review the informed consent document with the subject or legal representative. Informed consent should be obtained from the subject, or if a subject is judged by the investigator to be unable to provide informed consent, consent should be obtained from his/her legally authorized representative. The use of a surrogate for consent may only be done if allowed by local regulation and must be in accordance with local requirements.

As a guideline, children age 7 years or older should be presented the study assent (in addition to consent being obtained from his/her legally authorized representative). This age cutoff for assent may be adjusted for individual situations, and per institutional or site IRB policies/stipulations.

Subjects who were previously unable to provide informed consent, but are able to do so on a given study day, should be re-consented for continued study participation. If a subject becomes able to provide consent between visits, the subject should return to the center at the next study visit to provide consent unless institutional policies allow telephone consent.

6.3.1.2 Demographics

After informed consent is obtained, the following information should be recorded:

- Age
- Sex
- Ethnicity
- Race

6.3.1.3 Medical History

The investigator (or designee) will take a medical history following consent and conduct a complete physical examination. The following information should be recorded:

- Day of onset of influenza symptoms
- Influenza vaccination in the current season
- History of chronic medical conditions
- Any acute medical problems from the current hospitalization (including laboratory abnormalities)
- Current use of prescription and OTC medications
- History of allergies
- Additional history to be obtained from pregnant women includes last menstrual period, ultrasonic gestational age (if known), and complications during pregnancy.
- For women who are not of childbearing potential, the reason for this (if known) should be documented.

Medical conditions noted at the time of consent should be recorded in the eCRF. Any conditions that develop after consent should be recorded on the AE page of the eCRF.

6.3.1.4 Clinical Data at Screening

- Vital signs, including SpO_2. These values may be obtained from clinically collected information if they were obtained within 4 hours of the screening study assessment. Vital signs should be collected in a complete set within 30 minutes of each other.
- Early warning score
  - NEW score for age ≥ 18 years
  - PEW score for age < 18 years

6.3.1.5 Influenza Testing

The subject should have a respiratory specimen (nasal, throat, sputum, etc.) tested for influenza either by rapid antigen, PCR, or DFA that can detect influenza A. Test results from specimens that were collected within 48 hours before randomization may be used. If an influenza test was collected outside of the 48-hour window, it must be repeated.

6.3.1.6 Laboratory Testing

The following tests should be performed and/or prior results collected at screening:

- Blood typing (ABO).
  - Prior blood typing results may be utilized for screening and randomization (as ABO typing is unlikely to change for a given individual). Results from an active blood typing (as per each institution's policy) must be available prior to release and administration of study plasma.

- Urine or serum pregnancy test for females of childbearing potential.
    - Results from laboratory tests obtained up to 7 days before enrollment may be used for the pregnancy test. No exclusion is made based on pregnancy, but as this may alter a subject's assessment of risks/benefits, it should be done at the time of screening.
    - If a female subject is not of childbearing potential, the underlying cause (i.e. premenarche, post-menopause, hysterectomy, etc.) should be included in source documentation.

6.3.2 Determination of Eligibility

After the previously mentioned screening evaluations have been completed (i.e., medical history, laboratory tests), the investigator or designee is to review the inclusion/exclusion criteria and determine the subject's eligibility for study randomization.

Baseline evaluations will not be completed for screen failures. Only the following information will be collected on screen failures: demographics (age, screen number, sex, birth date, ethnicity, and race) and reason for ineligibility. Subjects who are found to be ineligible will be told the reason for ineligibility, and may not be re-screened for the study.

Those subjects found eligible will have baseline data collected prior to randomization and plasma administration. Day 0 is considered the day of randomization. Baseline data should be collected just prior to randomization.

6.3.3 Study Day 0: Baseline Evaluation and Randomization

6.3.3.1 Baseline Clinical Data

- Clinical data must be collected just prior to randomization (no more than 4 hours between collection and randomization).
- Vital signs, including SpO_2. These values may be obtained from clinically collected information if they were obtained within 4 hours prior to randomization. Vital signs should be collected in a complete set within 30 minutes of each other.
    - This can be the same set of vital signs as used at screening as long as they were obtained within 4 hours prior to randomization.
- Clinical data:
    - Assessment of clinical status (6-point ordinal scale)
    - Glasgow coma scale
    - Early warning score
        - NEW score for age ≥ 18 years
        - PEW score for age < 18 years
    - Organ dysfunction score
        - SOFA score for age ≥ 18 years
        - PELOD score for age < 18 years
    - Measures of clinical support:
        - Hospitalization
        - Oxygen requirement
        - Mechanical ventilator requirement
        - ICU requirement
        - ECMO requirement
    - Chest x-ray finding if performed for clinical indication within 24 hours prior to randomization
- Assessment of ARDS using PaO_2 (or SpO_2), FiO_2, and recent chest x-ray data.

- Note: If the above-listed clinical data assessments were performed for screening and collected < 4 hours prior to randomization, they do not need to be repeated for baseline assessment unless the investigator believes there has been a likely change in the score based on clinical assessment.
- Review new medical conditions, concomitant medication, and evaluate for AEs that have occurred after informed consent.

6.3.3.2 Baseline Clinical Safety Laboratory Tests

Blood draws for the following laboratory tests will be performed prior to randomization and plasma administration. The results do not need to be available prior to randomization or plasma administration.

- CBC with differential white cell count (to include neutrophil and lymphocyte percentages), hemoglobin, hematocrit, and platelets
- Blood chemistries (creatinine, glucose, total protein, ALT/GPT, AST/GOT, total bilirubin)
- PT/INR

Any tests not collected clinically, should be obtained according to the protocol. Results from laboratory tests performed for clinical indications < 12 hours before randomization may be used if available.

6.3.3.2.1 Pediatric Blood Collection Guidelines

Refer to institutional policies and recommendations on safe blood sample volume limits for pediatric patients. Sites may reduce the volume of research blood sample collection in order to remain within these limits.

6.3.3.3 Baseline Research Procedures on Day 0

The following baseline research procedures will be performed after screening evaluations and determination of eligibility have been completed but prior to randomization and plasma administration. These must be performed just prior to randomization (no more than 4 hours between collection and randomization). Baseline research samples should not be collected for screen failures.

- OP (throat) swab for quantitative PCR
- 25.5 mL of blood for adults or 8.5 mL of blood for children should be obtained. This will be used for the following laboratory tests:
  - HAI to influenza A/H1N1 and A/H3N2
  - Stored samples for other research tests evaluating the immune response to influenza as defined in Section 7.5

6.3.3.4 Randomization

Those subjects found eligible for randomization in this study will be randomized in the IWRS system as noted in Section 5.2, after baseline assessments have been completed.

6.3.4 Study Plasma Administration

After all baseline evaluations and randomization have been completed, subjects will receive the study plasma. Refer to Section 5.7 for dosing information. The calendar date and 24-hour clock time for study plasma administration should be recorded for the start and end of each infusion.

Vital signs should be obtained during plasma infusion according to institutional policies. Vital signs during infusion are not collected in the database. All grade 3 or grade 4 AEs (e.g. hypotension) or other events (chest pain, rash, etc.) and all SAEs occurring during study plasma administration should be captured as noted in Section 10.2.

6.3.5 Study Day 1

Day 1 is always the calendar day following the day of randomization, even if the plasma infusion starts late in the day of randomization or if the plasma infusion starts on the calendar day following the day of randomization. However, plasma administration should be completed prior to collecting the Day 1 visit procedures.

6.3.5.1 Clinical Data on Day 1

- Vital signs, including SpO_2. These values may be obtained from clinically collected information if they were obtained within 4 hours of the study assessment. Vital signs must be collected in a complete set within 30 minutes of each other.
- Clinical data
  - Assessment of clinical status (6-point ordinal scale)
  - Glasgow coma scale
  - Organ dysfunction score
    - SOFA score for age ≥ 18 years
    - PELOD score for age < 18 years
  - Measures of clinical support:
    - Hospitalization
    - Oxygen requirement
    - Mechanical ventilator requirement
    - ICU requirement
    - ECMO requirement
  - Chest x-ray finding if performed for clinical indication
  - Assessment of ARDS using PaO_2 (or SpO_2), FiO_2, and recent chest x-ray data.
- Review new medical conditions, concomitant medication, and evaluate for AEs

6.3.5.2 Clinical Laboratory Tests on Day 1

The following laboratory test will be performed:

- CBC with differential white cell count (to include neutrophil and lymphocyte percentages), hemoglobin, hematocrit, and platelets
- Blood chemistries (creatinine, glucose, total protein, ALT/GPT, AST/GOT, total bilirubin)
- PT/INR

Results from laboratory tests performed for clinical indications < 12 hours before the visit may be used if available. Any tests not collected clinically, should be obtained according to the protocol.

