A multicenter, randomized, double-blinded, placebo-controlled study of CYT107 to restore absolute lymphocyte counts in sepsis patients

IRIS-7B
(Immune Reconstitution of Immunosuppressed Sepsis patients)

PROTOCOL VERSION 1.4

Trial codification:	NCT02640807
Active ingredient tested:	recombinant glycosylated human CYT107 (CYT107)
Development phase:	Phase II
Study chairmen	Dr. Edward Sherwood M.D., PhD Vanderbilt University School of Medicine
Sponsor:	RevImmune
Date:	12/14/2015

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INVESTIGATORS AGREEMENT

I,……the undersigned, understand that the study will not start without the prior written approval of a properly constituted Ethics Committee. No changes will be made to the study protocol without the prior written approval of the sponsor and the Ethics Committee.

I have read, understood, and agreed to abide by all the conditions and instructions contained in this protocol. I agree to comply with the National regulations and ICH Harmonized Tripartite Guideline for Good Clinical Practice for conducting clinical trials and local regulations and will conduct the above study under these standards.

_____

Principal Investigator (print name)

_____ _____

Principal Investigator (signature) Date (day/month/year)

_____

Sponsor Representative (print name)

_____ _____

Sponsor Representative (signature) Date (day/month/year)

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1. Protocol Synopsis

TITLE	**A multicenter, randomized, double-blinded, placebo-controlled study of two dosing frequencies of glycosylated recombinant Interleukin-7 (CYT107) treatment to restore absolute lymphocyte counts in sepsis patients; IRIS-7B (Immune Reconstitution of Immunosuppressed Sepsis patients)**
Study chairman	Edward Sherwood, PhD, MD; Vanderbilt University School of Medicine
Clinical phase	Phase II
Sponsor(s)	RevImmune
Product	CYT107
Active Ingredient	CYT107 – Glycosylated recombinant human CYT107 (CHO-S)
Parallel study	A parallel study will be performed in France to allow a common statistical analysis of the primary end points and analysis for the enrolled patient population.

Introduction

Sepsis is the leading cause of death in critically ill patients in most intensive care units in Europe and the US. Despite advances in supportive treatment and encouraging findings in the laboratory, mortality has changed little in the past decades.(1) Recently, evidence has accumulated that sepsis progresses from a state of hyper-inflammation to a state of immunosuppression. This immunosuppressive phase is characterized by increased incidence of secondary infections often with relatively avirulent opportunistic type pathogens.(4),(5) Currently, new therapeutic approaches to sepsis are occurring using immuno-adjuvants that boost host immunity. One of the most promising agents is interleukin-7 (IL-7, CYT107).(6),(7)

IL-7 is an essential, non-redundant, pluripotent cytokine produced mainly by bone marrow and thymic stromal cells that is required for T-cell survival.(8)(9)(10) In addition to its anti-apoptotic properties, IL-7 induces potent proliferation of naïve and memory T-cells potentially supporting replenishment of the peripheral T-cell pool which is severely depleted during sepsis(8). IL-7 also reverses T cell "exhaustion" and increases expression of cell adhesion molecules which improve the ability of T-cells to traffic to sites of infection.(11)(12)(13)

In clinical trials at the National Cancer Institute, IL-7 caused a doubling of circulating CD4 and CD8 T cells and an increase in size of spleen and peripheral lymph nodes by ~ 50%.(14),(15) Similarly, a recent trial of IL-7 in HIV-1 infected patients who had persistently low CD4 T cells despite effective viral suppression demonstrated that Il-7 induced a 2-3 fold increase in circulating CD4 and CD8 T cells (12). *Thus, IL-7 reverses a major pathologic abnormality in sepsis, i.e., profound lymphopenia.*(16) IL-7 has many additional actions that are highly beneficial in sepsis (Fig.3). Il-7 increases the ability of T cells to become activated, potentially restoring functional capacity of hypo-responsive or "exhausted" T cells which typify sepsis. (8), (12) Additionally, IL-7 increases expression of cell adhesion molecules which enhance trafficking of T-cells to sites of infection. (16) Finally, IL-7 increases T cell receptor diversity leading to more potent immunity against pathogens. (12),(13)

IL-7 has shown efficacy both clinically and in animal infectious models. Multiple recent case reports of patients with idiopathic low CD4 T cells suffering from progressive focal leukoencephalopathy (PML) have shown that IL-7 caused rapid increases in circulating lymphocytes, markedly decreased circulating JC virus (often to undetectable levels), and led to disease resolution.(17)
Our collaborators (Drs. Hotchkiss, Sherwood, and Crouser) demonstrated that IL-7 restored the delayed type hypersensitivity response, decreased sepsis-induced lymphocyte apoptosis, reversed sepsis-induced depression of interferon gamma (IFN-γ), a cytokine that is essential for macrophage activation, and improved survival in murine polymicrobial sepsis. (16). This group also reported that IL-7 is beneficial in a fungal sepsis model that reproduces the delayed secondary infections typical of ICU patients.(18) Importantly, as demonstrated in Figure 1, data in septic patients shows a highly significant correlation between the absolute lymphocyte count and survival in patients with sepsis.

This proposal will test the ability of glycosylated recombinant IL-7 (CYT107) to restore the absolute lymphocyte counts in septic patients who have markedly reduced levels of circulating lymphocytes.

Objectives	Primary Objective To study the biological activity and safety of two dosing regimens of CYT107 at 10 µg/kg twice a week for the first week, followed by: • Once a week for the low frequency regimen • Twice a week for the high frequency regimen • Control group: will receive placebo (NaCl 0.9%) twice a week Activity The primary objective is to demonstrate that treatment of lymphopenic septic patients with CYT107 will reverse their low absolute lymphocyte count (ALC) which is a marker of immunosuppression and which correlates with mortality. This primary end point, increased, ALC by >50% in septic patients with lymphopenia, will be measured in the control (placebo) and two "dosing frequency" groups at approximately day 42 following randomization and initiation of study drug treatment. Safety To characterize the short term safety in a context of a single cycle of CYT107 (4 weeks) administered according to these two dosing regimens in lymphopenic sepsis patients. Based on the data collected from previous CYT107 studies in Oncology and HIV infection,(12)(13)(14)(15) treatment of lymphopenic sepsis patients with CYT107 is expected to increase the absolute lymphocyte count (ALC) by greater than 50% from baseline by day 42 after therapy is initiated. To date, CYT107 has not been tested in patients with sepsis. Septic patients frequently have decreased kidney and liver function, changes in circulating blood volume, and loss of vascular integrity with leakage of proteins into the interstitial space.(2) These alterations might impact the pharmacokinetics and pharmacodynamics of CYT107. Thus, we will administer CYT107 using a protocol that is highly similar to a previous protocol that was effective in improving the ALC in patients with cancer, HIV, and hepatitis C and was well tolerated with a low incidence of serious adverse effects.(12)(13)(14)(15)(17). The incidence, type and severity of adverse events occurring during the 6 weeks study period will be analyzed for the control arm and two "dosing frequency" groups. Secondary Objectives To characterize CYT107 pharmacokinetics (PK) in patients with sepsis To characterize CYT107 immunogenicity by detection and quantification of binding antibodies and detection of neutralizing antibodies in positive samples To characterize CTY107 pharmacodynamics, specifically to determine if CYT107 can restore depressed functional activity of immune effector cells in patients with sepsis To determine if CYT107 decreases the incidence of secondary infections in septic patients

Study Design	This is a phase II, double-blinded, randomized, placebo-controlled study aimed at comparing, two dosing frequencies in lymphopenic patients with sepsis. The study will be performed in the US. A twin study will be performed in France using the same study design so that a common statistical analysis could be performed at the end of the two studies. Patients will be randomized to three arms; CYT107 High Frequency Arm, CYT107 Low Frequency Arm, and Control (standard of care), with a ratio of 1:1:1 BLINDING: Patients and care-providers will be blinded as well as outcome assessors.
Sample Size	A total of 30 evaluable septic patients from US and French sites will be randomized 1:1:1 to receive study drug treatment over 4 weeks: • High frequency: intramuscular (IM) administration of CYT107 at 10µg/kg x2/week x 4 wks (total of 8 doses of CYT107) • Low frequency: IM administration of CYT107 at 10µg/kg x2/ the first week followed by CYT107 and Placebo weekly for 3 wks(total of 5 doses of CYT107 + 3 doses of Placebo) • Control: Will receive 8 administrations of Placebo (twice a week for 4 wks)

Selection Criteria	**Inclusion Criteria:** 1) Patients of age ≥ 18 yrs to 80 yrs 2) Patients with persistent suspected sepsis at 48-120 hrs after admission 3) Two or more criteria for the systemic inflammatory response syndrome (SIRS) (see reference #19 for SIRS criteria) and a clinically or microbiologically suspected infection. 4) At least one organ failure as defined by a SOFA score of ≥2 at any time point during the 48-120 hrs after admission to the ICU 5) Requirement of vasopressor treatment as follows: i) epinephrine or norepinephrine at ≥ 0.05 µg/kg/min ideal body weight; ii) vasopressin, or iii) dopamine at ≥ 4-5 µg/kg/min ideal body weight, continuously for 4 hrs or more, provided that at least 20 ml/kg of ideal body weight of crystalloid or an equivalent volume of colloid was administered during the 24-hour interval surrounding the start of vasopressor treatment, to maintain systolic pressure ≥ 90 mmHg or a mean arterial pressure ≥ 60 mmHg at any time point during their sepsis course preceding enrollment into the CYT107 study. 6) Lymphopenia with an absolute lymphocyte count ≤ 900 cells/mm^3 at either the day of consent or the day prior to consent during their ICU stay. 7) Predicted length of stay in the ICU of up to two weeks after starting drug therapy treatment in the trial 8) Ability to obtain a signed informed consent from patient or LAR consent.

Selection Criteria (Con't)	**Exclusion Criteria:** 1) Cancer with current chemotherapy or radiotherapy and/or .receipt of chemotherapy or radiotherapy within the last 6 weeks. All patients with current, or history of, hematologic malignancy (including, but not limited to, ALL, AML, CLL, CML, etc.) or lymphoma will be excluded, regardless of receipt of recent chemotherapy. 2) Cardiopulmonary resuscitation within the previous 4 weeks without objective evidence of full neurologic recovery) or patients who have minimal chance of survival and are not expected to live > 3-5 days as defined by an APACHE II score of ≥ 35 at time of consideration for study eligibility 3) Patients with a history of or who currently have evidence of autoimmune disease including for example: myasthenia gravis, Guillain Barre syndrome, systemic lupus erythematosis, multiple sclerosis, scleroderma, ulcerative colitis, Crohn's disease, autoimmune hepatitis, Wegener's etc. 4) Patients who have received solid organ transplant or bone marrow transplant 5) Patients with active or a history of acute or chronic lymphocytic leukemia 6) AIDS-defining illness (category C) diagnosed within the last 12 months prior to study entry 7) History of splenectomy 8) Any hematologic disease associated with hypersplenism, such as thalassemia, hereditary spherocytosis, Gaucher's Disease, and autoimmune hemolytic anemia 9) Pregnant or lactating women 10) Participation in another investigational interventional study within the last 6 months prior to study entry, with the exception of studies aimed at testing sedation products belonging to standard of care such as Propofol, Dexmedetomidine, Midazolam. 11) Patients receiving immunosuppressive drugs, e.g., TNF-alpha inhibitors, for rheumatoid arthritis, inflammatory bowel disease or any other reason, or systemic corticosteroids other than hydrocortisone at a dose of ≤ 300 mg/day 12) Patients receiving concurrent immunotherapy or biologic agents including: growth factors, cytokines and interleukins, (other than the study medication); for example IL-2,growth factors, interferons, HIV vaccines, immunosuppressive drugs, hydroxyurea, immunoglobulins, adoptive cell therapy 13) Prisoners

Study Treatment

Each dose of CYT107 will be administered in the ICU or hospital setting. If the patients are discharged from the hospital at which treatment was begun, they will not receive additional treatment with CYT107.

CYT107 will be administered by the intramuscular route at the dose of 10µg/kg based on the patient's ideal body weight, twice a week for the first week, followed by:

- Once a week for the low frequency regimen
- Twice a week for the high frequency regimen
- *Control is a placebo (denoted by "P") and is the saline diluent. To maintain blinding, all 3 combinations, i.e., low and high frequency CYT107 and placebo are administered at the same frequency.

	Week 1		Week 2		Week 3		Week 4	
days	1	4	8	11	15	18	22	25
CYT107 - High	X	X	X	X	X	X	X	X
CYT107 - Low	X	X	X	P	X	P	X	P
Placebo	P	P	P	P	P	P	P	P

Primary Biological Activity & Safety Study Endpoints

1) Weekly measures of ALC to determine the kinetic of restoration of adequate absolute lymphocyte count and to validate that CYT107 therapy can increase the ALC by >50% from baseline. The main indicators to be determined are:

i) the relative increase in ALC at day 42 expressed as a percentage of baseline value determined at the time point that CYT107 was first administered (day 1)

2) assessment of the clinical, biological and immunological tolerance of CYT107 administration in each group: incidence and scoring of all adverse events (AE)

i) biological effects and tolerance (see Appendix Flowchart #3 for all biological/laboratory tests that are to be assessed for CYT107 effects)

ii) adverse events (AEs) and serious adverse effects (SAEs) during the duration of the study period will be recorded, ending day 42 +/- 3

Secondary Biological Activity Study Endpoints	1) To determine pharmacokinetics (PK) including Cmax, area under the curve,(AUC) and $T^{1/2}$ life of CYT107 in patients with severe sepsis and multiple organ failure who are treated with 10 μgs/kg ideal body weight using 2 different dosing regimens 2) Assessment of CYT107 immunogenicity by detection and quantification of binding antibodies and detection of neutralizing antibodies in positive samples 3) To determine pharmacodynamics (PD) including effects of CYT107 therapy to: i) improve absolute numbers and function of immune effector cells ii) changes in the absolute numbers of CD4+ and CD8+ T cells over the study period, standardized on the duration in the study. iii) Changes in the expression of the CYT107 receptor (CD127) on the surface of CD4+ and CD8+ T cells. iv) Effects of CYT107 on circulating monocyte HLA-DR expression v) Effects of CYT107 on whole blood LPS-stimulated TNF-α vi) Effects of CYT107 on anti-CD3/anti-CD28 stimulated peripheral blood mononuclear cells to produce IFN-γ. 4) To determine effect of CYT107 to decrease the incidence of secondary infections. CYT107 has been highly effective in clinically relevant bacterial and fungal animal models of sepsis.(16)(18) We will compare the effect of CYT107 to reduce hospital-acquired secondary infections in CYT107 treated septic patients versus a control group of septic patients who receive placebo. The rate of hospital-acquired secondary infections at 42 days is roughly 35-40%.(20) We speculate that CYT107 will reduce the rate of secondary infections from 35-40% % to 25%.