6.3.5.3 Research Procedures on Day 1

The following research procedures will be performed:

- 17 mL of blood for adults or 8.5 mL for children should be obtained. This will be used for the following laboratory tests:
  - HAI to influenza A/H1N1 and A/H3N2
  - Stored samples for other research tests evaluating the immune response to influenza as defined in Section 7.5

6.3.6 Study Day 2

If the subject is no longer hospitalized, this visit may occur by telephone. In this case, the assessments below should be restricted to the ordinal scale, oxygen requirements, new medical conditions, concomitant medication, and evaluation for AEs.

6.3.6.1 Clinical Data on Day 2

- Vital signs, including SpO_2. These values may be obtained from clinically collected information if they were obtained within 4 hours of the study assessment. Vital signs must be collected in a complete set within 30 minutes of each other.
- Clinical data
  - Assessment of clinical status (6-point ordinal scale)
  - Measures of clinical support:
    - Hospitalization
    - Oxygen requirement
    - Mechanical ventilator requirement
    - ICU requirement
    - ECMO requirement
  - Chest X-ray finding if performed for clinical indication
  - Assessment of ARDS using PaO_2 (or SpO_2), FiO_2, and recent chest x-ray data.
  - Review new medical conditions, concomitant medication, and evaluate for AEs

6.3.7 Study Day 3

The subject's ability to provide consent should be evaluated. Any subject who was unable to previously provide consent independently should be administered the informed consent according to institutional policies.

6.3.7.1 Clinical Data on Day 3

This visit must occur in the hospital or clinic, or be conducted as a home visit if authorized in accordance with Section 6.2. During this visit the following will occur:

- Vital signs, including SpO_2. These values may be obtained from clinically collected information if they were obtained within 4 hours of the study assessment. Vital signs should be collected in a complete set within 30 minutes of each other.
- Clinical data
  - Assessment of clinical status (6-point ordinal scale)
  - Glasgow coma scale
  - Early warning score
    - NEW score for age $\geq$ 18 years
    - PEW score for age < 18 years
  - Organ dysfunction score
    - SOFA score for age $\geq$ 18 years
    - PELOD score for age < 18 years
  - Measures of clinical support:
    - Hospitalization
    - Oxygen requirement
    - Mechanical ventilator requirement
    - ICU requirement
    - ECMO requirement
  - Chest x-ray finding if performed for clinical indication
  - Assessment of ARDS using PaO_2 (or SpO_2), FiO_2, and recent chest x-ray data.
- Review new medical conditions, concomitant medication, and evaluate for AEs

6.3.7.2 Clinical Safety Laboratory Tests on Day 3

The following laboratory test will be performed:

- CBC with differential white cell count (to include neutrophil and lymphocyte percentages), hemoglobin, hematocrit, and platelets
- Blood chemistries (creatinine, glucose, total protein, ALT/GPT, AST/GOT, total bilirubin)
- PT/INR

Results from laboratory tests performed for clinical indications < 12 hours before the visit may be used if available. Any tests not collected clinically, should be obtained according to the protocol.

6.3.7.3 Research Procedures on Day 3

The following research procedures will be performed:

- OP (throat) swab for quantitative PCR
- 17 mL of blood for adults or 8.5 mL for children should be obtained. This will be used for the following laboratory tests:
  - HAI to influenza A/H1N1 and A/H3N2
  - Stored samples for other research tests evaluating the immune response to influenza as defined in Section 7.5

6.3.8 Study Day 7 (±1)

The subject's ability to provide consent should be evaluated. Any subject who was unable to previously provide consent independently should be administered the informed consent according to institutional policies.

6.3.8.1 Clinical Data on Day 7

This visit must occur in the hospital or clinic, or be conducted as a home visit if authorized in accordance with Section 6.2. During this visit the following will occur:

- Vital signs, including SpO_2. These values may be obtained from clinically collected information if they were obtained within 4 hours of the study assessment. Vital signs should be collected in a complete set within 30 minutes of each other.
- Concomitant medications
- Clinical data
  - Assessment of clinical status (6-point ordinal scale)
  - Glasgow coma scale
  - Early warning score
    - NEW score for age ≥ 18 years
    - PEW score for age < 18 years
  - Organ dysfunction score
    - SOFA score for age ≥ 18 years
    - PELOD score for age < 18 years
  - Measures of clinical support:
    - Hospitalization
    - Oxygen requirement
    - Mechanical ventilator requirement
    - ICU requirement
    - ECMO requirement
  - Chest x-ray finding if performed for clinical indication
  - Assessment of ARDS using PaO_2 (or SpO_2), FiO_2, and recent chest x-ray data.

- Review new medical conditions, concomitant medication, and evaluate for AEs

6.3.8.2 Clinical Safety Laboratory Tests on Day 7

The following laboratory test will be performed:

- CBC with differential white cell count (to include neutrophil and lymphocyte percentages), hemoglobin, hematocrit, and platelets
- Blood chemistries (creatinine, glucose, total protein, ALT/GPT, AST/GOT, total bilirubin)
- PT/INR

Results from laboratory tests performed for clinical indications < 24 hours before the visit may be used if available. Any tests not collected clinically, should be obtained according to the protocol.

6.3.8.3 Research Procedures on Day 7

The following research procedures will be performed:

- 17 mL of blood for adults or 8.5 mL for children should be obtained. This will be used for the following laboratory tests:
  - HAI to influenza A/H1N1 and A/H3N2
  - Stored samples for other research tests evaluating the immune response to influenza as defined in Section 7.5

6.3.8.4 Day 7 Clinical Status

If the Day 7 visit does not occur, in person, attempts should be made to call the subject to obtain information about their disposition and functional status (in order to score the Day 7 Clinical Status [primary endpoint]). If the subject cannot be reached by phone, the hospital record should be reviewed to determine if/when the subject was discharged, and to determine the clinical status on Day 7. This missed visit may still be a protocol deviation, but this would allow the primary endpoint to be ascertained.

6.3.9 Study Day 14 (±2)

If the subject is no longer hospitalized, this visit may occur by telephone. In this case, the assessments below should be restricted to the ordinal scale, oxygen requirements, new medical conditions, concomitant medication, and evaluation for AEs. The subject's ability to provide consent should be evaluated. Any subject who was unable to previously provide consent independently should be administered the informed consent according to institutional policies.

6.3.9.1 Clinical Data on Day 14

- Vital signs, including SpO_2. These values may be obtained from clinically collected information if they were obtained within 4 hours of the study assessment. Vital signs should be collected in a complete set within 30 minutes of each other.
- Clinical data
  - Assessment of clinical status (6-point ordinal scale)
  - Measures of clinical support:
    - Hospitalization
    - Oxygen requirement
    - Mechanical ventilator requirement
    - ICU requirement
    - ECMO requirement
  - Assessment of ARDS using PaO_2 (or SpO_2), FiO_2, and recent chest x-ray data.
- Review new medical conditions, concomitant medication, and evaluate for AEs

6.3.10 Study Day 28 (Day 28-32)

If the subject is no longer hospitalized, this visit may occur by telephone. In this case, the assessments below should be restricted to the ordinal scale, oxygen requirements, new medical conditions, concomitant medication, and evaluation for AEs.

6.3.10.1 Clinical Data on Day 28

- Vital signs, including SpO_2. These values may be obtained from clinically collected information if they were obtained within 4 hours of the study assessment. Vital signs should be collected in a complete set within 30 minutes of each other.
- Clinical data
  - Assessment of clinical status (6-point ordinal scale)
  - Measures of clinical support:
    - Hospitalization
    - Oxygen requirement
    - Mechanical ventilator requirement
    - ICU requirement
    - ECMO requirement
  - Assessment of ARDS using PaO_2 (or SpO_2), FiO_2, and recent chest x-ray data.
- Review new medical conditions, concomitant medication, and evaluate for AEs

6.4 Special Pregnancy Follow-up Visit

Pregnant subjects should have additional follow-up to evaluate outcomes of the pregnancy. These visits may occur in the hospital, clinic, by chart review, or by telephone, and should occur every month (±2 weeks, by calendar day of each month) starting one month after discharge until after delivery. The purpose is to assess for SAEs and for pregnancy outcome including gestational age at outcome and live births.

7 MEASURES OF EFFICACY, SAFETY, AND PHARMACOKINETICS

7.1 Pharmacokinetic Measures

Anti-influenza A/H1N1 and A/H3N2 antibodies will be measured by HAI in all subjects on Study Day 0 (pre-infusion), 1, 3, and 7.

7.2 Influenza A Diagnostics - Viral Shedding

OP swabs will be processed to determine quantitative PCR for influenza A.

7.3 Efficacy Measures

7.3.1 Ordinal Scale

The ordinal scale is an assessment of the clinical status at the first assessment of a given study day (i.e., at approximately 8AM). The clinical status should be recorded as the subject currently exists, without consideration as to need (i.e., is the subject in the ICU, and not does the subject have medical needs to be in the ICU, or has the subject been discharged home and not is the subject capable at that moment of being discharged to home). The scale is as follows:

1. Death
2. In ICU
3. Non-ICU hospitalization, requiring supplemental oxygen
4. Non-ICU hospitalization, not requiring supplemental oxygen

5. Not hospitalized, but unable to resume normal activities
6. Not hospitalized with full resumption of normal activities

For the purposes of this study, resumption of normal activities refers to resumption of activities that the subject could undertake prior to first onset of symptom(s) of influenza.

7.3.2 NEW Score

The NEW Score has demonstrated an ability to discriminate patients at risk of poor outcomes. [15] This score is based on 7 clinical parameters. The NEW Score is being used for inclusion criteria, and for efficacy (change from baseline to Day 3 and Day 7). For inclusion criteria, the NEW score is used to define severe influenza. Therefore, the subject must have had a NEW score ≥ 3 within 12 hours prior to randomization. For efficacy, baseline and follow-up days, the score should be calculated as it is at the time of assessment, i.e., no attempt should be made to find the best or worst score in a period of time.

All parameters of the NEW score, including vitals, must be collected as a complete set within 30 minutes of each other. From Day 1 onwards, this should be evaluated at the first assessment of a given study day (i.e., at approximately 8AM). These parameters can be obtained from the hospital chart if performed within 4 hours of the time of assessment, or can be measured at the time of assessment.

Table 6: NEW Score

National Early Warning Score (NEWS)*

PHYSIOLOGICAL PARAMETERS	3	2	1	0	1	2	3
Respiration Rate	≤8		9 - 11	12 - 20		21 - 24	≥25
Oxygen Saturations	≤91	92 - 93	94 - 95	≥96			
Any Supplemental Oxygen		Yes		No			
Temperature	≤35.0		35.1 - 36.0	36.1 - 38.0	38.1 - 39.0	≥39.1	
Systolic BP	≤90	91 - 100	101 - 110	111 - 219			≥220
Heart Rate	≤40		41 - 50	51 - 90	91 - 110	111 - 130	≥131
Level of Consciousness				A			V, P, or U

*The NEWS initiative flowed from the Royal College of Physicians' NEWS Development and Implementation Group (NEWSDIG) report, and was jointly developed and funded in collaboration with the Royal College of Physicians, Royal College of Nursing, National Outreach Forum and NHS Training for Innovation.

Please see next page for explanatory text about this chart.

Royal College of Physicians

NHS Training for Innovation

7.3.3 PEW Score

The PEW score is a numerical scoring system to define pediatric patients at risk of clinical deterioration. [16] This score is based on 7 clinical parameters. The PEW Score is being used for inclusion criteria, and for efficacy (change from baseline to Day 3 and Day 7). For inclusion criteria, the PEW score is used to define severe influenza. Therefore the subject must have had a PEW score ≥ 3 within 12 hours prior to randomization. For efficacy, baseline and follow-up days, the score should be calculated as it is at the time of assessment, i.e., no attempt should be made to find the best or worst score in a period of time.

All parameters of the PEW score, including vitals, must be collected as a complete set within 30 minutes of each other. From Day 1 onwards, this should be evaluated at the first assessment of a given study day (i.e., at approximately 8:00 AM). These parameters can be obtained from the hospital chart if performed within 4 hours of the time of assessment, or can be measured at the time of assessment.

Table 7: PEW Score

		Component subscore			
Component	**Age group**	**0**	**1**	**2**	**4**
Heart rate, beats/min	<3 months	>110 and <150	≥150 or ≤110	≥180 or ≤90	≥190 or ≤80
	3 to <12 months	>100 and <150	≥150 or ≤100	≥170 or ≤80	≥180 or ≤70
	1 to 4 years	>90 and <120	≥120 or ≤90	≥150 or ≤70	≥170 or ≤60
	5 to <12 years	>70 and <110	≥110 or ≤70	≥130 or ≤60	≥150 or ≤50
	≥12 years	>60 and <100	≥100 or ≤60	≥120 or ≤50	≥140 or ≤40
Systolic blood pressure, mmHg	<3 months	>60 and <80	≥ 80 or ≤60	≥100 or ≤50	≥130 or ≤45
	3 to <12 months	>80 and <100	≥100 or ≤80	≥120 or ≤70	≥150 or ≤60
	1 to 4 years	>90 and <110	≥110 or ≤90	≥125 or ≤75	≥160 or ≤65
	5 to <12 years	>90 and <120	≥120 or ≤90	≥140 or ≤80	≥170 or ≤70
	≥12 years	>100 and <130	≥130 or ≤100	≥150 or ≤85	≥190 or ≤75
Capillary refill, s		<3			≥3
Respiratory rate, breaths/min	<3 months	>29 and <61	≥61 or ≤29	≥81 or ≤19	≥91 or ≤15
	3 to <12 months	>24 or <51	≥51 or ≤24	≥71 or ≤19	≥81 or ≤15
	1 to 4 years	>19 or <41	≥41 or ≤19	≥61 or ≤15	≥71 or ≤12
	5 to <12 years	>19 or <31	≥31 or ≤19	≥41 or ≤14	≥51 or ≤10
	≥12 years	>11 or <17	≥17 or ≤11	≥23 or ≤10	≥30 or ≤9
Respiratory effort		Normal	Mild increase	Moderate increase	Severe increase/any apnea
Oxygen saturation, %		>94	91–94	≤90	
Oxygen therapy		Room air		Any: <4 L/min or <50%	≥4 L/min or ≥50%

7.3.4 SOFA Score

The SOFA score is a scoring system to determine the extent of a person's organ function or rate of failure. [17, 18] The score is based on six different scores, one each for the respiratory, cardiovascular, hepatic, coagulation, renal, and neurological systems. Baseline assessment should be based on data collected closest to (but also prior to) randomization and plasma administration. Each subsequent day should use the worst value for each parameter in the preceding 24 hour period.

<u>Respiratory System</u>

When using PaO_2:

PaO_2/FiO_2 (mmHg)

0 = $PaO_2/FiO_2 \geq 400$

1 = $PaO_2/FiO_2 < 400$

2 = $PaO_2/FiO_2 < 300$

3 = $PaO_2/FiO_2 < 200$ and mechanically ventilated

4 = $PaO_2/FiO_2 < 100$ and mechanically ventilated

PaO_2 should be used if available. However, if PaO_2 is not available, the following table can be used to generate the SOFA score with SpO_2 [19]:

Table 8: Use of SpO_2/FiO_2 in SOFA Score

SOFA Respiratory System Points (using SpO_2)	SpO_2/FiO_2 Ratio		
	PEEP < 8 or not intubated	PEEP 8-12	PEEP > 12
0	≥ 457	≥ 515	≥ 425
1	< 457	< 515	< 425
2	< 370	< 387	< 332
3	< 240	< 259	< 234
4	< 115	< 130	< 129

Note: The original SpO_2/FiO_2 Ratio as published would require a SpO_2 > 110% to achieve a SOFA score of 0. Therefore, this score has been modified to accept a SpO_2 of 96% or greater on room air as normal (i.e. a SpO_2/FiO_2 Ratio of ≥ 457 would be a SOFA score = 0).

Nervous System

Glasgow coma score

0 = GCS score of 15
1 = GCS score of 13-14
2 = GCS score of 10-12
3 = GCS score of 6-9
4 = GCS score of < 6

Cardiovascular System

Mean arterial pressure (MAP) OR administration of vasopressors required (vasopressor drug doses are in mcg/kg/min)

0 = No hypotension
1 = MAP < 70 mmHg
2 = dopamine ≤ 5 or dobutamine (any dose)
3 = dopamine > 5 OR epinephrine ≤ 0.1 OR norepinephrine ≤ 0.1
4 = dopamine > 15 OR epinephrine > 0.1 OR norepinephrine > 0.1

Liver

Total Bilirubin (mg/dL)

0 = Total Bilirubin < 1.2
1 = Total Bilirubin 1.2 - 1.9
2 = Total Bilirubin 2.0 - 5.9
3 = Total Bilirubin 6.0 - 11.9
4 = Total Bilirubin ≥ 12.0

Coagulation

Platelets$\times 10^3/\mu L$

0 = Platelets $\geq$ 150 $\times 10^3/\mu L$
1 = Platelets < 150 $\times 10^3/\mu L$
2 = Platelets < 100 $\times 10^3/\mu L$
3 = Platelets < 50 $\times 10^3/\mu L$
4 = Platelets < 20 $\times 10^3/\mu L$

Renal System

Creatinine (mg/dL) (or urine output)
0 = Creatinine < 1.2 mg/dL
1 = Creatinine 1.2 - 1.9 mg/dL
2 = Creatinine 2.0 - 3.4 mg/dL
3 = Creatinine 3.5 - 4.9 mg/dL, or urine output < 500 mL/d
4 = Creatinine $\geq$ 5.0, or urine output < 200 mL/d, or dialysis requirement

7.3.5 PELOD Score

The PELOD score is an organ dysfunction score that can be used in the pediatric population to sequentially evaluate organ dysfunction and predict mortality. Studies have noted that to calculate the PELOD score, each organ dysfunction received points for the variable associated with the highest points. For example, if the worst heart rate of the day was 200 beats/min (10 PELOD points) and systolic blood pressure remained at 30 mmHg (20 PELOD points), then 20 PELOD points were assigned. The maximum number of points for an organ is 20, and the maximum PELOD score 71 [20, 21]. Baseline assessment should be based on data collected closest to (but also prior to) randomization and plasma administration. Each subsequent day should use the worst value for each parameter in the preceding 24 hour period.