Clinical and biological assessment	*Medical History*: A complete medical history of the patient will be obtained either by patient self-report, discussion with family if the patient is unable to provide a history, and review of the electronic medical records (EMR) of the patient. *Physical Examination*: The physical exam findings will be obtained by examination of the electronic medical records (History & Physical). *Laboratory Studies*: Results from standard of care laboratory tests as necessary to evaluate safety and efficacy of CYT107 will be obtained either by the EMR or Principal Investigator (PI) orders for research labs as detailed in Appendix 2- Flow Chart. • APACHE II and SOFA scores will be obtained by review of EMR and/or by the CYT107 Clinical Research Nurse Coordinator • Respiratory: oxygen saturation and inspired oxygen concentration • Imaging: ECG and spleen ultrasound • Hematology: hemoglobin, hematocrit, red blood cells, mean corpuscular volume, white blood count and differential, platelets, Prothrombin Time (PT) and partial thromboplastin time (PTT) • Chemistry: electrolytes (i.e., sodium, potassium, chloride, bicarbonate, calcium, and phosphorus), creatinine, glucose, albumin, liver function tests (total bilirubin, AST, ALT, Alkaline Phosphatase), and CRP (C-reactive protein). • Urinalysis: red blood cells • Pregnancy Tests in women of childbearing age without known contraceptive method (Serum or urine) • CYT107 Pharmacokinetics. Plasma levels of CYT107 will be assayed at various time points as specified (see Section 4.3.2 for details of timing of blood draws for PK studies. • Specific CYT107 binding and neutralizing antibody (at specialized labs only)

Statistical methods and analysis	Descriptive statistics will include: Quantitative variables: n, mean, standard deviation, minimum, maximum, 95% confidence intervals, median and quartiles will be presented when considered relevant Qualitative variables: n and percent, and 95% confidence intervals when relevant Inferential analyses Categorical variables: The Fisher exact test or Pearson Chi-2 test (and Cochran-Mantel Haenszel procedure for adjusted inferences) as appropriate, will be used to compare rates of categorical variables between the two dosing frequency levels. Continuous variables: parametric ANOVA (alternatives: non parametric ANOVA or transformation of the data / ANOVA for adjusted inferences) will be used to compare continuous variables between the two dosing frequency levels. *Primary Analysis of the Primary Objective* Primary endpoint: increase in absolute lymphocyte count (ALC) by 50% at Day 42
Duration of Patient participation	Patients are treated with CYT107 or control for 4 weeks and laboratory studies obtained for an additional 2 weeks up to approximately 42 days (Appendix 2, Flow Chart). Patient outcomes will be followed at approximately 29, 42, 60, 90, 180 and 365 days to record data including incidence of new secondary infections, patient disposition, (i.e., discharged to home, skilled care facility, or long term health care facility), and readmissions to hospital, ICU days, and mortality.

Abbreviations

ADA	Anti-Drug Antibodies
AICD	Activation induced cell death
AE	Adverse effects
ARV	Anti-retroviral
CCR5	CC chemokine receptor type 5
CD	Cluster of differentiation
CHO	Chinese hamster ovary
CMV	Cytomegalovirus
CRF	Case report form
CRO	Contract research organization
CROI	Conference on retroviruses and opportunistic Infections
CRP	C reactive protein
CTL	Cytotoxic T lymphocytes
CXCR4	CXC chemokine receptor type 4
CYT107	CHO recombinant-human-Interleukin-7
CYT 99 007	*Escherichia Coli* recombinant human-Interleukin-7
D	Day
DAIDS	Division of acquired immunodeficiency syndrome
eCRF	Electronic case report form
EDC	Electronic data capture
ELISA	Enzyme-linked immunosorbent assay
EMR	Electronic medical record
FAS	Full analysis activity dataset
GCP	Good clinical practice
GI	Gastro-intestinal
HAART	Highly active antiretroviral therapy
HCT	Hematopoietic cell transplantation
HIV	Human immunodeficiency virus
ICH	International conference on harmonization
CYT107(R)	Interleukin 7 (receptor)

INR	Immune non responders
IRIS	Immune reconstitution inflammatory syndrome
DSMB	Independent safety monitoring committee
IWRS	Interactive web response system
LPD	Lymphoproliferative disorder
LTi	Lymphoid tissue inducer
M	Month
NADA	Neutralizing Anti-Drug Antibodies
NK	Natural killer
NKT	Natural killer thymic
PBMC	Peripheral blood mononuclear cells
Pbo	Placebo
PD	Pharmacodynamics
PD-1	Programmed death-1
PI	Principal investigator
PK	Pharmacokinetic
PP	Per protocol activity data set
QTc	Corrected QT Interval
r-hCYT107	Recombinant human CYT107
SAE	Serious adverse effects
SIV	Simian immunodeficiency virus
TCR	T cells receptor
TGF-β	Tumor growth factor
TREC	T cells receptor rearrangement excision circles
Treg	Regulatory T cells
TSH	Thyroid-stimulating hormone
W	Week

Definitions

Study entry: signature of informed consent

Study period: time interval between study entry (enrollment) and end of study

2.0 Background and Rationale

2.1 Sepsis

2.1.1 Epidemiology of Sepsis

In 2012, over 20 million patients world-wide are estimated to be afflicted by sepsis annually and recent epidemiological analyses showed that mortality from severe sepsis and septic shock is still elevated – around 30% both in Europe and US.(1) Both pro and anti-inflammatory responses are initially induced in septic shock patients with the secondary occurrence of sepsis-induced immunosuppression.(2)

2.1.2 Pathogenesis of Sepsis, critical immune paralysis

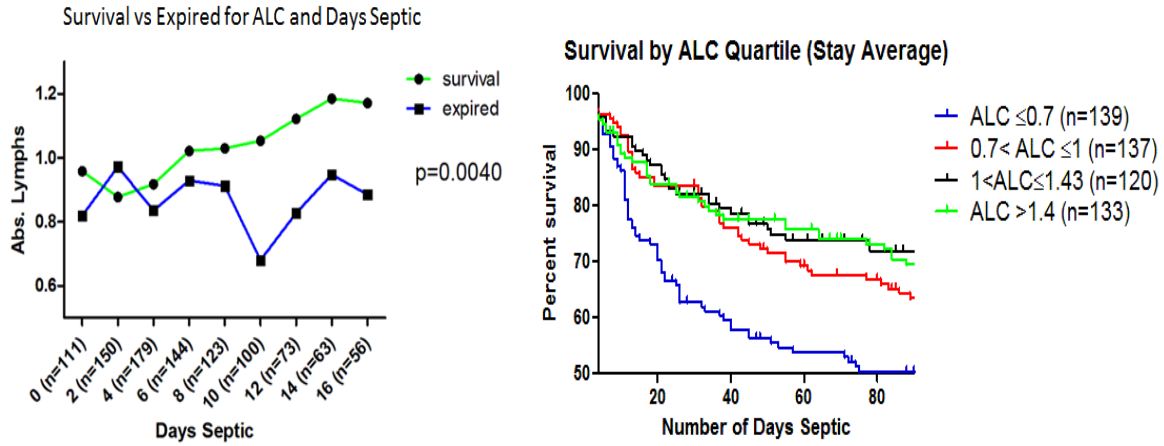
In contrast to the theory that morbidity and mortality in sepsis are due to unrelenting inflammation, our group recently demonstrated that patients dying of sepsis have marked immunosuppression.3 In the first detailed immunologic study of organs from patients dying of sepsis, spleens and lungs were harvested in the ICU within 30-180 minutes after death in 40 septic patients. Stimulated cytokine secretion studies and immune phenotyping of cell surface receptor/ligand expression profiles were conducted. Isolated splenocytes were stimulated with lipopolysaccharide (LPS) or anti-CD3/anti-CD28 and production of cytokines quantitated. Stimulated splenocytes from septic patients had profoundly decreased production of both pro- and anti-inflammatory cytokines, values which were often <10% of those from critically-ill non-septic patients. Similar findings were observed in immune effector cells isolated from lung (unpublished data). Immune effectors cells from spleen and lung demonstrated upregulated expression of inhibitory receptors including PD-1, expansion of T regulatory cells and myeloid derived suppressor cells, and down-regulation of activation pathways. Collectively, these results show that sepsis induces numerous overlapping mechanisms of immune suppression involving both innate and adaptive immunity.

2.1.2.1 Low ALC is associated with increased incidence of bacteremia and mortality

Sepsis induces widespread apoptosis of CD4 and CD8 T cells leading to a low ALC. The absolute CD4 T cell count in many septic patients is as low as observed in patients with AIDS.(21)(22) Numerous investigators have shown that septic patients with the lowest ALC have the highest mortality in the disorder.(23)(24) CYT107 induces proliferation of naïve and memory T cells thereby supporting replenishment of lymphocytes which are needed to combat infection.

Recently, our group examined the relationship between the ALC in sepsis survivors versus patients dying of sepsis. As shown in Figure 1. below, septic patients who died had a persistently low ALC compared to patients who survived sepsis.

Figure 1 Septic patients with persistently low ALC have higher mortality



We prospectively monitored a group of patients with a diagnosis of severe sepsis. We examined the ALC in patients who survived sepsis versus patients who died of sepsis. Both groups of septic patients tended to have an ALC which was lower than the lower limit of normal for our hospital, i.e., 1.2 cells/μL x 103. In Figure 1 (left hand panel), the Y axis equals the ALC in cells/μL x 10^3. X axis equals the number of days septic; the number in parentheses is the number of patients who are septic on that particular day. Note that patients who survived sepsis had a gradual increase in the ALC count while those septic patients who died of sepsis tended to have a persistently low ALC. In Figure 1 (right hand panel), the averaged ALC over patient length-of-stay was divided into 4 quartiles. Note that patients with the lowest averaged ALC had the highest mortality. It is also important to note that mortality continued to increase from days 30 to 80 and was ~50% in the patients with the lowest ALC. These data provide a strong rationale for use of CYT107 therapy in sepsis because it will increase the ALC and thereby improve patient host defenses.

2.2 Current treatments and their limitations

Current therapies have focused on agents which block the host immune response. Over 30 trials of immune blockers have failed to improve survival in sepsis.(25) These failures have led to a reevaluation of the pathophysiology of sepsis and new therapies consisting of agents that boost host immunity.(2) New trials with GM-CSF and IFN-γ, drugs which enhance immune function are underway in sepsis.(26)(27)

2.3 Study product: glycosylated recombinant human CYT107 "CYT107"

CYT107 has critical roles in T cell development and may restore at multiple levels the immunologic situation specifically by restoring adequate T cells count.(8)(9)(10). CYT107 that will be employed in the present clinical trial is the current glycosylated form of recombinant human CYT107 and is produced in engineered CHO mammalian cells. This form shows less immunogenicity in primates and is expected to be less/non-immunogenic in humans. Furthermore, as discussed below, its pharmacokinetic and dynamic profile may allow for a greater interval between administrations. CYT107 has been assessed in humans and results of

toxicity studies performed in cynomolgus macaques after one cycle of treatment are summarized in section 4.3 of the investigator brochure.

2.3.1 Quantitative and functional effects of CYT107

The quantitative and functional effects of CYT107 are comprehensively reviewed in the Investigator's Brochure and briefly summarized in the table below, demonstrating how administration of CYT107 may help to meet several of the unmet medical needs.

Table 1 : Main immunological properties of IL7

Quantitative impact

- Development and expansion of CD4+ and CD8+ αβ T cells: naive, and memory subpopulations
- Development and expansion of γδ T cells and natural killer thymic (NK T) cells

Functional impact

- Decrease frequency of "exhausted" PD-1 CD4+ and CD8+ T cells
- Decrease apoptosis (increase of anti-apoptotic Bcl-2 protein, decrease of pro-apoptotic Bim protein)
- Decrease frequency of regulatory T cells (Tregs)
- Increase cytotoxicity of CD8+ T cells
- Co stimulation of T cells:
  - Increase reactivity of T cells against weak immunogens / subdominant epitopes
  - Increase differentiation and survival of central memory T cell

Pre-clinical studies

The available preclinical and clinical results of CYT107 underscore its therapeutic potential to restore adequate CD4+ T cells counts and improve immune protection.

IL-7 plays a key role in thymopoiesis

IL-7 has critical and non-redundant roles in T cells development, hematopoiesis, and post-developmental immune functions as a prototypic homeostatic cytokine.(8-10) IL-7 also supports T cells survival by up-regulating members of the anti-apoptotic Bcl-2 family, and by down-regulation of the pro-apoptotic activity In contrast to IL-2, CYT107 therapy in humans induces robust and functional CD4+ T cells expansion without selective expansion of Treg cells.(10) In addition, CYT107 therapy increases T cells receptor (TCR) repertoire diversity and increases circulating TCR rearrangement excision circles (TRECs) numbers, which have been used as an indirect measure of thymic output because they reflect TCR gene recombination events and are enriched within the RTE fraction (13). CYT107 signals through the CYT107 receptor, which is composed of the CYT107Rα (CD127) and the common gamma (γ) chains.

2.3.1.1 Inverse correlation between CD4+ T cells lymphopenia and circulating IL-7

An inverse correlation between CD4+ T cells lymphopenia and circulating IL-7 has been consistently documented after any infectious or iatrogenic lymphopenia, implicating endogenous IL-7 in the regulation of T cell homeostasis(28). In HIV-infected patients, elevated CYT107 levels gradually decline as CD4+ T cells recovery occurs following effective anti-retroviral therapy. Importantly, several studies suggest that IL-7 drives CD4+ T cells restoration subsequent to the suppression of HIV replication. (12) IL-7 circulating levels are particularly high in patients benefiting from an exceptionally successful and fast immune restoration.[28]

2.3.1.2 Experimental effects of CYT107 in HIV/SIV infection

a) Expansion of CD4+ T cells

A sustained increase in CD4+ T cells and a parallel expansion of CD8+ T cells has been consistently demonstrated in SIV-infected monkeys treated by CYT107,(29) before being confirmed in humans which were performed using CYT 99 007 (1st generation non-glycosylated CYT107/ E.Coli r-hCYT107) and CYT107 (glycosylated r-hCYT107 CYT107).(30)(12).

b) Enhancement of anti-HIV/SIV T cells responses

The pleiotropic quantitative or functional effects of CYT107 converge to improve T cells response against HIV:

- CYT107, because of its co-stimulatory effect, may increase the magnitude and breadth of anti HIV CD4+ and CD8+ T cells responses;
- CYT107 increases cytotoxicity of specific anti-HIV CD8+ T cells, as shown *ex vivo* with T cells derived from HIV-infected donors;
- CYT107 may foster the differentiation, of protective memory CD4+ and CD8+ T cells, which is typically blocked and/or outnumbered by the accumulation of exhausted $CD127^{low}$ effector memory T cells. Thus r-hCYT107 may alter the balance between $CD127^{low}$ exhausted and $CD127^{high}$ protective T cells in favor of the latter subpopulation.

2.3.1.3 Results of trials with CYT107 and ongoing trials with CYT107

(See section 5 of the Investigator brochure for exhaustive information of human experience with CYT107)

CYT107 the glycosylated form of CYT107 being developed by Revimmune was tested in several indications (oncology, HCV, HIV, HCT). The safety profile and preliminary activity as measured in the ongoing clinical studies were satisfactory. The total number of patients treated with CYT107 today is 275 in 13 trials (12)(13)(31). In addition more than ten patients received CYT107 for compassionate use (17).

Safety

Most frequent adverse reactions reported in clinical trials with CYT107 (see section 5.2.2) are:

- injection site reaction

- lymphadenopathy
- pyrexia
- rash
- fatigue

Most frequent severe adverse reactions that are reported in clinical trials with CYT107 are: (see section 5.2.2 Investigator Brochure)

- Rashes: overall rashes related to CYT107 do not require systemic treatment except antihistaminic and some require topical corticosteroids. Secondary prophylaxis is recommended
- ALT/AST elevations: most are mild to moderate. Two asymptomatic and isolated transient grades 3 and 4 transaminase elevations were reported. Rules for decreasing CYT107 dose in case of transaminases elevation in HIV studies with repeated cycles of CYT107 are in place.
- Allergic reactions – four patients (out of over 300 patients) developed grade 3 allergic reactions of generalized urticarial without significant systemic reactions. Treatment consisted of antihistimines and corticosteroids and patients resolved symptoms without complications. All reactions occurred on the second treatment cycle of CYT107.
- Anaphylactoid reaction: One patient out of over 300 patients had a grade 2 anaphylactoid reaction consisting of itching and swelling of the left side of her tongue and uvula. There was no accompanying bronchospasm. She was treated with intravenous corticosteroids and promethazine. She was monitored for 4 hours and recovered uneventfully and was discharged.
- Immunogenicity: as of January 2013 ADA were detected in 66/274 patients after one cycle of CYT107 and 6 had neutralizing antibodies. After a second cycle, ADA were detected in 59/77 patients and 27 had neutralizing anti-drug antibodies (NADA). Occurrence of NADA is higher in case of repeated cycles. Nevertheless, occurrence of NADA was not associated with an increase in the frequency or severity of AEs.

The drug is well tolerated and the most frequent adverse events observed are local erythema at injection site. (12-14, 31)

Activity

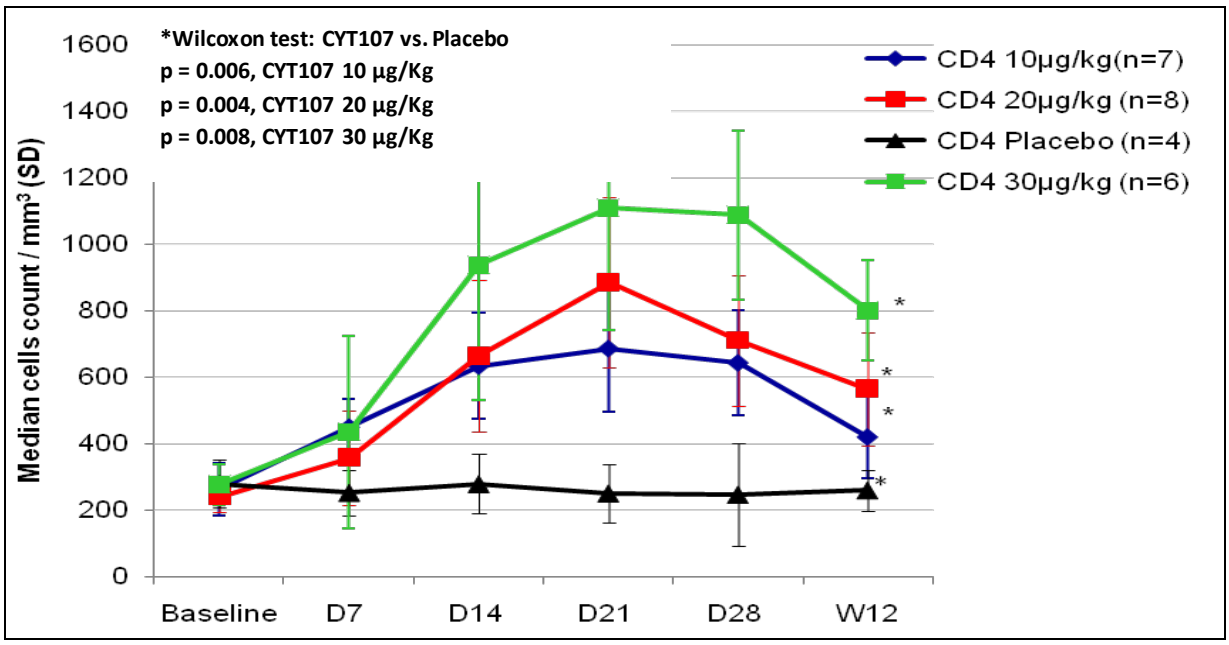
Recent trials in HIV-infected patients have demonstrated a sustained dose-dependent increase in naïve and memory CD4+ and CD8+ T cells after administration of r-hCYT107 either with CYT 99 007 or CYT107.(12,30,31) In a prospective randomized placebo-controlled study, a single subcutaneous (SC) dose of r-hCYT107 was well tolerated with biologic activity demonstrable at 3 µg/kg and a maximum tolerated dose (i.e. without SAE or dose limiting toxicity) of 30 µg/kg. Single-dose of recombinant r-hCYT107 increased the numbers of circulating CD4+ and CD8+ T cells, predominantly of central memory phenotype. Levy et al.[1] published recently the preliminary results of the CYTHERIS study. These results were based on 13 patients who received a total of 8 subcutaneous injections of 2 different doses of r-hCYT107 (CYT 99 007); 3

or 10 µg/kg, 3 times per week (w) over a 16-day period. R-hCYT107 was well tolerated and induced a sustained increase of naive and central memory CD4+and CD8+ T cells. (12)

We have now confirmed and extended our initial results with the completion of the INSPIRE study conducted in USA, Canada, France, Italy, utilizing the current glycosylated version of r-hCYT107 (CYT107) in HIV patients whose disease was well controlled by HAART but who remained immune non-responders with CD4+ T cells counts below 400/µL.

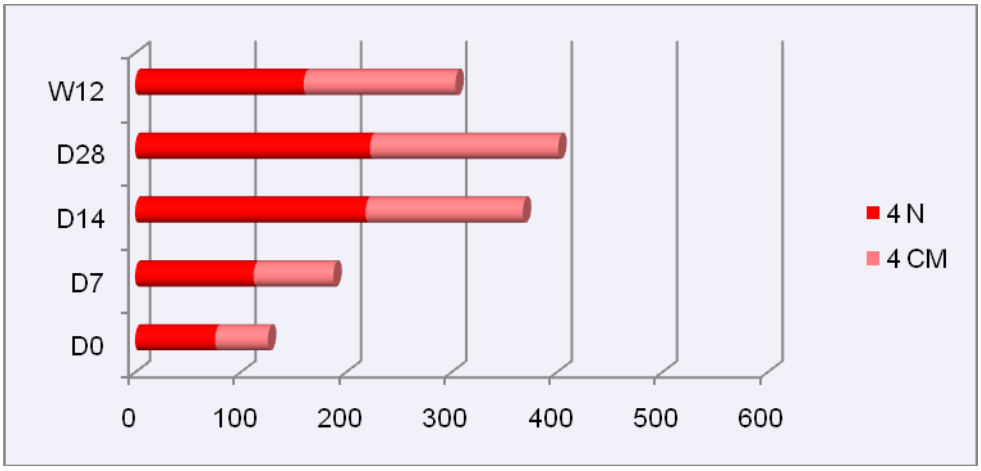
Results from INSPIRE patients confirmed the ability of CYT107 to produce a high quality immune recovery as shown in figure 2. The increases of CD4+ T cells counts induced by the three dosages of CYT107 administrations (10, 20 and 30 µg/kg/week) were significant when compared to the levels observed in the placebo arm (12).

Figure 2 Dose-dependent effect of CYT107 (CYT107) on CD4+T cells



At 20 µg/kg, CYT107 (CYT107) preferentially expanded Naïve (N) and Central memory (CM) CD4+ as shown in figure 3.

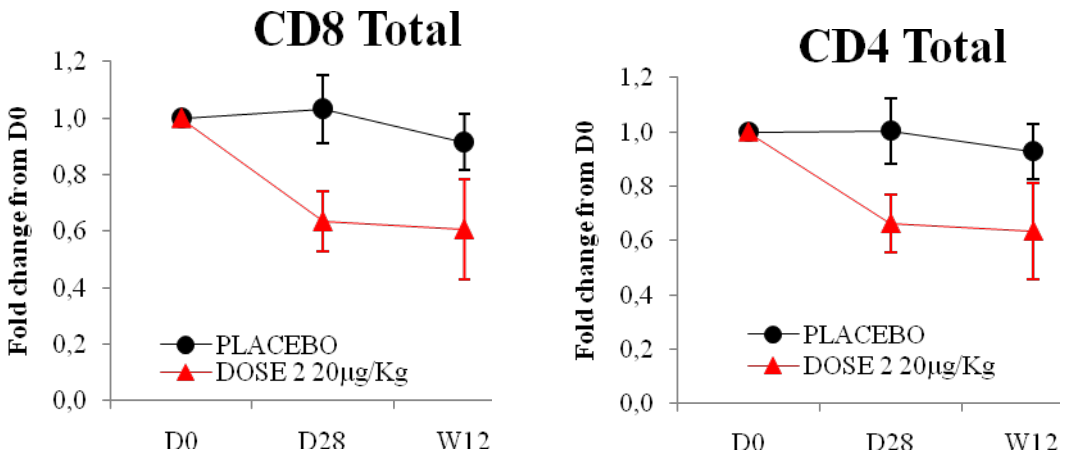
Figure 3 Expansion of Naive (N) and Central Memory (CM) T cells after CYT107, 20 µg/kg



X axis is the ALC/µL. Y axis represents Days (D) or Weeks (W).

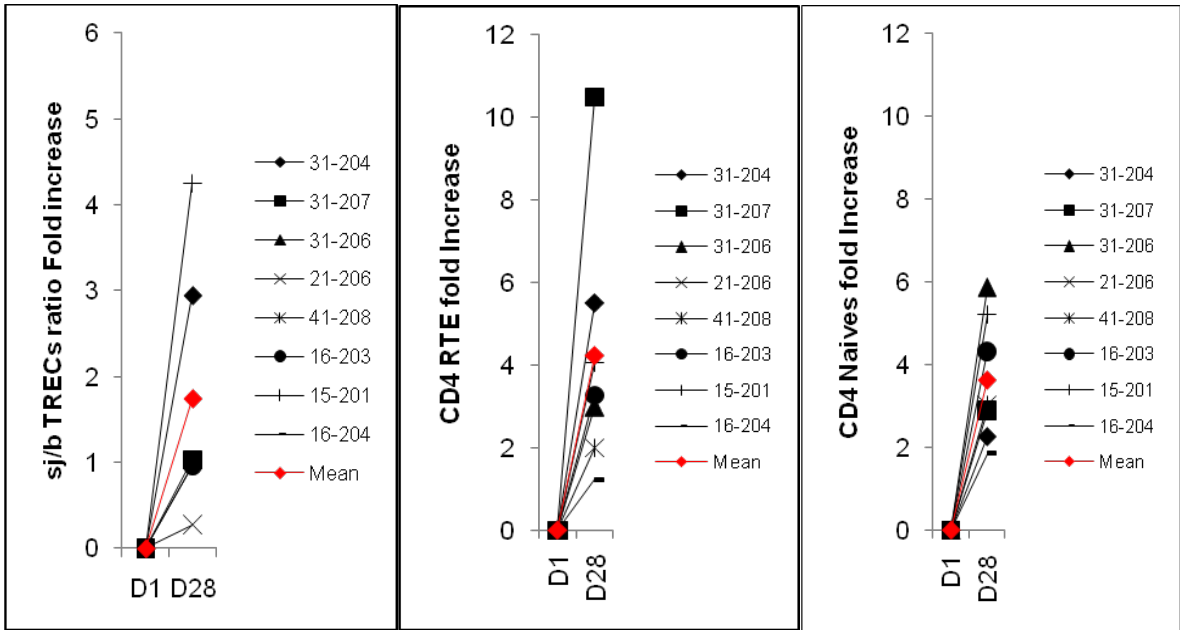
This expansion was associated with a significant decrease of the "PD-1^{+}" exhausted CD4+ and CD8+ T cells as shown in figure 4:

Figure 4 Decrease of exhausted (PD1+) CD4+ and CD8+ T cells after CYT107



In addition, a clear support of thymopoïetic production, was demonstrated by the increase in TREC ratio, recent thymic emigrants and naïve T cells (see figure 5)

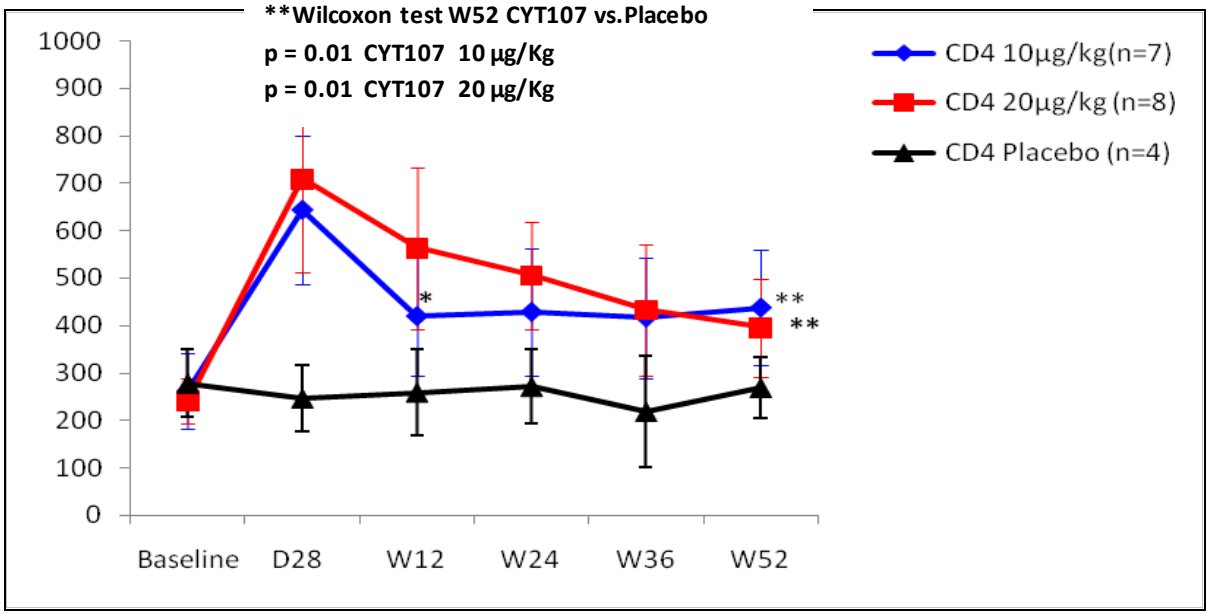
Figure 5 increase in TREC ratio, recent thymic emigrants and naïve T cells



Various results from the INSPIRE study demonstrated the quality of CYT107-induced T cells reconstitution in these "Immune Non Responding" patients:

- *CYT107 administration was clinically and biologically well tolerated*
- *20 µg/kg/week was the dose with the best efficacy/safety ratio*
- *A single cycle (3 subcutaneous injections) induced a rapid and sustained increase of CD4+ and CD8+ T cells, with most patients (5/8) treated with 20 µg/kg of CYT107 reaching CD4+ T cells counts > 500 cells/µL at W12*
- *CYT107 induced a brisk expansion of T cells subsets – increasing RTE, naïve, central memory and effector T cells*
- *The increase in CD4 and CD8 T cells is long lived and persists for a number of months (Figure 6)*

Figure 6 Long-term increase of CD4+ after CYT107 administration.