Special Considerations for Respiratory Score:

- If blood gas testing was not performed for clinical reasons (e.g., as part of medical care) and the subject is not intubated, it can be assumed that the PaO_2/FiO_2 ratio is >70 and $PaCO_2$ is $\leq$ 90, and the respiratory score would be 0.
- If the subject is intubated then there will likely be, as part of routine care, an ABG or VBG to determine if the score is 1 or 10 points.
    - A PaO_2/FiO_2 ratio is $\leq$ 70 and $PaCO_2$ is > 90 is very severe disease. If no ABG or VBG is available, the clinical team will be able to estimate if the PaO_2/FiO_2 ratio is $\leq$ 70 or $PaCO_2$ is > 90 based on severity of disease, in which case the respiratory score would be 10.

Table 9: PELOD Scoring System

Scoring system				
	0	**1**	**10**	**20**
Organ dysfunction and variable				
Neurological*				
Glasgow coma score	12–15	7–11	4–6	3
	and		or	
Pupillary reactions	Both reactive	NA	Both fixed	NA
Cardiovascular†				
Heart rate (beats/min)				
<12 years	≤195	NA	>195	NA
≥12 years	≤150	NA	>150	NA
	and		or	
Systolic blood pressure (mm Hg)				
<1 month	>65	NA	35–65	<35
1 month−1 year‡	>75	NA	35–75	<35
1–12 years‡	>85	NA	45–85	<45
≥12 years	>95	NA	55–95	<55
Renal				
Creatinine (mg/dl)				
<7 days	<1.58	NA	≥1.58	NA
7 days−1 year‡	<0.39	NA	≥0.39	NA
1–12 years‡	<1.13	NA	≥1.13	NA
≥12 years	<1.58	NA	≥1.58	NA
Respiratory§				
PaO2 (mmHg)/FIO2 ratio	>70	NA	≤70	NA
	and		or	
PaCO2 (mmHg)	≤90	NA	>90	NA
	and			
Mechanical ventilation§	No ventilation	Ventilation	NA	NA
Hematological				
White blood cell count (×109/L)	≥4.5	1.5–4.4	<1.5	NA
	and	or		
Platelets (×109/L)	≥35	<35	NA	NA
Hepatic				
Aspartate transaminase (IU/L)	<950	≥950	NA	NA
	and	or		
Prothrombin time (or INR)	>60 (<1.40)	≤60 (≥1.40)	NA	NA

Notes:
PaO2=arterial oxygen pressure. FIO2=fraction of inspired oxygen.
PaCO2 =arterial carbon dioxide pressure. INR=international normalized ratio.

**** Glasgow coma score: use lowest value. If patient is sedated, record estimated Glasgow coma score before sedation. Assess patient only with known or suspected acute central nervous system disease.***

† Heart rate and systolic blood pressure: do not assess during crying or iatrogenic agitation.
‡ Strictly less than.
§ If arterial measurement of PaO2 unavailable, estimate fPaO2/FIO2 ration from SaO2. Mechanical ventilation: the use of mask ventilation is not counted as mechanical ventilation.measurement only. PaCO2 may be measured from arterial, capillary, or venous samples.

7.3.6 ARDS

The incidence of ARDS will be assessed at each study day using the revised Berlin definition of ARDS. [22]

Table 10: Berlin Criteria for ARDS

	Acute Respiratory Distress Syndrome
Timing	Within 1 week of a known clinical insult or new or worsening respiratory symptoms
Chest imaging[a]	Bilateral opacities—not fully explained by effusions, lobar/lung collapse, or nodules
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload Need objective assessment (eg, echocardiography) to exclude hydrostatic edema if no risk factor present
Oxygenation[b]	
Mild	200 mm Hg < PaO_2/FIO_2 ≤ 300 mm Hg with PEEP or CPAP ≥5 cm H_2O[c]
Moderate	100 mm Hg < PaO_2/FIO_2 ≤ 200 mm Hg with PEEP ≥5 cm H_2O
Severe	PaO_2/FIO_2 ≤ 100 mm Hg with PEEP ≥5 cm H_2O

Abbreviations: CPAP, continuous positive airway pressure; FIO_2, fraction of inspired oxygen; PaO_2, partial pressure of arterial oxygen; PEEP, positive end-expiratory pressure.
[a] Chest radiograph or computed tomography scan.
[b] If altitude is higher than 1000 m, the correction factor should be calculated as follows: [PaO_2/FIO_2 × (barometric pressure/760)].
[c] This may be delivered noninvasively in the mild acute respiratory distress syndrome group.

If PaO_2 is not available, the Manual of Operations (MOP) provides instructions for estimating the PaO_2/FiO_2 ratio from the FiO_2 and SpO_2.

The oxygenation assessment should be within 4 hours of the assessment of ARDS. If there is no chest imaging on the day of assessment, the most recent imaging may be used. Subjects meeting all criteria meet criteria for ARDS. Severity should be graded based on the oxygenation in the above table.

7.3.7 Measures of clinical support

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

- Hospitalization
- Oxygen requirement
- Mechanical ventilator requirement
- ICU requirement
- ECMO requirement

If any of these are present, it should be determined if these are new or ongoing from previous study visits. In the case of a new requirement, the date started should be recorded (not just the date of assessment).

If there were previous interventions that are no longer needed, the date of discontinuation should be noted (not just the date of assessment).

7.3.8 Efficacy Measures Not Used

In IRC002, symptom resolution provided no discriminating power in this population. Additionally, it is notoriously inaccurate, and therefore will not be collected in this study. Most subjects in IRC002 did not have fever, and therefore resolution of fever will not be used in this study.

7.4 Safety Evaluations

As both arms are receiving plasma, the study is not designed to determine the safety of plasma. The safety profile of plasma, however, is well known from decades of transfusions. This study will be able to determine the safety of specific anti-influenza plasma to determine if the rate of AEs is higher, lower, or no different than standard (low-titer) plasma.

7.4.1 Laboratory Evaluations

All laboratory evaluations (except reference endpoint assays) will be performed at the local CLIA, CLIP, or CAP certified clinical laboratory (comparable certifications that are not listed maybe be used after approval by the sponsor). The same laboratory should be used throughout the study for a given subject. Blood samples will be collected at screening if necessary for ABO typing or pregnancy testing, and on Study Days 0, 1, 3, and 7, for evaluation of routine laboratory safety (chemistry, complete blood count with differential). The investigator is to use the "Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events" Version 2.0, November 2014 which can be found in Appendix A.

7.5 Research Tests

At visits noted in Section 6, blood will be obtained for other laboratory tests that help characterize subjects' immune response to influenza. This could include antibody response to influenza and other biomarkers of severity of disease. Additionally, OP swabs are obtained for the purpose of assessing viral replication, and any remaining samples may be used to assess other aspect of the virus infection (such as sequencing). No genetic testing of the subject is done on these samples. Any use of these specimens beyond the above stated purposes will require review of the planned research by the IRB/IEC.

8 RISKS/BENEFITS

8.1 Potential Risks

8.1.1 Risk of Plasma Transfusions

Possible transfusion reactions and adverse events are outlined below. The management of all reactions to plasma should follow institutional policies and clinician's judgement.

Common risks of plasma transfusions may include one or more of the following: fever, rash, hives, or headache.

Allergic transfusion reactions can occur when patients have antibodies that react with proteins in the plasma. They can present with urticaria, itching, and/or fevers that occur soon after starting a transfusion. Symptoms are usually controlled by slowing the transfusion and giving antihistamine. Generally, the transfusion may be continued if the symptoms are self-limited.