These results demonstrate that the effect of CYT107 to increase the ALC persists for a number of weeks after cessation of therapy. Given the high rate of recurrent infections that persist for up to one year in septic patients following hospital discharge (Journal of Intensive Care 29:87-95 2014), this CYT107 induced increase in ALC may be highly beneficial.

Going forward, RevImmune is currently considering various new studies with CYT107 (CYT107) in oncology as well as in sepsis.

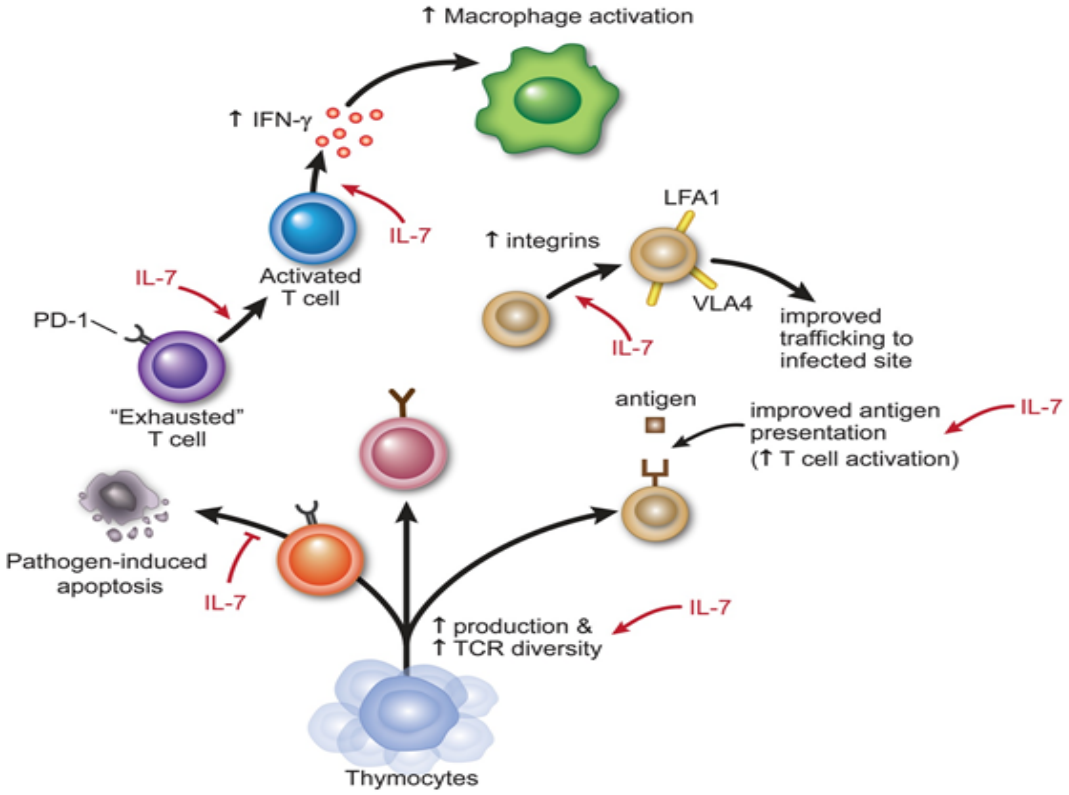
2.4 Rational for an CYT107 clinical trial in sepsis

CYT107 is an essential, non-redundant, pluripotent cytokine produced mainly by bone marrow and thymic stromal cells that is required for T-cell survival.(8,9) In addition to its anti-apoptotic properties, CYT107 induces potent proliferation of naïve and memory T-cells potentially supporting replenishment of the peripheral T-cell pool which is severely depleted during sepsis.(10) CYT107 also reverses T cell "exhaustion" and increases expression of cell adhesion molecules which improve the ability of T-cells to traffic to sites of infection.'12) Unlike other closely related common γ-chain cytokines such as IL-2, CYT107 does not induce a hyper-inflammatory "cytokine storm" response and has been remarkably well-tolerated.(8)

Because of CYT107's ability to boost and restore CD4 and CD8 T cell function, key components of the adaptive immune system, it was hypothesized that CYT107 would be efficacious against invading pathogens. Subsequent studies have proven this hypothesis to be correct. (16, 18) CYT107 has shown efficacy in improving morbidity and mortality against diverse pathogens. (16,18) CYT107 restored cytokine secretion, improved T cell trafficking to the site of infection, and decreased tissue viral load in a mouse model of lymphocytic choriomeningitis.(11) CYT107 reversed lymphopenia and T cell exhaustion and improved T cell repertoire diversity in rhesus macaques with simian immunodeficiency virus.(29) Several independent laboratories have shown that CYT107 improves survival in a clinically relevant bacterial peritonitis model.(16,32) Most recently, CYT107 has been shown to improve survival in a clinically-relevant, two-hit model of fungal sepsis.(18)

Importantly, there is increasing evidence that CYT107 will be highly effective in clinical infectious disease. CYT107 reversed T cell exhaustion and restored T cell functionality in HIV patients who were non-responders to HAART.12,(32) CYT107 increased T cell receptor diversity in HIV patients with lymphopenia and in patients who had lymphopenia following radiation or chemotherapy for treatment of acute myelogenous leukemia.(12,13) In a subset of patients with hepatitis C who were non-responders to conventional therapy, CYT107 lead to complete clearing of virus from the blood (33). CYT107 also lead to clearing of the JC virus in a lymphopenic patient with progressive multifocal leukoencephalopathy (PML).(17) Subsequently, 10 additional patients with PML have been treated with CYT107 on a compassionate basis and have had beneficial responses including clearing of virus (personal communication Dr. Michel Morre – former CEO of Cytheris).

Figure 7 Beneficial Effects of CYT107 in Sepsis



CYT107 has numerous and diverse effects to improve immune function in sepsis including: i) decreases in sepsis-induced apoptosis, ii) increased production of T cells with diverse T cell receptors (TCRs), iii) decreasing PD-1 expression with reversal of T cell exhaustion, iv) increased IFN-γ production, v) increased integrin expression leading to improved T cell trafficking to infected sites.[6,7,16,18] *Thus, CYT107 is ideally suited to treat patients with sepsis because it directly reverses key immunologic defects in the disorder.*

2.4.1 Rationale of the Clinical Study: the need to reconstitute adequate lymphocyte counts in sepsis In conclusion, CYT107 reverses a fundamental defect in sepsis, i.e., the massive loss of lymphocytes. CYT107 has been highly effective in improving survival in clinically relevant animal models of sepsis.(16,18,34). It offers great promise in the treatment of septic patients.

Therapeutic immune reconstitution should be:

- *Strong and stable*: meaning to allow patients to recover peripheral CD4+ T cells counts to above the 500 cells /µL for the duration of the study period,
- *Deep*: meaning not only involving peripheral CD4+ T cells recovery but also inducing a T cells repopulation of lymph nodes and gut mucosa,
- *Diverse*: involving both CD4+ and CD8+ T cells, naïve and memory,
- *Functional*: limiting over-activation (CD38+HLADR+), exhaustion (PD1+), and production of Treg cells while preserving functionality (CD28+).

- *Broad*: supporting thymic activity and facilitating the recovery of the peripheral TCR repertoire.

2.4.2 Study Population

Patients selected in this trial will have severe sepsis with documented lymphocytopenia consisting of an absolute lymphocyte count (ALC) of less than 900 cells per microliter. (ALC <900/ μL). Also see Inclusion criteria.

2.4.3 Investigational Medicinal Product – glycosylated recombinant human IL-7 (CYT107)

2.4.3.1 Route

CYT107 will be administered intra-muscularly or subcutaneously based on hemostasis results and whether patients are receiving anticoagulant and antifibrinolytic treatments. If the patient receives anticoagulant and/or antifibrinolytic therapy, intramuscular route is contraindicated. In that case, CYT107 will be administered subcutaneously.

By subcutaneous route, according to the monkey studies results, CYT107 bioavailability is satisfactory (> 75%) with an extended kinetic profile which may favor prolonged CYT107 activity. But the sub-cutaneous route is known to favor recombinant proteins immunogenicity, accordingly following the recent example of beta interferon, these new CYT107 studies will be mainly performed by intra-muscular administration. Depending on haemostasis results (INR greater than 2.5 or a platelet count less than 35,000), CYT107 will be administrated subcutaneously. This explains why the present study also includes the assessment of PK profile in these patients (35)(36)(37).

2.4.3.2 Dose

To date, CYT107 has not been tested in patients with sepsis. Septic patients frequently have decreased kidney and liver function, changes in circulating blood volume, and loss of vascular integrity with leakage of proteins into the interstitial space.(2) These alterations might impact the pharmacokinetics and pharmacodynamics of CYT107. Thus, we will administer CYT107 using a protocol that is highly similar to a previously protocol that was effective in improving the ALC in patients with cancer, HIV and HCV infections and was well tolerated with a low incidence of serious adverse effects. (12-14)

The dose of 10 μg/kg, ideal body weight, administered either once or twice a week, will be evaluated in this study. CYT107 at a dose of 10 and 20μg/kg once a week has shown an excellent safety profile and biological activity in previous studies in HIV and oncology patients. (12-14, 32)

2.4.3.3 Dosing frequency:

Specificities of CYT107/CYT107Rα pathway regulation and data gathered now in humans favor injections once a week and short therapeutic cycles. According to monkey and human data, a three to seven day interval between each injection of CYT107 should lead to full

recovery of CYT107Rα expression (CD127) at the surface of T cells which responded to a previous dose of CYT107. Furthermore, a sustained response of T cells to CYT107 should be fully restored, as measured one week later by Ki-67 expression. A weekly interval was successfully tested in the HIV and post HSCT studies aimed at inducing an immune reconstitution- Short cycles, limited to 4 weeks, should avoid inducing exhaustion of T cells responsiveness to high doses of CYT107, which could occur despite the recovery of CD127 expression.

In the specific case of sepsis, there is a real urgency to allow the patients recovering an adequate number of T lymphocytes. Accordingly we will use a twice a week regimen for the very first week of treatment in both groups. Although the recovery of the CYT107 receptor at day 4 is not 100%, it is sufficient to re-stimulate the pool of T cells available for expansion. In the low frequency group we will use this twice a week regimen for the first week only, while it will be used for the full 4 weeks cycle in the high frequency group.

A staggered approach to CYT107 treatment will be used in which treatment will not be initiated in a newly enrolled patient until 24 hours after the previously enrolled patient has received the second dose of CYT107 without evidence of serious adverse event (SAE) occurrence. In cases of a suspected adverse event, a scheduled dose will be postponed by 3 days to allow the adverse event to resolve. For any severe or serious adverse event during the treatment period (as judged by the Investigator), dosing will be suspended and the DSMB will be consulted for case review and advice as to termination of treatment.

2.5 Risk-Benefit Ratio

Sepsis causes extensive apoptotic death of immune effector cells leading to an impaired immune system. CYT107 prevents sepsis induced cell death and improves survival in many clinically-relevant animal models of sepsis. We postulate that by improving host immunity, CYT107 will improve the ability of septic patients to eradicate their primary infection and decrease the incidence of new secondary infections. Given the low risk of adverse effects in patients treated with CYT107 and the potential for benefit, we believe that the risk benefit ratio lies greatly toward use of CYT107 in patients who have a highly likelihood of dying from sepsis; the expected mortality in septic patients who meet the entry criteria for this CYT107 study is approximately 45% at 60 days.

3.0 Objectives of the Trial

3.1 Primary Objective

To study the **biological activity** and **safety** of two dosing regimens of CYT107 at 10µg/kg over a treatment period of 4 consecutive weeks:

Twice a week for the first week, followed by:

- Once a week for the low frequency regimen (plus placebo once a week)
- Twice a week for the high frequency regimen
- Control group: will receive placebo (NaCl 0.9%) twice a week

Activity

The primary objective is to demonstrate that treatment of lymphopenic septic patients with CYT107 will reverse the drop in absolute lymphocyte count (ALC) which is a marker of immunosuppression and which correlates with mortality (see Figure 1 and references 4, 21, 23). The effect of CYT107 on the kinetics of the recovery of ALC will be recorded. CYT107 has been demonstrated to increase ALC in a diverse array of patient populations including patients with HIV, patients who underwent bone marrow transplantation, and patients with cancer. Based on the data collected from previous CYT107 studies in Oncology and HIV infection, the treatment of lymphopenic sepsis patients by CYT107 is expected to produce:

- Primary endpoint: increase of ALC by greater than 50% from baseline by day 42 after CYT107 therapy initiated.
- We will also evaluate the number of patients who restore ALC to >1200 cells/mm^3 (the lower limit of normal for ALC at most hospitals) by day 42 with the aim of observing this recovery in more than 50% of the patients

These end points will be measured in the three treatment groups (2 dosing frequencies + 1 placebo) at day 42 in both the French and US studies. All patients will undergo randomization for study drug treatment and undergo all study related tests and procedures.

Safety

To characterize the short term safety in a context of a single cycle of CYT107 (4 weeks) administered according to these two dosing regimens in lymphopenic sepsis patients.

3.2 Secondary Objectives

3.2.1 To characterize CYT107 pharmacokinetics (PK) in this patient population

Another secondary objective of the study is to examine the pharmacokinetics (PK) and pharmacodynamics (PD) of CYT107 in patients with sepsis. Septic patients frequently have changes in circulating blood volume, impaired vasculature integrity with leakage of proteins into the interstitial space, and organ dysfunction. Although the investigators expect that CYT107's main PK parameters in septic patients will not be substantially affected by these alterations, it is important to define CYT107 AUC, Cmax and half-life in this patient population. This information can be used to guide the proper dosing of CYT107 in a larger trial.

Up to now, CYT107 was administered by sub-cutaneous route to patients, but this route of administration is susceptible to increase the immunogenicity of the drug and patient's edema which is frequent in sepsis patients might alter the bioavailability. In this study the CYT107 product will be preferentially given by intra muscular administration. As exemplified for Interferon by Munafo et al(35)., FSH by Steinkampf (36) et al., Alefacept by Sweetser (37) et al. and Onercept by Trinchard-Lugan (38) et al., the IM route is known to deliver pharmacokinetic profiles very similar to the S.C. route. Nevertheless the PK profile of CYT107 administered by I.M. will be determined during the study. Nevertheless, septic patients are susceptible to development of coagulopathy, which increases the risk of complications associated with

intramuscular drug administration. Thus, CYT107 will be administered via the subcutaneous route in coagulopathic (INR>2.5, platelet count less than 35,000/ul) patients.

3.2.2 To characterize CYT107 Pharmacodynamics - To determine if CYT107 can restore depressed functional activity of immune effector cells in patients with sepsis.

We will look at the ability of CYT107 to improve common markers of immune function that have been shown to correlate with outcome in patients with sepsis. Note that these tests are not standardized clinically available tests but rather tests that may be useful in the future to help guide administration of immunotherapeutic agents. These tests will be performed in the 3 investigators laboratories by their laboratory technicians.

- Effects to increase monocyte HLA-DR expression
- Effects to increase whole blood LPS-stimulated TNF-α
- Effects on anti-CD3/anti-CD28 stimulated peripheral blood mononuclear cells to produce IFN-γ.
- Effects to increase patient ALC to >1200 and to increase absolute CD4 and CD8 T cell counts.

3.2.3 To assess the impact on number and type of secondary infections:

CYT107 has been highly effective in improving survival in clinically relevant bacterial and fungal animal models of sepsis.(16,18,34) In addition, CYT107 has been effective in boosting immunity and reducing viral loads in patients with HIV and JC virus induced progressive multi-focal leukoencephalopathy.(18,12,32) Most recently, CYT107 led to restoration of gut associated lymphoid tissue in HIV patients with a subsequent reduction in markers of inflammation.(31) Thus, we believe that CYT107 will improve host immunity and thereby lead to a decrease in hospital acquired secondary infections in septic patients.

Consequently, we hope to see a decrease in secondary opportunistic type bacterial and fungal infections in septic patients treated with CYT107 compared to septic patients who are treated with placebo (Control group). We will use the Center for Disease Control criteria for definition of ICU-acquired infections. Because it is often difficult to determine if a secondary hospital acquired infection is present, a panel of 3 experienced ICU physicians, "The Secondary Infection Evaluation Committee", will be used to evaluate and grade whether a secondary hospital acquired infection is present. These 3 physicians will be blinded to patient treatment with CYT107 versus placebo. These 3 physicians will not be members of the investigative team but rather colleagues of the investigators. They will meet either in person or by teleconference once a month or as deemed necessary by the Investigator to judge secondary infections.

4.0 Study Design

4.1 Overall Design

This is a phase II, multi-center, double-blinded, placebo-controlled, randomized study aimed at comparing, two dosing frequencies of CYT107 and placebo in patients with sepsis. The study will be performed in parallel at multiple sites in the United States and France using identical study design and data collection. The results of the French and US investigative teams will be

combined for preliminary and final analysis. A REDCap database will be used for data management for all sites. A central computerized randomization center will be used to allocate treatments in both the US and French studies. *The current protocol deals with the United States investigative study.* For this preliminary trial of CYT107, there will be 4 investigative sites in the US, however, study enrollment is planned to take place at the coordinating site, Vanderbilt University Medical Center. If this current study demonstrates that CYT107 is safe and effective in increasing the ALC in septic patients, a larger trial of CYT107 will be conducted and additional sites in both countries may be recruited. The list of investigators and trial sites in the US are the following:

- Edward Sherwood MD, PhD; Professor of Anesthesiology, Vanderbilt University School of Medicine
- Richard Hotchkiss MD; Professor of Anesthesiology, Medicine, and Surgery, Washington University School of Medicine
- Elliott Crouser MD, Professor of Medicine, Ohio State School of Medicine
- Lyle Moldawer Ph.D., Professor of Surgery, University of Florida, Gainesville, School of Medicine.

4.2 Study Treatment

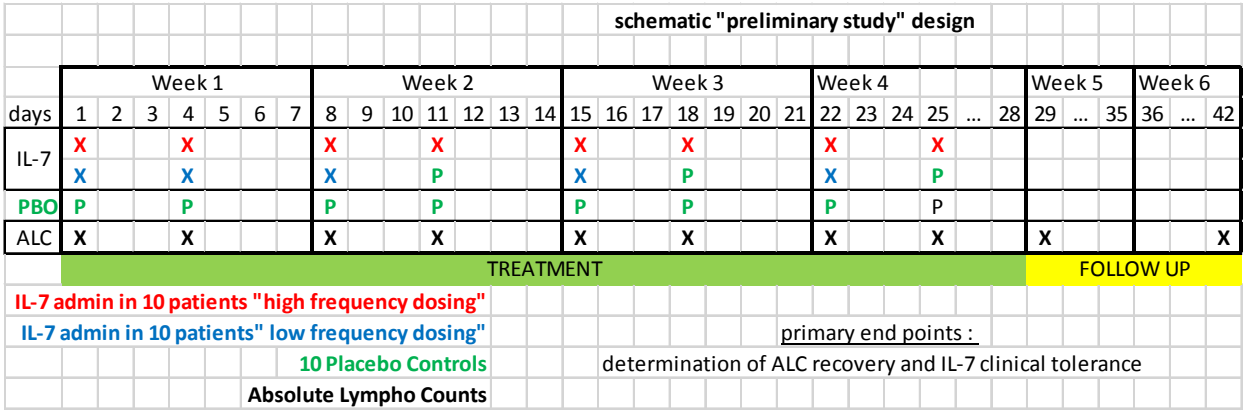
Up to thirty patients may be enrolled at 4 sites in the US, i.e., Vanderbilt, Ohio State, University of Florida, and Washington University School of Medicine, however, it is anticipated that the initial enrollment of 30 subjects will occur at Vanderbilt, Washington University and Limoges in France. (Note: the French sites may also enroll up to 30 patients such that a total of 30 evaluable patients will be available from the two countries). In the event that patient accrual is delayed in one country, all patients will be recruited from either the US or France alone. A computerized protocol will implement permuted block randomization in blocks of size three. This scheme ensures balance across the treatment groups for every third randomization. A maximum of 20 septic patients will be treated with CYT107 at a dose of 10μg/kg. There will be two dosing regimens as illustrated in Figure 8 (below). CYT107 will be administered intra-muscularly at the dose of 10μg/kg (IBW), once or twice a week for a four week cycle. Ten septic patients will have CYT107 administered twice a week for 4 weeks. Ten septic patients will have two doses of CYT107 administered during the first week followed by one dose of CYT107 in conjunction with 1 dose of placebo for 3 additional weeks. Ten septic patients will receive placebo twice a week for four weeks along with routine standard of care per the clinical providers.

At approx. 48-120 hrs **after admission** for sepsis, those septic patients who meet the Inclusion/Exclusion criteria (see previous section) and provided written informed consent, will be entered into the study. The first dose of CYT107 (as indicated below) is at approx. 48-120 hrs after sepsis onset. The initial dose of study drug will be administered only in the ICU by the PI or research nurse. If patients have an INR greater than 2.5 or a platelet count less than 35,000, CYT107 will be administered by the subcutaneous route rather than by intra-muscular injection.

A staggered approach to CYT107 treatment will be used in which treatment will not be initiated in a newly enrolled patient until 24 hours after the previously enrolled patient has received the second dose of CYT107 without evidence of serious adverse event (SAE) occurrence.

In patients with coagulopathy (INR greater than 2.5 or a platelet count less than 35,000), CYT107 will be administrated subcutaneously.

Figure 8 Schematic study design of CYT107 Protocol



Dosing regimen for CYT107 in septic patients showed administration details of the "high frequency dosing" – (shown in red color) or the low frequency dosing (shown in blue color). The ALC is quantitated weekly throughout 6 weeks duration. Given that CYT107 will boost host immunity, we predict that patients treated with CYT107 will have decreased incidence of new secondary infection, decreased hospital readmissions (which generally are due to new secondary infections), and improved 60 day, 90 day, 180 days and one year mortality. Thus, these parameters will be recorded in all patients. The 60 day, 90 day, and one year data will be obtained by telephone interview, physician records, or by interrogating appropriate databases and newspaper obituary records. The patients will not have to be seen in clinic.

Additional experimental details:

- Patients with severe sepsis and organ failure who are admitted to the ICU are candidates. Septic patients who are not being treated in the ICU are not candidates for study enrollment.

- Septic patients will continue to receive the CYT107 to complete the full dose schedule even if their sepsis has resolved if they remain in the acute care hospital setting. Patients will not receive additional doses of CYT107 if they are discharged from the hospital.

If patients who are enrolled in the study fail to complete two weeks of the protocol for any reason such as death, discharge or transfer from the hospital, or study withdrawal decision by family or physician, then the patient will not be considered to have had an acceptable course of therapy with CYT107 to allow evaluation of its effects. The next patient to be recruited into the CYT107 study will be assigned the next randomization treatment until all groups have enrolled at least 10 subjects. In this manner, the investigators will be able to maintain proper patient

randomization to evaluate CYT107's efficacy. Note: research data for all subjects that underwent randomization and treatment will be completed regardless of length of time on study and will be entered into the database and reported to all Committees including the DSMB, FDA, IRB, etc.

4.3 Endpoints

4.3.1 Primary Biological Activity & Safety Study Endpoints

1) *Weekly measures of absolute lymphocyte counts (ALC)* will be performed to determine the kinetics of restoration of ALC. Specifically, we will quantitate the increase of ALC at day 42 +/- 3 days, expressed as a percentage of baseline value determined at the time point that CYT107 was first administered (day 1). We will determine the number of patients who reach a 50% increase over the baseline value and we will also determine the percentage of patients reaching an ALC > 1200 at day 42 +/- 3 days in each group.
2) *Assessment of the clinical, biological, and immunological effects of CYT107* as well as its safety and tolerability, i.e., incidence and scoring of all adverse events: AEs and SAEs all over the study period, ending day 42 +/- 3 days, will be recorded and evaluated by the DSMB, Steering Committee, and secondary infections committee.

4.3.2 Secondary Biological Activity Study Endpoints

1) *PK Sessions:* Briefly each patient will be sampled on day 1 and day 22 at 0 (pre-dose), 1, 3, 5, 7, 9 and 24 hrs post CYT107 administration. Main PK parameters will be assessed including Model-independent PK analysis will be used to estimate Tmax, Cmax, half-life, clearance and area-under-the-curve (AUC).
2) *Pharmacodynamics effects on immune markers:* The investigative sites will also examine the effect of CYT107 to restore function of immune effector cells in patients with sepsis.
    i. Effect of CYT107 to increase the percentage of patients reaching an ALC>1200 in each dosing group. (An ALC of 1200 represents the lower limit of normal for most hospital laboratories.)
    ii. Effect of CYT107 on absolute numbers of $CD4^+$T and $CD8^+$T over the study period,
    iii. Effects on circulating monocyte HLA-DR expression
    iv. Effects on whole blood LPS-stimulated TNF-α
    v. Assessment of CYT107 immunogenicity by detection and quantification of binding antibodies and detection of neutralizing antibodies in positive samples.

3) Incidence and type of opportunistic secondary infections: We will compare the effect of CYT107 to reduce hospital-acquired secondary infections in CYT107 septic patients versus a control group of septic patients who receive placebo. The rate of hospital-acquire secondary infections at day 42 is ~ 40%.(20) We postulate that CYT107 will reduce the rate of secondary infections by ~ 30-40%. We will also examine other clinical measures in the CYT107-treated septic patients and compare outcomes including: SOFA scores, ICU free days, hospital free days, ventilator free days, decrease

in readmissions to ICU for recurrent infections. We will also record at approx. 60, 90, 180 and 365 day (1 year) general health status and survival.

All end points will be evaluated in a global analysis, pooling the results of the US and French sites using REDCap as the common patient database. Results will be visible to all investigative centers.

4.4 Duration of the study

Patients will be treated with CYT107 or placebo for 4 weeks duration and undergo laboratory monitoring of various organ functions as detailed previously for the 28 days following the initial treatment. The ALC will be followed for an additional 14 days due to continuing effects of CYT107. Primary end points will be measured at approximately Day 42 +/- 3 days. Patient outcomes regarding secondary infections, readmission to hospitals, mortality, etc. will be followed at approximately 60, 90, 180 and 365 days after CYT107 therapy is initiated. Long term survival (1 year survival) will be recorded using a variety of means including physician records, patient telephone communication, and searches of mortality databases.

5. Study Population

5.1 Inclusion Criteria:

1) Patients of age ≥ 18 yrs to 80 yrs

2) Patients with persistent suspected sepsis at 48-120 hrs after admission

3) Two or more criteria for the systemic inflammatory response syndrome (SIRS) (see reference (19) for SIRS criteria) and a clinically or microbiologically suspected infection.

4) At least one organ failure as defined by a SOFA score of ≥2 at any time point during the 48-120 hrs after admission to the ICU

5) Requirement of vasopressor treatment as follows: i) epinephrine or norepinephrine at ≥ 0.05 μg/kg/min ideal body weight; ii) vasopressin, or iii) dopamine at ≥ 4-5 μg/kg/min ideal body weight, continuously for 4 hrs or more, provided that at least 20 ml/kg of ideal body weight of crystalloid or an equivalent volume of colloid was administered during the 24-hour interval surrounding the start of vasopressor treatment, to maintain systolic pressure ≥ 90 mmHg or a mean arterial pressure ≥ 60 mmHg at any time point during their sepsis course preceding enrollment into the CYT107 study.

6) Lymphopenia with an absolute lymphocyte count ≤ 900 cells/mm^3 at either the day of consent or the day prior to consent during their ICU stay

7) Predicted length of stay in the ICU of up to two weeks after starting drug therapy treatment in the trial

8) Ability to obtain a signed informed consent from patient or LAR consent.

5.2 Exclusion Criteria

1) Cancer with current chemotherapy or radiotherapy (Receipt of chemotherapy or radiotherapy within the last 6 weeks). All patients with current, or a history of, hematologic malignancy (including, but not limited to, ALL, AML, CLL, CML, etc.) or lymphoma will be excluded, regardless of receipt of recent chemotherapy.

2) Cardiopulmonary resuscitation within the previous 4 weeks without objective evidence of full neurologic recovery) or patients who have minimal chance of survival and are not expected to live > 3-5 days as defined by an APACHE II score of ≥ 35 at time of consideration for study eligibility.

3) Patients with a history of or who currently have evidence of autoimmune disease including for example: myasthenia gravis, Guillain Barre syndrome, systemic lupus erythematosis, multiple sclerosis, scleroderma, ulcerative colitis, Crohn's disease, autoimmune hepatitis, Wegener's etc.

4) Patients who have received solid organ transplant or bone marrow transplant

5) Patients with active or a history of acute or chronic lymphocytic leukemia

6) AIDS-defining illness (category C) diagnosed within the last 12 months prior to study entry

7) History of splenectomy

8) Any hematologic disease associated with hypersplenism, such as thalassemia, hereditary spherocytosis, Gaucher's Disease, and autoimmune hemolytic anemia

9) Pregnant or lactating women

10) Participation in another investigational interventional study within the last 6 months prior to study entry, with the exception of studies aimed at testing sedation products belonging to standard of care such as Propofol, Dexmedetomidine, Midazolam

11) Patients receiving immunosuppressive drugs, e.g., TNF-alpha inhibitors, for rheumatoid arthritis, inflammatory bowel disease or any other reason, or systemic corticosteroids other than hydrocortisone at a dose of ≤ 300 mg/day

12) Patients receiving concurrent immunotherapy or biologic agents; including growth factors, cytokines and interleukins other than the study medication, for example IL-2, growth factors, interferons, HIV vaccines, immunosuppressive drugs, hydroxyurea, immunoglobulins, adoptive cell therapy.

13) Prisoners

5.3 Study ID Numbers

Study ID numbers are assigned using an alpha-numeric sequence. The 2 first letters will signify the study center followed by sequential assignment of ID number in numerical order (WU-01, WU-02, etc. - Center ID numbers are defined in the center list- see Appendix). Each subject consented and enrolled will be assigned the next available number for the individual sites.

6. Study Visits and Procedures

All protocol related procedures are described in *Appendix II*: Flow charts of the study.