TRALI may occur, but this risk is minimized by only using male-donated plasma in this study (as noted in Section 5.1). TRALI is characterized by a clinical constellation of symptoms including dyspnea, hypotension, and fever. Although the precise pathogenesis of TRALI remains unknown, it has been shown to be related to the transfusion of anti-HLA class I and anti-neutrophil antibodies most often from plasma from multiparous women (antibodies presumably generated during pregnancy) or donors who have received multiple blood transfusions. The risk of TRALI is reported as 1 out of 5000 transfusions. [23]

The infused plasma volume is roughly 450-700 mL, so there is the risk of volume overload in the recipient which could cause pulmonary edema. Transfusion-associated circulatory overload (TACO) has

been associated with plasma infusion and may be clinically indistinguishable from TRALI even though the physiologic mechanisms differ. TACO is hydrostatic not permeability edema and more responsive to diuresis when it occurs. [24] Subjects with preexisting conditions who may not tolerate this volume of plasma will be excluded from this study, but this condition could still occur in recipients.

There are case reports of pulmonary emboli occurring after administration of IVIG and plasma therapy, though definitive studies assessing risk are lacking. [25] In one series, 5 of 10 subjects critically ill with H1N1 were shown to have pulmonary emboli. [26] This high percentage has not been shown in other H1N1 series, and pulmonary emboli have been shown to develop in approximately 10%-15% of critically ill adults. [27] However, the potential risk of pulmonary embolism exists.

In IRC002, one SAE of liver injury was considered to be possibly related to the plasma administration. Acute liver injury has been observed in other subjects and considered not related to the plasma. In IRC005, one SAE of stroke was considered to be possibly related to the plasma administration. Strokes were seen at a similar frequency in both the plasma and the control arm of IRC002 (where no plasma was given) and were not considered related to study plasma in that study. Given the severity of illness in the population being treated, exact attribution of causality of these events is difficult.

Other more serious risks are rare and may include the following: serious allergic reactions including anaphylaxis, bacterial infections, or viral infections like hepatitis B, hepatitis C, human immunodeficiency virus (HIV) and Zika. Anaphylaxis, which can be life threatening, may be associated with skin reactions, swelling of face, lips, tongue or throat, constricted airways with wheezing and trouble breathing, weak and rapid pulse, nausea, vomiting, dizziness, fainting and/or shock.

In IRC002, the study population was very ill and therefore had many Serious Adverse Events (SAEs). The following are the list of SAEs seen in IRC002:

Table 11: SAEs seen in IRC002

Event	Total (N=98)	Treatment received P+S (N=46)	S (N=52)
Overall	**28 (29%)**	**9 (20%)**	**19 (37%)**
Respiratory, thoracic and mediastinal disorders	**9 (9%)**	**2 (4%)**	**7 (14%)**
Acute respiratory distress syndrome	3 (3%)	1 (2%)	2 (4%)
Pneumothorax	2 (2%)	0 (0%)	2 (4%)
Respiratory failure	2 (2%)	0 (0%)	2 (4%)
Pharyngeal haemorrhage	1 (1%)	1 (2%)	0 (0%)
Respiratory distress	1 (1%)	0 (0%)	1 (2%)
Pulmonary embolism	1 (1%)	0 (0%)	1 (2%)
Nervous system disorders	**6 (6%)**	**2 (4%)**	**4 (8%)**
Cerebrovascular accident	3 (3%)	1 (2%)	2 (4%)
Cerebral infarction	1 (1%)	1 (2%)	0 (0%)
Subarachnoid haemorrhage	1 (1%)	1 (2%)	0 (0%)
Seizure	1 (1%)	0 (0%)	1 (2%)
Sedation	1 (1%)	0 (0%)	1 (2%)
Mental impairment	1 (1%)	0 (0%)	1 (2%)
Infections and infestations	**6 (6%)**	**2 (4%)**	**4 (8%)**
Septic shock	2 (2%)	1 (2%)	1 (2%)
Clostridium difficile colitis	1 (1%)	1 (2%)	0 (0%)
Lower respiratory tract infection viral	1 (1%)	0 (0%)	1 (2%)
Sepsis	1 (1%)	0 (0%)	1 (2%)
Pneumonia	1 (1%)	0 (0%)	1 (2%)
Metabolism and nutrition disorders	**5 (5%)**	**2 (4%)**	**3 (6%)**
Hyperkalaemia	2 (2%)	0 (0%)	2 (4%)
Gout	1 (1%)	1 (2%)	0 (0%)
Hypernatraemia	1 (1%)	1 (2%)	0 (0%)
Acidosis	1 (1%)	0 (0%)	1 (2%)
Gastrointestinal disorders	**4 (4%)**	**0 (0%)**	**4 (8%)**
Intestinal ischaemia	1 (1%)	0 (0%)	1 (2%)
Gastrointestinal haemorrhage	1 (1%)	0 (0%)	1 (2%)
Abdominal compartment syndrome	1 (1%)	0 (0%)	1 (2%)
Retroperitoneal haemorrhage	1 (1%)	0 (0%)	1 (2%)
Cardiac disorders	**3 (3%)**	**1 (2%)**	**2 (4%)**
Atrial fibrillation	1 (1%)	0 (0%)	1 (2%)
Cardiac arrest	1 (1%)	0 (0%)	1 (2%)
Cardiogenic shock	1 (1%)	0 (0%)	1 (2%)
Supraventricular tachycardia	1 (1%)	1 (2%)	0 (0%)
Hepatobiliary disorders	**2 (2%)**	**1 (2%)**	**1 (2%)**
Portal vein thrombosis	1 (1%)	0 (0%)	1 (2%)
Liver injury	1 (1%)	1 (2%)	0 (0%)
Injury, poisoning and procedural complications	**2 (2%)**	**1 (2%)**	**1 (2%)**
Hepatic haematoma	1 (1%)	0 (0%)	1 (2%)
Post procedural haemorrhage	1 (1%)	1 (2%)	0 (0%)
Investigations	**2 (2%)**	**1 (2%)**	**1 (2%)**
Blood creatine phosphokinase MB increased	1 (1%)	1 (2%)	0 (0%)
Activated partial thromboplastin time prolonged	1 (1%)	0 (0%)	1 (2%)
Blood and lymphatic system disorders	**2 (2%)**	**0 (0%)**	**2 (4%)**
Leukocytosis	1 (1%)	0 (0%)	1 (2%)
Anaemia	1 (1%)	0 (0%)	1 (2%)
Surgical and medical procedures	**2 (2%)**	**0 (0%)**	**2 (4%)**
Endotracheal intubation	2 (2%)	0 (0%)	2 (4%)
Thoracostomy	1 (1%)	0 (0%)	1 (2%)
General disorders and administration site conditions	**1 (1%)**	**0 (0%)**	**1 (2%)**
Chest pain	1 (1%)	0 (0%)	1 (2%)
Psychiatric disorders	**1 (1%)**	**0 (0%)**	**1 (2%)**
Mental status changes	1 (1%)	0 (0%)	1 (2%)
Vascular disorders	**1 (1%)**	**0 (0%)**	**1 (2%)**
Hypotension	1 (1%)	0 (0%)	1 (2%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	**1 (1%)**	**0 (0%)**	**1 (2%)**
Acute myeloid leukaemia	1 (1%)	0 (0%)	1 (2%)

8.1.2 Risk of Phlebotomy

The primary risks of phlebotomy include local discomfort, occasional bleeding or bruising of the skin at the site of needle puncture, and rarely hematoma, infection, or fainting. At the time of enrollment and during study visits, each subject will be asked about participation in other research studies to ensure that blood draws do not exceed the following amounts for all research protocols combined: 10.5 mL/kg or 550 mL, whichever is smaller, over any 8-week period for adults, and no more than 5 mL/kg in a single day (no more than 9.5 mL/kg may be drawn over any 8-week period) for persons under the age of 18.

8.1.3 Risk of Oropharyngeal Swab

The primary risk of an OP swab is local discomfort. This swab can stimulate the gag reflex and, very rarely, vomiting.

8.2 Potential Benefits

8.2.1 Benefits of Treatment

The benefits of antiviral treatment with anti-influenza immune plasma in patients actively infected with influenza are unknown. However, the IRC002 study is suggestive that anti-influenza plasma in addition to standard antiviral therapy (i.e., oseltamivir) will more rapidly decrease severity of illness and improve outcomes after infection with influenza compared to not receiving anti-influenza plasma.

8.2.2 Benefits of Diagnosis

Knowing the diagnosis of influenza will allow appropriate precautions to be taken to prevent accidental transmission and infection to others (i.e., family members, co-workers, etc.). This knowledge may also allow appropriate public health interventions to possibly minimize additional cases.

8.2.3 Alternatives

The alternative to participation in this study is routine standard care. For suspected or confirmed severe influenza this will generally include an antiviral (amantadine, rimantadine, oseltamivir, or zanamivir) determined largely on availability and susceptibility.