6.1 Screening period

The following procedures will be performed at the screening visit or during the screening period:

- Dated and signed patient informed consent (by the patient or family member)
- Each site will assign the subject the next available study ID number
- Inclusion/exclusion criteria checklist review
- Demographics, relevant medical/surgical history, Sepsis history, APACHE II score, SOFA score, GLASGOW coma scale
- Physical examination and lymphoid system examination
- 12-lead electrocardiogram (EKG) and interpretation (pretreatment EKG)
- Spleen ultrasound (Pre-treatment)
- Pregnancy test if female of childbearing potential without known contraceptive method
- Cardiac check: presence of vasopressors, EKG, MAP, HR, pHa
- Respiratory check: PaO2, FiO2 (A-a Grad if FiO2 $\geq$ 0.5)
- Kidney functions: Creatinine, Urine analysis, 24hrs diuresis
- Liver function: ALT, AST, Bilirubin
- Sampling for:
    - Hematology (ALC, WBC, ANC, Mono, Platelets) and blood chemistry (including CRP, PT, PTT) as described in the flow chart,
    - PBMC for phenotyping and functionality test (CD3, CD4, CD8 T cells, HLA-DR expression, whole blood TNF-alpha production) as described in flow chart,

Data from laboratory tests and procedures performed as standard of care (SOC) during the screening period will be utilized for applying screening criteria. All remaining tests and procedures listed in Appendix 2 will be conducted for research purposes only and ordered by the PI.

6.2 Randomization

The randomization list will comprise three arms “CYT107 High frequency Arm” and “CYT107 Low frequency Arm”, and placebo Arm. A permuted-block randomization will be used in blocks of size of 3 and an allocation ratio of 1:1:1, stratified by study country (US and France). Subjects will be allocated randomly within each block. This procedure will be repeated until at

least 10 evaluable subjects are randomly assigned to each treatment group, totaling at least 30 evaluable subjects (between US and France).

Screening data, including lab results from screening and any relevant medical information, will be reviewed by the Research Nurse Coordinator and PI at each academic medical center for confirmation of all inclusion/exclusion criteria and randomization authorization.

After randomization authorization is granted by the PI at the responsible institution, the randomization assignment will be generated by computer. Each subject will be assigned the next available number. Subjects, investigators, and all study team members will be blinded to drug treatment. In the case of dropouts (either subject- or investigator-initiated), subjects will be replaced, to provide the required number of subjects. Randomization of replacements will be as follows:

1. Subjects withdrawn prior to randomization (prior to receiving CYT107 or placebo) will simply be replaced and the next subject will receive the same randomization assignment.

2. CYT107 treated subjects who are withdrawn after randomization while receiving the study drug treatments but prior to Day 14 will be replaced using with the next subject receiving the next randomization assignment. This will continue until a minimum of 10 patients have been randomized to each of the 3 cohorts and remained on study treatment through Day 13.

3. Subjects withdrawn or completing at least two weeks of study drug treatment will be considered evaluable and the following subject will receive the next scheduled randomization sequence.

6.3 Post Randomization Period

See Appendix 2 flowchart for designation of all study related procedures (standard of care and/or research purposes only)

6.3.1 Procedures for Day 0 baseline

6.3.1.1 Pre CYT107 administration

- History and Physical examination and lymphoid system examination
- Blood Sampling for : ALC count, Baseline PK and Immunogenicity assays

6.3.1.2 Post CYT107 administration Day 1 Week 1

- At 1, 3, 5, 7 and 9 hrs post treatment : Blood sampling for CYT107 PK assays (approximately 7 mL blood/timepoint from IV line)
- At 3 hrs post treatment :
  - Vital signs (Temperature, RR, Pulse and BP)
  - Review and record adverse events experienced
  - 12-lead electrocardiogram (ECG) and interpretation
- At 24 hrs post treatment (Day 2) : Blood sample for PK assay (total blood for PK over 24 hrs approx. 49mL)

6.3.2 Procedure for Days 4, 8, 15, 22, 29, 42

- APACHE II score, SOFA score, GLASGOW coma scale
- Physical examination and lymphoid system examination.
- Cardiac check: presence of vasopressors, EKG, MAP, HR,
- Respiratory check: PaO2, FiO2 (A-a Grad if FiO2 ≥ 0.5 and patient on mechanical ventilation)
- Kidney functions: Creatinine, Urine analysis
- Liver function: ALT, AST, Bilirubin
- Sampling for:
  - Hematology (ALC, WBC, ANC, Mono, Platelets) and blood chemistry (including CRP, PT, PTT) as described in the flow chart,
  - PBMC for phenotyping and functionality test (CD3, CD4, CD8 T cells, HLA-DR expression, whole blood TNF-α as described in flow chart,

6.3.2.1 Additional procedure for day 11

- Blood sample for Immunogenicity assay

6.3.2.2 Additional procedure for day 22

- Before administration : Baseline blood sample for PK assay and Immunogenicity assay
- At 1, 3, 5, 7 and 9 hrs post treatment : Blood sampling for CYT107 PK assays
- At 3 hrs post treatment :
  - Vital signs (Temperature, RR, Pulse and BP)
  - 12-lead electrocardiogram (EKG) and interpretation
- At 24 hrs post treatment (Day 23) : Blood sample for PK assay (total blood for PK over 24 hrs approx. 49 mL)

6.3.2.3 Additional Procedure for Day 42

(See Appendix 2 Flow Chart for complete lists of clinical and laboratory studies.)

- ALC
- Hematology labs

6.4 Procedure for Days 60, 90, 180 and One Year Follow-up Period

Patients will be followed for 60, 90, 180 and 365 day outcomes regarding disposition, i.e., incidence of new secondary infections, long term status, i.e., still hospitalized, discharged to long term care facility or nursing home, discharged to home, and mortality.
Systematic blood sample for Immunogenicity testing at D60, repeated at day 180 and 360 if not negative for anti-CYT107 antibodies.

7.0 Safety and Biological Assessments

7.1 Clinical Safety

The following assessments will be performed on specified days (see flowchart) prior, during, and following therapy:

- Complete medical history obtained from the electronic medical record which should include
  - Past relevant medical and surgical history whether or not related to Sepsis
  - A complete Sepsis diagnosis and treatment history including actual or estimated start and stop dates of anti-sepsis treatments, and a complete history of any

prescription medications taken for the treatment of secondary opportunistic infections including start and stop dates and dose regimens
- All home prescription and non-prescription medications and dietary supplements will be recorded. All new medications within the last 3 weeks will also be recorded. ,

- Full physical examination including vital signs (temperature, pulse, resting blood pressure and respiratory rate), weight, and particular attention to the CYT107 injection sites.
- Reporting of AEs to the DSMB through-out study participation according to the "Division of AIDS table for grading the severity of adult and pediatric Adverse Events, Version 2.0, November, 2014". Note that we are using the NIAID AIDS table for grading adverse effects because CYT107 (like IL-2) has been used in several clinical trials of AIDS patients and its potential adverse effects are well described and included in this table.
- ECG and Ultrasound

The following will be performed during the study according to the schedule described in Appendix II.

- ECG 12-lead with detailed analysis at screening, three hours after the first dose of CYT107 at Day 1 and three hours after CYT107 administration at approximately Day 22.
- Spleen ultrasound (3 dimensions): will be performed at screening, and at approximately D42. Laboratory Investigations per protocol (appendix 2).

7.1.1 Hematology and biochemistry (See Appendix 2 flow chart)

Hematology includes**:**
hemoglobin, hematocrit, red blood cells, mean corpuscular volume, white blood cells with differential (including ALC, platelets, Monocytes, Absolute Neutrophil Counts) Coagulation and inflammation markers: Prothrombin Time (PT), Partial Thromboplastin Time (PTT).

Chemistry includes:
Electrolytes (sodium, potassium, chloride, bicarbonate, calcium, magnesium and phosphorus), creatinine, glucose, albumin, liver function tests (total bilirubin, AST, ALT, Alkaline Phosphatase), and CRP (C-reactive protein).

7.2 CYT107 Pharmacokinetics

Pharmacokinetic profile will be determined in all patients.
Samples will be collected at D1 and D22 at pre-dose (0) and at approx:
1, 3, 5, 7, 9 and 24 hrs post dose
Samples will be analyzed for CYT107 content and data analyzed to determine the main PK parameters: Cmax, Tmax, AUC, half-life.

7.3 CYT107 Immunogenicity

Specific CYT107 binding and neutralizing antibody studies will be performed.
Samples will be collected at D1, D11, D22, and at approximately D60.

If the D60 binding antibodies test is positive, samples will be tested for the presence of neutralizing antibodies. Another sample will be tested for binding antibodies at approximately day 180. The same will be done at approximately D360 if D180 is positive.

7.4 CYT107 Pharmacodynamics/Immunology

The immune monitoring will include:
Differential count of T cells CD3, CD4, CD8
Monocyte HLA-DR expression
Whole blood TNF-α production

8.0 Investigational Medicinal Product (see details in Investigator's Brochure)

8.1 CYT107

8.1.1 Description

CYT107 (CYT107) is a recombinant protein belonging to the class of growth factors and more specifically to the class of cytokines. CYT107 is a heavily glycosylated and sialylated form of recombinant human interleukin-7 expressed from a CHO cell line, composed of 152 amino acids, with an average molecular mass as determined by mass spectrometry of 22 kDa and a mean pI of 7. The molecular formula of the peptidic sequence only (non glycosylated) is $C_{762}H_{1241}N_{213}O_{228}S_{11}$. The protein contains 3 disulfide bridges (Cys2- Cys 92, Cys 34- Cys 129, and Cys 47- Cys 141) and 4 glycosylation sites (3N, 1O).

8.1.2 Source

CYT107 used in this clinical trial was manufactured under good manufacturing practice (GMP) criteria at a 600 L fermentation and purification scale by PATHEON (Princeton, NJ), for REVIMMUNE Inc. At clinical site, the product will be made available to the hospital pharmacy by CSM , Fargo, ND58103.

8.1.3 Formulation

CYT107 is supplied in a 2 mL vial as 0.5 mL of CYT107 (1 mg) in 10 mM Sodium Acetate, 100 mM NaCl, 50 mM glutamic acid. The pH of the solution is 5 and the osmolality is 320 ± 40 mOsm. The concentration of CYT107 in solution is 2 mg/ml.

8.1.4 Stability

Stability studies will continue throughout the clinical study. Updated stability information will be periodically communicated to the hospital pharmacy.

8.1.5 Special Handling

There are no specific guidelines for safe handling of CYT107. Institutional guidelines for safe handling of proteins in general should be followed.

8.1.6 CYT107 Preparation

Syringes containing the CYT107 dose (placebo dose same volume of NaCl 0.9%) will be prepared by the hospital pharmacy and delivered to the clinic in a blinded fashion. The product

should be defrosted at least 1 hour before administration. Defrosted product (and possibly put in syringe) should be kept refrigerated at +4°C/+8°C until use and for no more than 12 hours.

CYT107 ampoule will only be used if the solution is clear (following visual inspection), the vial is undamaged and the use by (expiration) date (if marked on the vial) has not been passed.

Based on patient's ideal body weight on day of study entry, the appropriate number of vials of CYT107 drug product will be prepared and administered. CYT107 will be kept refrigerated until time of immediate administration to the patient. To minimize the chance for contamination, sterile or septic technique should be carefully observed during CYT107 solution preparation (filling of the syringe) and administration.

A vial is restricted for use by a single patient. Take no more than 0.5 mL into the syringe. The dose to be injected will be divided as necessary into multiple intramuscular injections so that each injection will not exceed 0.5 ml in volume. (Dosing guidelines for ideal body weight make the requirement for multiple injections highly unlikely).

8.1.7 Drugs interactions

Not known; are being presently investigated.

8.2 CYT107 Presentation and Packaging

CYT107 is supplied as a sterile colorless liquid in 2 cc glass vials that are packed individually in cardboard box. Labels are stuck on vial and box.

The labeling is following local and GMP rules. The detailed description of labeling is provided in the Pharmacy Manual.

8.3 CYT107 Storage Accountability and Return of Unused Product

Study product will be stored frozen at -20°C until used.

The investigator, his/her designee, or hospital pharmacist must maintain complete records of all study products received, stored and subsequently dispensed.

Study product is Revimmune property; at the conclusion of the study, all unused study drug must be returned to Revimmune or designee. All unused product should be kept in suitable storage conditions until shipped. Unused product should not be used on non-study patients.
The detailed procedures to be followed by Clinic and Pharmacy are provided in the Pharmacy Manual.

8.4 Study Drug Administration

Each dose of CYT107 or Placebo will be administered in the hospital by a trained research nurse or PI. CYT107 or Placebo will be administered by intramuscular route at the dose of 10 µg/kg based on the patient's ideal body weight unless the patient meets criteria for subcutaneous injection (see Sect 4.2 Study Treatment).

The volume to be administered will be identical in all groups: 0.005mL/kg: ex: 0.375mL for patients of 75kg. The control group will receive the same volume of saline, dosing to IBW.

The detailed procedures to be followed by Pharmacy and hospital are provided in the Pharmacy Manual including a table of volumes to be administered per patient total body weight.

8.5 Duration of treatment

- Patient randomized in the "CYT107 High Frequency Arm" will receive CYT107 treatment at 10µg/kg at days 1, 4, 8, 11, 15, 18, 22, 25
- Patient randomized in the "CYT107 Low Frequency Arm" will receive CYT107 treatment at 10µg/kg at days 1, 4, 8, 15, 22 and placebo (NS: NaCl 0.9%)) at days 11, 18 and 25.
- Patient randomized in the "CONTROL Arm" will receive Placebo (NS: NaCl 0.9%) at days 1, 4, 8, 11, 15, 19, 22 and 25.

8.5.1 Dose Adjustment According to Patient's Weight

8.5.1.1 Obese Persons

For a patient with a Body Mass Index (BMI) over 30, a corrected weight will be used to calculate the final dose the patient will receive.
The corrected weight will be calculated as follows:
Corrected weight (kg) = 30 x height^2 (in m)

8.6 CYT107 Administration Permanently Discontinued

8.6.1 Reasons for CYT107 administration permanently discontinued

The investigator may interrupt study treatment at any time if a definite contraindication to treatment appears such as:

- Appearance of a critical event as defined in section 12.1
- Development of an exclusionary condition
- Requirement for prohibited concomitant medications
- If the patient or patient's family decides to switch to comfort measure only and forgo life sustaining therapy.
- Patient request to terminate the study treatment
- Patient Informed Consent Withdrawal
- Patient withdrawn per other events at PI discretion

8.6.2 Procedure in case of CYT107 administration permanently discontinued

If a patient has a premature study treatment discontinuation for any reason, the subject may be retained in the study for data collection and testing procedures for remainder of the study period. If it is not possible, the last visit may include all tests and procedures scheduled for day29 visit plus a blood sample for detection of CYT107 immunogenicity (see appendix 2 Study flowchart). These data should be recorded in the research CRF.
The primary reason for withdrawal will be clearly documented in the patient's medical record and recorded in the CRF.

In the specific case of patient consent withdrawal, the investigator must make every effort to perform the safety assessments.
Patients who are withdrawn from the study will be assigned appropriate alternative treatment as recommended by the PI in collaboration with the clinical team.

8.7 BLINDING

8.7.1 Organization of Blinding

The study will be performed in a double-blind manner. The patient, the investigator and study center staff will be blinded to study drug allocation. The pharmacist will be unblinded to study drug and will prepare study treatment or placebo for a patient as specified by the randomization scheme.

8.7.2 Unblinding

Emergency unblinding of the patient should only be undertaken by the investigator when it is essential to treat the subject safely and efficaciously. Most often, study drug discontinuation and knowledge of the possible treatment assignments are sufficient to treat a study subject who presents with an emergency condition. The investigator will make be responsible for making a decision to unblind in case of a suspected unexpected serious adverse reaction (SUSAR). The investigator will obtain the randomization assignment and a blinded alert will be sent to RevImmune, the coordinating center and the study team that a patient has been unblinded. The patient will be followed until resolution of the adverse event. All "end of study" assessments must be performed.

8.7.3 Final Unblinding

For every patient, the main study end points will be reached at day 60. Accordingly when the last patient of both IRIS-7A and IRIS-7B studies (i.e. the 30th patients of the two cumulated studies) will reach day 60, the database will be locked and the study unblinded for analysis.

8.8 Concomitant treatments

8.8.1 Steroids

Patients enrolled in this clinical trial may be receiving steroids; this will be limited to hydrocortisone at a dose no higher than 300 mgs of hydrocortisone daily.

8.8.2 Prohibited Medications

Are as follows:

- Concurrent immunotherapy; including growth factors, cytokines and interleukins other than the study medication, for example IL-2, Interferons, HIV vaccines, immunosuppressive drugs, hydroxyurea, immunoglobulins, adoptive cell therapy;
- Any investigational agent;
- Systemic corticosteroids other than hydrocortisone at a dose of 300 mgs/day.
- Biological agent (other cytokines such as IL-2, growth factor, monoclonal antibody);
- Cytotoxic chemotherapy

If it becomes obvious, during the course of the study, that administering any of these therapies are not in the patient's best interest, due to the patient's imminent demise or the patient or the patient's family desire to switch to comfort measures only, (i.e., not to continue aggressive care), then study drug treatment will be discontinued. (See section 7.8.2 for details on procedures to follow in case of study drug discontinuation).

9.0 Statistical Analysis

9.1 Sample Size

Sample size justification

The primary endpoint of this trial is to compare two different dosing regimens of CYT107 to increase the absolute lymphocyte count (ALC), a marker of immunosuppression in sepsis which correlates with mortality. Although CYT107 has not been previously been used to treat patients with sepsis, there are data on the effect of CYT107 to increase the absolute numbers of CD4 and CD8 T cells in patients with cancer and in patients with HIV (see Figs 2&6 and reference #14). As demonstrated in Figs 2 & 6, CYT107 at a dose of 10 micrograms/kg (the dose that will be used in the present study) has a very predictable effect to cause a doubling of the number of CD4 T cells which are a major component of the absolute lymphocyte count (ALC) in patients with HIV. This effect to increase the absolute numbers of CD4 T cells was consistent in almost all patients who were treated with CYT107. CYT107 has a similar effect (although not quite as pronounced) on CD8 T cells. Therefore, we anticipate that greater than 80% of septic patients who are treated with CYT107 at a dose of 10 micrograms/kg (either high frequency dosing regimen or the low frequency CYT107 dosing regimen) will increase their ALC by over 50% by day 42, whereas less than 20% of patients who receive placebo will exhibit such a change. Under these assumptions, a simulation-based power calculation as implemented using the primary analytical plan listed in section 9.3.2. In particular, the proposed study design (10 subjects per treatment arm) was found to have greater than 85% power to detect an equal or more extreme treatment effect. Thus, we believe that 10 patients per group is sufficient to show a statistically significant effect of CYT107 to increase the ALC by over 50%.

Two other major goals of the study are to establish the safety of CYT107 in patients with sepsis and to determine that the pharmacokinetics and pharmacodynamics are not significantly altered in septic patients. CYT107 has been administered to over 300 patients and has been well tolerated with a low incidence of side effects. (see Investigator's brochure.)

9.2 Statistical Analysis

9.2.1 Analysis Datasets

9.2.1.1 Definition of protocol deviations

Major protocol deviations are defined as deviations liable to bias the evaluation of the main study endpoint. The following deviations will be considered major:

- Noncompliance with the inclusion or exclusion criteria
- Noncompliance with study treatment
- Intake/administration of prohibited medication(s)

All other deviations will, a priori, be considered as minor deviations. However, all deviations will be reviewed during a data review meeting before data base lock and statistical analysis. This data review will also serve to constitute the analysis datasets.

9.2.1.2 Analysis datasets

The following subsets of study participants will be defined and analyzed:

1) The **Safety dataset (SAF)** is defined as those subjects enrolled in the study, who have received at least one dose of study treatment.

2) The **Full Analysis Activity dataset (FAS)** is defined as all enrolled subjects who have received all the study treatment (5-low frequency, or 8-high frequency) administrations and have an evaluation of the primary Biological Activity Study End Point.

3) The **Per Protocol Activity dataset (PP)** is defined as all enrolled subjects who have been treated consistently with the protocol and have the evaluation of the primary Biological Activity Study End Point available. To be considered evaluable, patients in all study arms must complete at least 2 weeks of study drug treatment.

9.2.2 Statistical Methods

Descriptive statistics for baseline characteristics and study outcomes will be presented in aggregate and stratified by treatment arm, and separately for the SAF, FAS, PP datasets. Descriptive statistics will be implemented as follows:

1) Continuous variables will be summarized using the sample mean, standard deviation, and 95% confidence interval for the mean, median, range, and quartiles
2) Categorical variables will be summarized using sample proportions and the associated 95% confidence intervals

Simple inferential analysis will be implemented for certain study outcomes:

1) Categorical outcomes will be evaluated using simple, ordinal, or multinomial logistic regression.
2) Continuous outcomes will be evaluated using linear regression techniques. Quantitative and graphical regression diagnostics will be considered. Data transformations and nonparametric methods will be utilized when necessary.

9.2.3 Analysis of the Primary Objective

A significant improvement in absolute lymphocyte count (ALC) is defined as an increase of more than 50% from baseline at any subsequent measurement, up to day 42. All patients will be classified as having demonstrated significant improvement in ALC by this definition. The frequencies of the resulting dichotomous outcome will be compared across treatment arms using simple logistic regression. The odds ratios associated with either treatment, relative to placebo, will be estimated and presented with 95% confidence intervals. The overall effect of treatment will be assessed using a likelihood ratio test of the null hypothesis that the odds of significant

ALC improvement are identical across treatment arms. These analyses will be implemented separately for the PP and FAS datasets defined above (9.2.1.2).
As a secondary analysis, the full sequence of ALC measurements for all patients will be evaluated using mixed effect regression methods, adjusting for baseline ALC and study day, and accounting for correlation among sequential repeated measurements. This analysis will permit an examination of secular trends in ALC, the interaction of treatment frequency and duration, and the variability in treatment effect among study participants. These effects will be summarized using estimates and 95% confidence intervals.

9.2.4 Analysis of Other Biological Activity Endpoints

Descriptive statistics and simple inferential analyses (as described in 9.2.2) will be provided for all other biological activity endpoints.

9.2.5 Analysis of Safety Endpoints

The safety of r-hCYT107 will be evaluated on all SAF datasets.

9.2.5.1 Adverse Events

Adverse events reported during the study will be coded using the MedDRA terminology.
Treatment emergent adverse events (TEAE) will be defined as any adverse event which occurs or worsens on study treatment during the treatment period.

Numbers and percentages of patients with at least one reported treatment emergent adverse event will be tabulated by system organ class and preferred term for:

- all TEAE
  - all TEAE leading to study drug discontinuation
  - all TEAE of grade 3 or 4
  - all TEAE for which relationship with the study drug is recorded as possible or probable
- by grade
  - all related TEAE leading to drug discontinuation
  - all related TEAE of grade 3 or 4

Recurring adverse events (i.e., adverse events classified with the same preferred term) for a given patient will only be counted once and only their most severe intensity or most severe relationship to the study treatment will be tabulated.

9.2.5.2 Safety laboratory tests

Descriptive statistics for laboratory safety tests will be computed at each time points on the raw values. If relevant for some parameters, changes from baseline will also be tabulated.

Shift tables from baseline will be presented.

9.2.5.3 Vital signs

Descriptive statistics will be computed at each time point on the raw values.

9.2.5.4 Other safety variables

For Physical and lymphoid examination and ECG; descriptive statistics will be provided at each time point.

9.2.6 Concomitant Treatments

Concomitant treatments will be coded with the WHO-DRUG dictionary and the ATC classification. They will tabulated by the anatomic, therapeutic and clinical level.

9.3 Statistical Analysis Plan

A Statistical Analysis Plan (SAP) will be written, validated and signed before the first interim analysis. This document will describe the detailed methodology for statistical analyses of the data collected in both US and French studies.

An interim analysis will be performed when the last patient of both IRIS-7A and IRIS-7B studies (i.e. the 30th patients of the two cumulated studies) will reach day 60, then the database will be locked and the study unblinded for analysis.
Final analysis including follow up controls will be performed when the last patient of both IRIS-7A and IRIS-7B studies (i.e. the 30th patients of the two cumulated studies) will reach day 360, then the database will be definitively locked for final data analysis.

10. Trial Organization

10.1 Steering Committee

A steering committee will be set up with the following members:

- Study Chairman
- Investigator from each center
- Medical representative from Revimmune
- Other external participants, as needed

This committee will meet under various circumstances:

On an ongoing basis, the Steering Committee will review all Grade $\geq$ 3 Adverse Events, and Critical Event.

Upon request of the Sponsor and of the DSMB recommendation to evaluate:

- Potential amendment
- Statistical analysis plan
- Results of interim analyses
- Study continuation

At the end of the study to review the activity and safety data

The steering committee meetings can be organized as a conference call.

10.2 Data Safety Monitoring Board (DSMB)

The DSMB will be composed of 4 experts who are not involved in the conduct of this trial as investigators but are experienced in the management of Sepsis disease and immunotherapy:

- Opal S, MD, Brown University School of Medicine, USA
- Pandharipande P, MD, Vanderbilt University School of Medicine, USA
- Harrell F, PhD, Vanderbilt University School of Medicine, USA
- Moore F, MD, University of Florida School of Medicine, USA

If need be, the DSMB is free to ask for the advice of one or several other experienced physicians whose name and advice, however, must be communicated to the steering committee.

The mission and rules of DSMB is described in the DSMB charter which will be signed by DSMB member before study initiation.

On an ongoing basis, the DSMB will review:

- All Critical Events.
- All treatment emergent adverse events (TEAEs)
- All SAE assessed by the investigator, by the DSMB, UVEC or by the 3 panel expert committee which adjudicates whether the abnormal finding is secondary to CYT107 or part of the septic process.
- Individual data on the size of lymphoid organs.

The DSMB will review, confirm or discuss the severity grade suggested by the investigator assess the causality relationship to CYT107 and the experimental protocol.

The DSMB will advise the Sponsor regarding study continuation. On an ongoing basis and at the end of the study the DSMB, will review the safety data.

The experts of the DSMB should follow and complete the Causality Assessment Guidelines.

10.3 Secondary Infections Evaluation Committee

Because it is often difficult to determine if a secondary hospital acquired infection is present, a panel of 3 experienced ICU physicians will be used to evaluate and grade whether a secondary hospital acquired infection is present. These members include: Dr. John Mazuski, Professor of Surgery, Washington University School of Medicine; Dr. Anne Drewry, Assistant Professor of Anesthesiology and Critical Care Medicine; Washington University School of Medicine. Dr. Brian Fuller, Assistant Professor of Anesthesiolgy and Emergency Medicine. Washington University School of Medicine.

These 3 physicians will be blinded to patient treatment with CYT107 versus placebo. They will not be members of the investigative team but rather colleagues of the investigators. They will meet either in person or by teleconference once a month to judge secondary infections.

11. Procedures for Special Events

11.1 Patient Replacement Policy

For subjects that fail to reach the primary endpoint and for whom the dataset is considered as not evaluable (i.e. patients who have failed to complete at least two weeks of study drug treatment), the next randomization assignment will be assigned to the next patient to be enrolled on the study in order to reach a minimum of 10 evaluable subjects in all cohorts and the targeted sample size of 30 evaluable subjects overall..

11.2 Patients Lost to Follow-up

Participation is completely voluntary. At any time a patient and/or the PI may decide to discontinue study participation.

Every effort should be made to obtain information about patients who cannot be contacted for follow up. The research team should make reasonable attempts to collect at least minimum safety data on all randomized patients, to the extent of their abilities, in accordance with HIPAA regulations and IRB approval.

12. Adverse Events / Serious Adverse Events / Critical Events

12.1 Definitions

The following standard definitions for adverse events will be used:

Adverse event: An adverse event is any undesirable or untoward medical event (clinical or non-clinical e.g. abnormal laboratory/EKG/imaging results) which occurs in a patient or in a patient in a clinical trial, who is receiving a pharmacological product, and which is not necessarily causally related to the treatment.

Serious adverse event: A serious adverse event or reaction is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (the term “life-threatening” in the definition of “serious” refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.).
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital abnormality
- is medically significant (event that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition)

Unexpected Serious Adverse event: An unexpected adverse event is an event, the nature or severity of which is not consistent with CYT107 Investigator's Brochure (updated at least annually).

Critical event: The following events, occurring after the start of CYT107 and considered to be possibly or probably related to the study drug:

- Any grade ≥ 3 Adverse Event (clinical or biological)
- Rash ≥ grade 2
- Any diagnosis of lymphoma confirmed by a pathologist
- Any adenopathy compromising or threatening organ function (e.g. mediastinal adenopathy inducing a respiratory distress; inguinal adenopathy inducing an edema of the lower limb; or any adenopathy threatening skin breakdown).
- Splenomegaly that is accompanied by:
  1) Clinical hypersplenism
  2) Suspicion of a diagnosis of splenic infarct (left upper quadrant pain and imaging study compatible with the diagnosis)
  3) Splenic rupture

12.2 Reporting of Adverse Events

12.2.1 Clinical and Biological Adverse Events

Any Adverse Events occurring for the time period between the signature of informed consent and the End of Study Visit, spontaneously reported by the patient or observed by others, will be recorded in the CRF and reported to the DSMB, RevImmune and IRB.

12.2.1.1 Nature and severity

The records will describe the nature, and the severity, using term and severity categories of the DAIDs "division of aids table for grading the severity of adult and pediatric adverse Events, Version 2.0.

According to previous clinical studies, Injection site reaction is frequently less than 5X5 cm. In consequence, with the objective of precisely determine the occurrence of any Injection Site Reaction the DAIDS (Division of Acquired Immunodeficiency Syndrome) has been adapted for this adverse event as follows:

Table 2 Division of AIDS Table (DAIDS 2.0) Toxicity Scale

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Injection Site Pain or Tenderness *Report only one*	Pain or tenderness causing no or minimal limitation of use of limb	Pain or tenderness causing greater than minimal limitation of use of limb	Pain or tenderness causing inability to perform usual social & functional activities	Pain or tenderness causing inability to perform basic self-care function OR Hospitalization indicated
Injection Site Erythema or Redness[13] *Report only one* *> 15 years of age*	2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area AND Symptoms causing no or minimal interference with usual social & functional activities	≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social & functional activities	≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Symptoms causing inability to perform usual social & functional activities	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
≤ 15 years of age	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection Site Induration or Swelling *Report only one* *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*
≤ 15 years of age	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*
Injection Site Pruritus	Itching localized to the injection site that is relieved spontaneously or in < 48 hours of treatment	Itching beyond the injection site that is not generalized OR Itching localized to the injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA

[13] Injection Site Erythema or Redness should be evaluated and graded using the greatest single diameter or measured surface area.