9 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS AND DATA

9.1 Intended Use of the Samples/Specimens/Data

Samples and data collected under this protocol may be used to study the clinical features, immunology, and virology of human influenza.

9.2 Storage of Samples/Specimens/Data

As part of this protocol, and with consent from study subjects, samples will be stored for further research. Any future research (aside from research listed within this protocol) will require approval from the IRB/EC. Samples and data will be stored using codes assigned by the investigators or their designee(s). Data will be kept in password-protected computers which are located in locked rooms. Samples will be stored in locked facilities.

9.3 Storage of Genetic Sample

Samples will not be obtained or stored for genetic testing as part of this study.

9.4 Tracking Samples/Specimens/Data

Samples will be tracked by a commercial software program.

9.5 Use of Samples/Specimens/Data at the Completion of the Protocol

Samples will be maintained for further laboratory testing for up to 10 years after completion (or termination) of the protocol. This length of time for storing samples may be extended only after permission of the IRB/IEC.

9.6 Reporting Loss or Destruction of Samples/Specimens/Data

Any loss or unanticipated destruction of locally maintained samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) will be reported to the corresponding IRB/IECs.

Any loss or unanticipated destruction of centrally maintained samples or data will be reported to all IRB/IECs.

10 ASSESSMENT OF SAFETY

10.1 Definitions

Adverse Event (AE)

An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse Reaction (AR)

An AE that is caused by an investigational agent (drug or biologic).

Suspected Adverse Reaction (SAR)

An AE for which there is a reasonable possibility that the investigational agent caused the AE. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE. An SAR implies a lesser degree of certainty about causality than AR, which implies a high degree of certainty.

Serious Adverse Event (SAE)

An SAE is an AE that results in one or more of the following outcomes:

- death
- a life threatening event (places the subject at immediate risk of death from the event as it occurred)
- an inpatient hospitalization or prolongation of an existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect
- a medically important event*

* Medical and scientific judgment should be exercised in deciding events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Unexpected Adverse Event

An AE is unexpected if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed.

It is the responsibility of the IND Sponsor to make this determination.

Serious and Unexpected Suspected Adverse Reaction (SUSAR)

A SUSAR is an SAR that is both serious and unexpected.

Unanticipated Problem (UP)

A UP is any event, incident, experience, or outcome that is:

1. unexpected in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document, Investigator's Brochure, or other study documents; and
   b. the characteristics of the subject population being studied; and
2. possibly, probably, or definitely related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (Per the IND Sponsor, an AE with a serious outcome will be considered increased risk.)

Unanticipated Problem that is not an Adverse Event (UPnonAE)

A UP that does not fit the definition of an AE, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered non-serious UPs. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Protocol Deviation

Any change, divergence, or departure from the IRB-approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as:

1. Those that occur because a member of the research team deviates from the protocol;
2. Those that are identified before they occur, but cannot be prevented;
3. Those that are discovered after they occur.

Serious Protocol Deviation

A deviation that meets the definition of a SAE or compromises (or has the potential to compromise) the safety, welfare, or rights of subjects or others.

10.2 Documenting, Recording, and Reporting Adverse Events

All grade 3 or grade 4 AEs, and all SAEs occurring from the time the informed consent is signed through the Day 28 (end of study) visit will be documented, recorded, and reported. Additionally, any AE leading to plasma discontinuation (regardless of grade) will be documented, recorded, and reported.

At each contact with the subject, information regarding AEs will be elicited by appropriate questioning and examinations and will be:

- immediately documented in the subject's medical record/source document,
- recorded on the AE eCase Report Form (AE eCRF), and
- reported as outlined below.

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

All abnormal laboratory findings will be reviewed on a routine basis by the PI to identify potential safety signals. All worsening laboratory values should be evaluated as potential AEs:

- If an abnormal laboratory finding is captured as a component of a diagnosis AE term that has already been recorded, the individual abnormal laboratory finding is NOT recorded as a separate AE.
- A laboratory abnormality should be reported as an AE if it requires an intervention or is considered clinically significant by the investigator (all Grade 3 or higher are considered clinically significant for this study). Interventions include, but are not limited to, discontinuation of treatment, dose reduction/delay, additional assessments such as repeating the lab test, or concomitant treatment.

  An abnormal lab not included on the DAIDS Table for Grading the Severity of Adult and Pediatric Events (V.2.0, Nov, 2014) should be assessed based on the following grading criteria.

  - Grade 1 (Mild) [not reportable]
    Events causing no or minimal interference with daily activity
  - Grade 2 (Moderate) [not reportable]
    Events causing greater than minimal interference with daily activity but not requiring medical intervention
  - Grade 3 (Severe) [reportable]
    Events causing inability to perform daily activity, or suggests organ toxicity, and/or requires medical intervention
  - Grade 4 (Potentially Life-Threatening)* [reportable]
    Events causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
  - Grade 5 (Death) [reportable]

  *** Note:** A severity assessment of "potentially life-threatening" is not necessarily the same as a life-threatening event as described in the SAE criterion. The latter means that the event is an immediate threat to life as opposed to a potential threat to life.

- In addition, any medically important laboratory abnormality may be reported as an AE at the discretion of the investigator who considers the test result clinically significant. This could include a laboratory result for which there is no intervention but the abnormal value is of clinical concern or suggests disease or organ toxicity. Grading should be based on the toxicity table where applicable or per the above guidelines.

10.2.1 Assessment of Adverse Event

The Investigator (or designee) will evaluate all AEs with respect to Seriousness (SAE criteria listed above), Severity (grading), and Causality (relationship to study agent and relationship to research) according to the following guidelines.

10.2.2 Severity

The Investigator (or designee) will grade the severity of each AE according to the "Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events" Version 2.0, November 2014 which can be found in Appendix A.

10.2.3 Causality

Causality (likelihood that the event is caused by the study agent(s)) will be assessed considering the factors listed under the following categories:

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- evidence to suggest a causal relationship
- is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship

OR

- good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship

OR

- definitely due to an alternative etiology

Note: Other factors should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator (or designee) may revise the causality assessment as additional information becomes available.

10.3 Investigator Reporting Responsibilities to the Sponsor

10.3.1 Adverse Events

Line listings and/or cumulative listings of AEs will be generated from the centralized database and submitted to the Sponsor when needed for periodic safety assessments, review of IND safety reports, preparation of IND annual reports and final study reports.

10.3.2 Serious Adverse Events

All SAEs (regardless of relationship and whether or not they are also UPs) must be reported on the Safety Expedited Report Form (SERF) and sent to the Clinical Safety Office (CSO) by fax or email attachment. Deaths and immediately life threatening SAEs must be reported to the CSO within 1 business day after the site becomes aware of the event. All other SAEs must be reported within 3 business days of site awareness.

CSO CONTACT INFORMATION:

Clinical Safety Office

5705 Industry Lane
Frederick, MD 21704
Phone 301-846-5301
Fax 301-846-6224
E-mail: rchspsafety@mail.nih.gov

SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE eCRF and the SERF.

10.3.3 Unanticipated Problems

UPs that are also AEs must be reported to the CSO and sent by fax or email attachment no later than 7 calendar days of site awareness of the event. UPs that are related to plasma administration, regardless of whether an AE occurred or not, should be reported to the CSO. All other UPs that are not associated with an AE are not reported to the CSO but may require sponsor and IRB notification.

If reporting UPs to the CSO, use the SERF or a local IRB UP form.

10.4 Safety Reporting for Off Study Subjects

After the end of the protocol-defined AE reporting period (Consent through the Day 28 visit), SAEs that are thought possibly, probably or definitely related to the plasma should be reported as SAEs if the site becomes aware of the event. However, aside from pregnancy follow-up detailed in Section 6.4, there is no expectation to actively evaluate all subjects for SAEs after protocol-defined AE reporting period ends.

Any SAEs that occur after the Day 28 visit will be noted in the final study report, though will not be used for the primary efficacy and safety analysis.

10.5 Investigator Reporting Responsibilities to the IRB

Investigators are responsible for reporting per their local IRB's requirements. The IRB-specific reporting requirements are attached as an appendix [if applicable].

10.6 Sponsor's Reporting Responsibilities

SUSARs as defined in 21 CFR 312.32 and determined by the IND Sponsor will be reported to FDA and all participating investigators as IND Safety Reports.

The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

AEs that are also UPs will be summarized by the IND Sponsor and distributed to investigators if they are relevant to other sites.

10.7 Safety Oversight

10.7.1 Protocol Team Monitoring Plan

The protocol team will review the enrollment and study-related safety data (AEs and SAEs) from all sites periodically during the study. These reviews will occur at a minimum twice monthly during active enrollment and follow-up. During periods of low influenza activity when no enrollment is occurring, these reviews do not need to occur.

10.7.2 Sponsor Medical Monitor

A Medical Monitor, representing the IND Sponsor (OCRPRO), has been appointed for oversight of safety in this clinical study. The Sponsor Medical Monitor will be responsible for performing periodic safety assessments.

10.8 Data and Safety Monitoring Plan

The NIAID Intramural DSMB includes independent experts that do not have direct involvement in the conduct of the study and have no significant conflicts of interests as defined by NIAID policy. The Board reviewed the study prior to initiation and will review the study yearly thereafter, unless otherwise recommended by the Board. Given the seasonal nature of influenza (generally November through April) the interim reviews will occur after each influenza season. The Board may convene additional reviews as necessary. The Board will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. All SAEs, all UPs, and all IND Safety Reports will be reported to the DSMB by the CSO. The DSMB will be notified of any cases of intentional or unintentional unblinding as soon as possible.

As a guideline for monitoring results comparing randomized treatments for the primary endpoint, as it is unlikely that this trial will be replicated, a high level of evidence concerning any favorable effect of high-titer versus low-titer anti-influenza plasma is considered desirable before considering early termination of the study. For this reason, the Peto-Haybittle stopping guideline will be used; this requires a nominal p-value of < 0.001 at an interim analysis in order for termination to be considered. Such a level of evidence would also facilitate more precise comparison of effects in patient subgroups and for secondary endpoints. Although application of this guideline is focused on the primary endpoint, if a similar level of evidence is achieved first in comparing mortality rates between randomized arms, then termination of the study might also be considered.

If accrual is slower than anticipated such that the study is not fully enrolled within three years, then a futility analysis may be conducted at that time (i.e. after about three years of enrollment). The DSMB may recommend termination or modification of the study if it appears unlikely that continuation of the study will demonstrate a benefit of high titer plasma on the primary endpoint. Conditional power provides a measure for evaluating futility: specifically, conditional on the results observed at an interim analysis, it is the power of the study to show a significant benefit of high titer plasma assuming an underlying true difference between the randomized arms (that will determine what future data might be observed). Conditional power will be evaluated both (a) under the assumption that high titer plasma truly affects the primary endpoint by the amount assumed in designing the study (providing 90% power—see Section 1.2); and (b) for a range of true effects that appear plausible based on the observed rates at the time of the interim analysis.

Consideration of futility with respect to conditional power requires consideration by the DSMB of the broad array of information available at an interim analysis and of the value of continuing the study even if conditional power for the primary endpoint might be low. For example, this might be because of effects being observed on mortality or other secondary endpoints or because continuing the study might be valuable in showing definitively a lack of an effect of high titer versus low titer plasma. As guidance,

however, if the conditional power to show an effect of high versus low titer plasma if the study continued to its planned sample size is lower than 20% under a range of plausible situations covered by (a) and/or (b) above, then the DSMB might recommend study termination.

Each site PI will submit the written DSMB summary reports with recommendations to their IRB(s).

10.9 Pausing Rules

The population enrolled in this study is a critically ill population, and based on the IRC002 data will likely have many SAEs due to the underlying illness. The medical monitor's review of the safety data is most likely to detect signals suggesting subject safety is compromised. However, below are absolute criteria for which the study will be paused to further enrollment until assessed by the sponsor medical monitor:

- Two or more of the same unexpected SAE (unexpected AE in terms of previously described risks in the Investigator's Brochure) that are probably or definitively attributed to the study plasma.
- Four or more of the same SAE that can be possibly, probably, or definitively attributed to the study drug.

The sponsor medical monitor will ultimately make the decision to either resume the study, ask for formal DSMB review, or stop the study.

11 STUDY MONITORING

As per ICH-GCP 5.18 and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID will visit the clinical research site to monitor all aspects of the study in accordance with the appropriate regulations. The objectives of a monitoring visit will be:

1) to verify the existence of signed informed consent documents and documentation of the informed consent process for each monitored subject;

2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs;

3) to compare abstracted information with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and

4) to help ensure investigators are in compliance with the protocol.

The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA, and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The site investigator (and/or designee) will make study documents (e.g., consent forms) and pertinent hospital or clinical records readily available for inspection by the local IRB, the U.S. FDA, the site monitors, and the NIAID staff for confirmation of the study data.

12 STATISTICAL CONSIDERATIONS

12.1 General Considerations

This is a randomized, double-blind study comparing high-titer versus low-titer anti-influenza immune plasma for the treatment of subjects hospitalized with severe influenza A infection. The primary analysis population for the evaluation of both efficacy and safety outcomes will include all subjects who have the

study plasma infusion started. This is an intention-to-treat analysis with the modification that subjects who are randomized but who do not have the study plasma infusion started will be excluded. Most subjects that do not receive plasma withdraw from the study early (Day 0-2 based in IRC002 data). Therefore, use of the modified intention-to-treat population is thought reasonable for this study. Supplementary intention-to-treat analyses including all randomized subjects for mortality, primary endpoint, and main secondary endpoints will be provided in the final analysis, though there may be limited follow-up data on many of the subjects who did not receive plasma.

12.2 Sample Size and Power Considerations

The planned sample size for the trial is 150 subjects, randomized in an approximately 2:1 ratio to high-titer versus low-titer anti-influenza plasma. To evaluate the power of the study, the following assumptions were made:

a. The primary analysis will compare the high-titer and low-titer plasmas using a proportional odds model and a two-sided Type I error rate of 0.05.

b. It is anticipated that very few of these subjects will be randomized and not start study plasma infusion (and so be excluded from the primary analysis) or be lost to follow-up prior to Day 7 (and so have missing data for the primary endpoint). In addition, power is reduced slightly and hence the effective sample size is reduced by interim analyses reviewed by the DSMB. Conservatively, a 10% reduction in the total sample size from 150 to 135 subjects was made to allow for these issues.

c. Data from the control arm of IRC002 for adults with influenza A infection, NEW score ≥ 3, and duration of time from onset of symptoms of influenza to randomization ≤ 6 days, suggests the distribution of subjects in the low-titer arm for the primary endpoint shown in the following table:

Table 12: Anticipated Distribution of Subjects in the Ordinal Scale

	Low-Titer Arm	**High-Titer Arm**	
	(Based on IRC 002)	80% Power	90% Power
Died	10%	4.3%	3.7%
In ICU	35%	20.4%	18.4%
Non-ICU hospitalization, requiring supplemental oxygen	25%	23.6%	22.6%
Non-ICU hospitalization, not requiring supplemental oxygen	10%	13.3%	13.4%
Not hospitalized, but unable to resume normal activities	5%	7.9%	8.2%
Not hospitalized, but with full resumption of normal activities	15%	30.6%	33.7%
Proportional odds ratio comparing high titer to low titer		2.50	2.88

Based on the above assumptions, the table also shows the type of true difference (assuming proportional odds) that would be detected with 80% or 90% power. For example, the study would have 80% power to detect a proportional odds ratio of 2.50. This is the odds ratio that arises when comparing the proportion not hospitalized and able to resume normal activities in the high-titer arm (30.6%) to that in the low-titer arm (15%). The proportional odds model assumes that the same odds ratio applies to the cumulative proportions moving up the ordinal scale: for example, for the proportion not hospitalized in the high-titer arm (30.6+7.9=38.5%) to that in the low-titer arm (15+5=20%); or for the proportion alive and not in an ICU in the high-titer arm (30.6+7.9+13.3+23.6=75.4%) to that in the low-titer arm (15+5+10+25=55%).

12.3 Statistical Analysis

A detailed statistical analysis plan will be developed before undertaking any comparative analyses of outcomes for the randomized high and low plasma titers. The following provides a brief summary of approach to analysis for the primary endpoint.

As noted above, the primary analysis will use a modified intention-to-treat approach which excludes randomized subjects who do not have an infusion of study plasma started. Statistical inferences will use a two-sided Type 1 error rate of 0.05, and corresponding 95% confidence intervals.

Analysis of the primary endpoint (ordinal outcome of clinical status at Day 7) will use a proportional odds model with an indicator variable for randomized treatment. It is useful to note that the score test of the treatment effect from a proportional odds model is equivalent to a Wilcoxon rank sum test even if the proportional odds assumption does not hold. If there are missing data for the primary endpoint, then sensitivity analyses will be undertaken to evaluate the sensitivity of study conclusions to the handling of the missing data. The proportional odds model will be extended to undertake comparisons of randomized treatments adjusted for variables that might be predictive of the outcome, for example, the ordinal clinical status at baseline. The model will also be extended to evaluate possible differences in the comparison of randomized treatments between subgroups of subjects (treatment by subgroup interaction), including subgroups defined by sex, age (adult/child), race/ethnicity, ordinal clinical status at baseline, NEW score at baseline, and days since onset of influenza symptoms to randomization.