12.2.1.2 Causality

Causality defined as the relationship between a study drug and an adverse event assessed according to chronological criteria, pharmacological properties of the drug and a search for other explanations or contributing factors. The causality is to be evaluated initially by the investigator, using the following likelihood scale:

None	Timing incompatible; appearance before study drug introduction, delay before appearance of adverse event too long, or symptoms appeared before event; or due to causes other than study drug administration (e.g. disease, environment etc.).
Unlikely	A clinical event with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.
Possible	A clinical event with a reasonable temporal relationship to drug administration, and which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Probable/Likely	A clinical event occurring in a reasonable temporal relationship to drug administration, unlikely to be due to concurrent disease or other drugs or chemicals. The response to withdrawal (dechallenge) should be clinically reasonable.

It will be specified whether the event is **Serious** or not and if the event is **Critical** or not according to the relevant definition and information regarding **Date of Onset, Date of Resolution**, **Actions Taken** and **Outcome**.

Ongoing adverse events should be reviewed at each subsequent assessment. If resolved, the details should be recorded in the CRF. If any AE changes for the worse, in frequency of attacks/symptoms or in severity, a new record of the event must be started (i.e. distinct reports are required for differing frequencies and/or severity of the same event to enable comprehensive safety reports and later analysis).

All adverse events, serious or causally related to the treatment or to the research protocol and still evolving at the end of the study are to be followed up until their resolution by the investigator.

12.2.2 Case of Routine Laboratory Measurements

A list of the laboratory normal ranges should be supplied at the start of the study by the implied laboratories or by the investigator to Revimmune.
The results of these will be in the CRF, and if they are abnormal a comment or explanation should be given, where possible using the following categories:

- abnormality not clinically significant (NCS)

- abnormality clinically significant, but normal for the study population (CS/N)
- abnormality clinically significant and is an adverse event (CS/AE)

It should be noted that all clinically significant abnormalities, not normal for the study population, count as Adverse Events even if they are not related to the use of study medication. An Adverse Event Form needs to be completed for all abnormalities falling into this category with all its items and the event must be followed in accordance to the same rules

12.3 Reporting Serious Adverse Events

All Serious Adverse Events will be reported to the institutional IRB and sent by e-CRF system, e-mail, or fax to the DSMB and Sponsor within 24 hours of being notified of the event using the SAE Report Form (Initial) in the Study Manual, whatever the relationship to the study drug. All information about the adverse event obtained later by the investigator (outcome, more precise medical details, results of investigations, copy of discharge summary etc.) is to be sent as soon as possible to the monitor completing an SAE Report Form (follow-up). When this information is passed on, care must be taken to continue to respect patient anonymity.

The medical monitor from the coordinating site may contact, or visit the investigator, in order to obtain details of the event.
Following collection of information the case should be reviewed separately by the investigator, by Sponsor, by the DSMB. All Serious Adverse Events must be followed by the investigator until resolution or stabilization, and a final assessment sent to Sponsor as a SAE Report Form (follow-up).

In addition, if the investigator becomes aware of the occurrence of an unexpected Serious Adverse Event/ reaction which appears after the end of the study, he or she should inform Sponsor and the institutional IRB following the same procedures described above.

SAEs should be reported to IRB/DSMB and FDA according to institutional and federal regulations.

12.4 Assessment of SAE Expectedness and reference document

The sponsor is responsible of assessing the expectedness of an AE according to the CYT107 Investigator's Brochure (latest version v7) used as the reference document.

12.5 Reporting of Critical Events

All Critical events have to be sent by e-CRF system, e-mail or fax to Sponsor within 24 hours of being notified of the event using the appropriate form (Critical Event report Form).
The medical Monitor may contact, or visit the investigator, in order to obtain details of the event. Following collection of information the case should be reviewed separately by the investigator, by Sponsor and by DSMB.

13.0 Collection and validation of data, and trial monitoring

13.1 Data Collection

Electronic Data Capture (EDC) will be used to collect individual data. An electronic Case Report Form (eCRF) is designed to contain information necessary for the evaluation of the patient and investigational agent. The eCRF must be completed in a timely manner following the visit and no more than a week after. The eCRF must be accurate and complete. All information required by the protocol is to be recorded in the eCRF based on source data. All REDCap data will be made available to the Sponsor in read-only format to review safety data and verify the validity and completeness of the forms.

13.2 Source Data

The investigator and the study nurse coordinator will identify any data that will be recorded directly on the CRF and considered as source data (i.e., no prior written or electronic record of the data). The study coordinator will document this on the study initiation report and revisit and document the use of the CRF as source documents as necessary during the course of the study.

13.3 Trial Monitoring

For the duration of the trial, the respective PI's at the 4 academic medical centers:

- Edward Sherwood MD, PhD; Professor of Anesthesiology, Vanderbilt University School of Medicine
- Richard Hotchkiss MD; Professor of Anesthesiology, Medicine, and Surgery, Washington University School of Medicine
- Lyle Moldawer PhD, Professor of Surgery, University of Florida, Gainesville, School of Medicine
- Elliott Crouser MD, Professor of Medicine, Ohio State School of Medicine

These investigators will ensure that all clinical monitoring, data management and data quality assurance follow local regulation and International Conference on Harmonization (ICH) Good Clinical Practices (GCP).
At the end of the study, the monitoring team will check that the investigators have all documents necessary for archiving.

13.4 Data Management/Statistics

Vanderbilt will be functioning as the CRO for the study and it will be responsible for all data management and statistical analysis. The data will be managed through the common database, i.e., Red Cap that is available to all academic centers in the United States.

The clinical data are sent from the clinical sites to the database via a web-based e-CRF and are stored on a server located at the CRO under ORACLE format. Once the clinical data is cleaned and the database is locked, the data will be transferred to SAS for statistical analysis at the CRO in charge of the analysis.

13.5 Quality Assurance

In accordance with local regulations and ICH Good Clinical Practice guidelines, the coordinating center may decide that the clinical trial should be audited in order to examine whether the quality control procedures are sufficiently and correctly adhered to. The audit can take place either at the clinical site or at any other trial drug evaluation site (e.g. laboratory). The investigator will be informed beforehand if an auditor (internal or external) is going to visit.

The local regulations and the ICH Good Clinical Practice guidelines also allow for an inspection by the health authorities. This inspection is similar to an audit but can take place either at the sponsor's or at the investigator's premises.

14.0 Ethical considerations

14.1 Informed Consent

Prior to entering the study, the investigator or designated co-investigator or study nurse, will explain to each patient or, in the case that the patient is not able to sign for himself/herself, their family member or legal authorized representative (LAR) the nature of the study, its purpose, procedures, expected duration, alternative therapy available, and the benefits and risks involved in study participation.

Patients/LAR will be provided a consent document and the opportunity to ask questions; information will be provided regarding voluntary participation and the ability to withdraw at any time from the study without prejudice. After this explanation and before any study-specific procedures have been performed, the patient or LAR will voluntarily sign and date an informed consent document.
The patient/LAR will be provided with a copy of the signed informed consent document. The original document will be maintained in the patient's research file, in accordance with all HIPAA and IRB regulations.

14.2 Potential Benefits of CYT107 to Patients with Sepsis

Sepsis is a disease with a high mortality for which there is no specific treatment other than traditional therapy of antibiotics and source control. The expected mortality in the septic patient population in the current study is approximately 40-45% at 60-90 days. Many of these deaths are due to failure to control the primary infection or development of new secondary infections. CYT107 reverses many of the sepsis-induced defects in host immunity and this improvement in host immunity will likely translate into decreased secondary infections, reduced ICU stay, and ultimately, improved survival. Although the present study is not powered to detect an improvement in survival, it is powered to demonstrate an effect of CYT107 to decrease new secondary infections which are a major cause of morbidity and mortality in sepsis.

Based upon data from both animal studies and clinical trials, we are extremely hopeful that CYT107 will improve morbidity and mortality in sepsis (12, 13, 14, 16, 18, 34). Studies from multiple independent laboratories using clinically relevant animal models of sepsis have shown that CYT107 improves survival (16, 18, 34). Finally, clinical trials of CYT107 in patients with cancer, bone marrow reconstitution, HIV, and JC virus induced progressive multi-focal

leukoencephalopathy have shown that CYT107 is well tolerated and efficacious. Clinical trials of CYT107 in oncology and in bone marrow reconstitution are being planned. Therefore, we believe that the risk benefit ratio is greatly in favor of use of CYT107 in patients with sepsis. As our group has argued passionately, we believe that CYT107 represents one of the most exciting immunotherapeutic agents in infectious disease in the last decade (6, 27, 39). If this proposed study is successful, it could radically change the way that physicians treat many infectious disorders including sepsis.

15.0 Regulatory considerations

15.1 Conditions for Conducting the Trial

15.1.1 Study Initiation Requirements

The protocol will have to be approved and signed by the representatives of the sponsor and by the investigators, as well as having been approved by each appropriate Ethics Committee or Institutional Review Board (IRB) in writing, before it can be initiated.

Prior to shipment of study drug, the investigator must provide Sponsor with at least the following documents:

- Signed final version of the protocol
- Curriculum vitae or other statement of qualification of the Principal Investigator (PI), any sub-investigator, and study coordinator.
- Ethics Committee/IRB approval letter, list of Ethics Committee (EC)IRB members if the PI is the contact of the EC
- Signed and dated Clinical Trial Agreement between Sponsor and the institution and the investigator
- Completed local forms as required (Pre-study Financial Disclosure, etc…)

15.1.2 Study Personnel

The investigator should maintain a list of appropriately qualified persons who are delegated to perform significant study-related duties. In addition, the investigator should maintain a study delegation sheet to document signatures and initials of all persons authorized to perform any research related duties.

All medically-related decisions should be performed by a person medically qualified.

Information to the patient and/or LAR prior to consent should be given by a qualified physician or nurse member of the research team.

The PI is responsible for informing the patient's attending physician(s) when a patient has provided informed consent for study participation.

15.2 Protocol Amendments

Neither the investigators nor Revimmune can modify the study protocol without mutual agreement. Any change must be made following GCP and must be approved by the appropriate

Research Ethics Committee and Regulatory Authorities before implementation, with 2 exceptions:

- when necessary to eliminate apparent immediate hazard to the patient
- when the change involves logistical or administrative aspects of the study

15.3 Study Funding

The costs necessary to perform the study will be agreed with the investigator and/or the management of the study facility, and will be documented in a separate financial agreement that will be signed by the investigator and/or the management of the study facility Sponsor and any other Financial Co-Sponsor of the trial.

16. Reporting and publication

16.1 Confidentiality Agreement

Investigators agree to hold confidential all information regarding the product being tested, including the study methodology, and information developed during the course of this study, unless the information has previously been published. Investigators will ensure that all employees respect the same rules.

17. Archiving

The investigator is to keep the identification lists of patients for at least 15 years after the completion or premature termination of the study. The medical records of the patients in the trial together with other data are to be kept for as long as the hospital, or institution allows, but for a minimum of 15 years or according the local regulatory requirement.

The sponsor or subsequent owner is required to keep all other documentation for the life of the product studied. The archived data can be kept in electronic form, provided that a back-up copy is kept and that a paper copy can be provided if necessary.

The protocol, ethical and government approvals, together with all other documents concerning the study, including any audit and inspection certificates are all to be kept as part of the trial master reference file. All data about adverse events also needs to be kept in this trial master file.

All data should be available for inspection by the appropriate authorities on demand.

Appendices:

Appendix 1: Good Clinical Practice

ICH Topic E6

Guideline for Good Clinical Practice

The document is available on the following web site:
http://www.emea.eu.int/pdfs/human/ich/013595en.pdf

A copy of 'ICH Topic E6 Guideline for Good Clinical Practice', step 5 consolidated guideline will be provided in the study manual.

Appendix 2: Flow Chart

IL-7 admin in 5 patients "high frequency dosing"

IL-7 admin in 5 patients" low frequency dosing"

flowchart / roadmap

Placebo 5 Controls	SCREEN			STUDY PERIOD																																Post Study FollowUp			
	screen			Week 1							Week 2							Week 3							Week 4							Week 5			Week 6			W9	W13	W26	W52
	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	32	35	36	39	42	60	90	180	360
CYT107 ADMINISTRATION HIGH				X			X				X			X				X			X				X			X				+/- 4 days						+/- 1 week			
CYT107 ADMINISTRATION LOW				X			X				X			P				X			P				X			P													
PLACEBO				P			P				P			P				P			P				P			P													
ALC primary end point	X		X	X			X				X			X				X			X				X			X				X					X				
Informed Consent			X																																					
physical clinical incldg weight			X				X				X							X							X							X									
APACHE II score			X				X				X							X							X							X									
SOFA score			X				X				X							X							X							X									
GLASGOW Coma Scale			X				X				X							X							X							X									
Cardiac																																									
Vasopressors			X				X				X							X							X							X									
EKG 12 leads 3hrs post CYT107			X	X																					X															
Splenic Ultrasound Echo			X																													X									
EKG MAP HR			X				X				X							X							X							X									
respiratory																																									
oxygen saturation Pa O2			X				X				X							X							X							X									
FiO2 (+ A-a Grad if FiO2 ≥ 0.5)			X				X				X							X							X							X									
kidney function																																									
blood chem including CRP			X				X				X							X							X							X									
creatinine			X				X				X							X							X							X									
urine analysis			X				X				X							X							X							X									
liver function																																									
ALT			X				X				X							X							X							X									
AST			X				X				X							X							X							X									
Bilirubin			X				X				X							X							X							X									
hematology																																									
white blood cell count			X				X				X							X							X							X						X			
absolute neutrophil count			X				X				X							X							X							X						X			
absoulte monocyte count			X				X				X							X							X							X						X			
platelet count			X				X				X							X							X							X						X			
D dim PT, PTT			X				X				X							X							X							X									
Research Labs Only																																									
CD3, CD4, CD8 T cell counts			X				X				X							X							X							X									
monocyte HLA-DR expression			X								X							X							X							X									
whole blood TNF-α production			X								X							X							X							X									
CYT107PK at specif time points				X6	X1																				X6	X1															
CYT107 antibodies befor admin				X										X											X													X		X*	X*
Follow ups Health status survey																																						X	X	X	X

Blood chemistries, CRP, creatinine, ALT, AST, hematologic values, and EKG will only be ordered if not already performed as part of routine ICU care by primary ICU physicians X if positive at D60 or 180

	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	32	35	36	39	42	60	90	180	360
	screen			Week 1							Week 2							Week 3							Week 4							Week 5			Week 6			W9	W13	W26	W52
	SCREEN			STUDY PERIOD																																		Post Study FollowUp			

Appendix Table 3: Correlates of Immune Response to CYT107

New secondary infections	Yes/No	# Days after sepsis onset	Infection site	Microbiology	
Recurrence of primary infection	Yes/No	Site of infection			
Readmission to ICU	Yes/No	Reason for readmission	# Days following ICU discharge		
Days in ICU					
Days on Ventilator					
ICU disposition	Survive/Die	Discharge Home	Discharge LTAC	Discharge Nursing home	Discharge Rehab facility
Mortality	(28 days):	(60 days):	(90 days):	(365 days):	

Appendix 4: References

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