Binary variables such as 28-day mortality, in-hospital mortality, incidence of new ICU admissions, etc., are expected to occur infrequently and will be compared between randomized arms using exact logistic regression. Quantitative variables such as duration of ICU stay and change from baseline in NEW score, etc., are expected to have reasonably skewed distributions and may be subject to censoring (e.g., for subjects in an ICU on Day 28); these will be compared between randomized arms using non-parametric tests (e.g., Wilcoxon's Test adapted, if necessary, to handle censoring).

Analysis of AE data will primarily be descriptive based on MedDRA coding of events. The proportion of subjects experiencing an SAE and the proportion experiencing a Grade 3 or higher AE will be compared between randomized arms using Fisher's Exact Test.

Analysis of HAI titers will also primarily be descriptive, comparing the geometric mean titers at Days 0, 3, and 7 between randomized arms, to confirm (or otherwise) a difference in titers for subjects receiving high-titer versus low-titer anti-influenza plasma.

For enrolled subjects who were not randomized (i.e. screen failures) or who were randomized but did not receive plasma, the final analysis will detail safety (deaths and SAEs), and reasons they were not randomized or did not received plasma respectively.

12.4 Endpoints

12.4.1 Primary Endpoint

The primary end point is the subject's clinical status at Day 7 (6-point ordinal scale):

1. Death
2. In ICU
3. Non-ICU hospitalization, requiring supplemental oxygen
4. Non-ICU hospitalization, not requiring supplemental oxygen
5. Not hospitalized, but unable to resume normal activities
6. Not hospitalized with full resumption of normal activities

12.4.2 Secondary Endpoints

- Ordinal outcome assessed at Days 1, 2, 3, 7, 14, and 28
- Date of hospital admission and discharge
- Date and cause of death (if applicable)
- NEW/PEW score at Day 0, 3, and 7
- Date/time of supplemental oxygen initiation and discontinuation (if applicable)
- Date/time of ICU admission and discharge (if applicable)
- Date/time of mechanical ventilation initiation and discontinuation (if applicable)
- Date/time of meeting ARDS criteria, date of resolution, and severity (if applicable)
- Date/time of ECMO initiation and discontinuation (if applicable)
- SOFA score for age ≥ 18 years and PELOD score for age < 18 years on Days 0, 3, and 7
- Disposition (home, rehabilitation, chronic nursing facility) following the initial hospitalization
- Quantitative PCR for influenza in oral swab on Day 0 and 3.
- Grade 3 and 4 adverse events
- SAEs
- Safety laboratory tests will be collected on Days 0, 1, 3, and 7

o CBC with differential white cell count (to include neutrophil and lymphocyte percentages), hemoglobin, hematocrit, and platelets

o Blood chemistries (creatinine, glucose, total protein, ALT/GPT, AST/GOT, total bilirubin)

- Hemagglutination Inhibition (HAI) titer Days 0, 1, 3, 7
- Grade 3 and grade 4 adverse events (AEs)
- Serious adverse events (SAEs)

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 IRB/IEC Approval

This protocol, informed consent document, relevant supporting information, and all types of subject recruitment or advertisement information must be submitted to the IRB/IEC for review and must be approved before the study is initiated.

Any amendments must also be approved by the IRB/IEC prior to implementing changes in the study.

The site investigator is responsible for keeping the IRB/IEC apprised of the progress of the study as deemed appropriate, but in any case at least once a year. The site investigator must also keep the IRB/IEC informed of any significant AEs.

13.2 Compliance with Good Clinical Practices (GCP)

This study will be conducted in compliance with the conditions stipulated by NIAID and local IRB/IEC, informed consent regulations, U.S. FDA regulations, and ICH/GCP Guidelines. In addition, all local regulatory requirements will be adhered to, in particular those which afford greater protection to the safety of the trial participants.

13.3 Informed Consent

The informed consent form for this study must be signed by the study subject or legal representative before participation in the study. A copy of the signed consent must be provided to the study subject. Signed consents must remain in each study subject's study file and be available for verification by study monitors at any time.

In the case of illiterate subjects, the consent form can be read to the subjects. IRB approval for use of the oral consent process will be obtained if required by the policy at the individual site.

If the subject is too ill to consent, the Legally Authorized Representative may consent for the subject. Once the subject is able, the subject will be consented for continuation in the study.

13.4 Rationale for Research Subject Selection

13.4.1 Inclusion of Children

Children represent a significant portion of those infected with influenza, and children can develop severe influenza. The optimal treatment of these children is not clear, and significant morbidity and mortality exist despite treatment with antivirals. Furthermore, standard plasma is frequently used in a critically ill pediatric population.

The close monitoring of subjects in this study will allow for early detection of complications from influenza, and therefore contributing to the subject's well-being (45 CFR 46.405). Additionally, this research study presents a reasonable opportunity to further the understanding of the treatment of severe influenza, which is a serious problem affecting the health or welfare of children (45 CFR 46.406). For these reasons children are eligible for enrollment into this protocol.

13.4.2 Inclusion of Pregnant Women

Pregnant women represent a significant portion of those infected with influenza. The optimal treatment of this group is also not clear, and significant morbidity and mortality exist despite treatment with antivirals. Furthermore, standard plasma is frequently used in a critically ill pregnant population. All study plasma has tested negative for Zika Virus (as well as other pathogens as previously described in Section 5.1). For these reasons, pregnant women are eligible for enrollment into this protocol.

13.4.3 Inclusion of Subjects Unable to Provide Informed Consent

While there are some risks associated with the plasma, given the potential for direct benefits of anti-influenza plasma to the individual subjects (as suggested by the previous study of anti-influenza plasma), participation in the study is allowed for subjects unable to provide informed consent.

13.4.4 Justification of Exclusions

The exclusion criteria are primarily to increase subject safety.

13.5 Anonymity and Confidentiality

The information obtained during the conduct of this clinical study is confidential. The results of the research study may be published, but subject names or identities will not be revealed. Records will remain confidential. To maintain confidentiality, the site investigators will keep records in locked cabinets and the results of tests will be coded to prevent association with the subject's names.

13.6 Compensation

Each site is responsible for generating a compensation scheme accounting for travel cost and time lost from work for outpatient visits, and that fits the local legal and regulatory requirements as well as the cultural norm.

14 DATA MANAGEMENT AND MONITORING

14.1 Source Documents

The primary source documents for this study will be the subjects' medical records. If the investigators maintain separate research records, both the medical record and the research records will be considered the source documents for the purposes of auditing the study. The investigator will retain a copy of source documents. The investigator will permit monitoring and auditing of these data, and will allow NIAID, SSS, IRB/IEC, and regulatory authorities access to the original source documents, regardless of media.

The investigator is responsible for ensuring that the data collected are complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of information) should support the data collected and entered in to the study database and must be signed and dated by the person recording and/or reviewing the data. All data submitted should be reviewed by the site investigator and signed as required with written or electronic signature, as appropriate. Data entered into the study database will be collected directly from subjects during study visits or will be abstracted from subjects' medical records. The subjects' medical records must record their participation in the clinical trial and what medications (with doses and frequency) or other medical interventions or treatments were administered, as well as any AEs experienced during the trial.

14.2 Data Management Plan

Study data will be collected at the study site(s) and entered into the study database. Data entry is to be completed on an ongoing basis during the study.

14.3 Data Capture Methods

Clinical data will be entered into a 21 CFR 11-compliant Internet Data Entry System (IDES). The data system includes password protection and internal quality checks to identify data that appear inconsistent, incomplete, or inaccurate.

14.4 Study Record Retention

The site investigator is responsible for retaining all essential documents listed in the ICH GCP Guidelines. The FDA requires study records to be retained for up to 2 years after marketing approval or disapproval (21 CFR 312.62), or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational agent for a specific indication. These records are also to be maintained in compliance with IRB/IEC, state, and federal medical records retention requirements, whichever is longest. All stored records are to be kept confidential to the extent provided by federal, state, and local law. It is the site investigator's responsibility to retain copies of source documents until receipt of written notification to the contrary from OCRPRO/DCR/NIAID. No study document should be destroyed without prior written agreement between OCRPRO/DCR/NIAID and the Principal Investigator.

Should the investigator wish to assign the study records to another party and/or move them to another location, the site investigator must provide written notification of such intent to OCRPRO/DCR/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. NIAID must be notified in writing and written NIAID permission must be received by the site prior to destruction or relocation of research records.

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16 APPENDIX A

IRC005 will use the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014 to grade AEs and laboratory abnormalities.

This table can be found at: https://nirc.s-3.com/irc005/node/1835