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Dompé pha.r.ma s.p.a.

CLINICAL STUDY PROTOCOL - CONFIDENTIAL

Study Number: REP0104

Investigational Product: Repertaxin

Title: A phase 2, multi-center, randomized, double-blind, placebo-controlled, parallel-group study of repertaxin in the prevention of primary graft dysfunction after lung transplantation.

Protocol Version Final Revision No. 3

Date of protocol: 20 March 2006

This protocol results from revision of "Final Revised Protocol No. 2 (30 August 2005)" according to Amendment No. 2 dated 20 March 2006.

STATEMENT OF CONFIDENTIALITY

CONTACT INFORMATION

SPONSOR	Dompé pha.r.ma s.p.a. [Dompé] Via Campo di Pile; 67100 L'Aquila, Italy Milan Offices: Via S. Martino 12-12/A, 20122 Milan, Italy Phone: +39 02 583831; Fax: +39 02 58383324
Medical Director, Coordinator	PPD
Clinical Trial Manager	PPD
CLINICAL RESEARCH ORGANIZATION	PPD
Project Leader	
Pharmacovigilance Contact	
PRIMARY INVESTIGATOR	PPD

INVESTIGATOR AGREEMENT SIGNATURES:

CLINICAL CENTER: PRINCIPAL INVESTIGATOR

I have read study protocol REP0104 "A phase 2, multi-center, randomized, double-blind, placebo-controlled, parallel-group study of repertaxin in the prevention of primary graft dysfunction after lung transplantation" and agree to conduct the study as outlined in the protocol, and in accordance with ICH-GCP and any local regulations.

Name of Principal Investigator: _____

Signature: _____ Date: _____

PROTOCOL APPROVAL SIGNATURES:

DOMPÉ

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Signing of this page of the protocol by the Sponsor and CRO indicates that the protocol format and content have been agreed and approved by both parties, and that it is agreed that no further changes to the protocol are required at the time of signing.

A photocopy of this fully signed page will be provided to all participating Investigational Sites for filing.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ALT	Alanine Aminotransferase
AE	Adverse Event
ADR	Adverse Drug Reaction
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BAL	Bronchoalveolar Lavage
BOS	Bronchiolitis Obliterans Syndrome
BP	Blood Pressure
°C	Degrees Celsius
CLcr	Calculated Creatinine Clearance (Cockcroft - Gault formula)
Cmax	Maximum Plasma Concentration
CO	Cardiac Output
CRA	Clinical Research Associate
CRF	Case Report Form
Css	Steady State Concentration
CVP	Right Atrial Pressure
CXCL8	CXC ligand 8 [formerly interleukin (IL)-8]
DGF	Delayed Graft Function
DMC	Data Monitoring Committee
EMEA	European Medicines Evaluation Agency
ESRD	End Stage Renal Disease
FDA	Food and Drug Administration
FEV_1	Forced Expiratory Volume in One Second
FiO_2	Fraction of Inspired Oxygen
FVC	Forced Vital Capacity
g	Grams
GCP	Good Clinical Practice
HR	Heart Rate
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IND	Investigational New Drug
IRB/REB	Institutional Review Board/Research Ethics Board
ISHLT	International Society of Heart and Lung Transplantation
i.v.	Intravenous
kg	Kilogram
mg	Milligram
mL	Millilitre
mmHg	Millimeters mercury
ng	Nanogram
NSAID	Non Steroidal Anti-Inflammatory Drug
PA	Pulmonary Artery
PaO_2	Partial Pressure of Arterial Oxygen
PCWP	Pulmonary Capillary Wedge Pressure
PEEP	Positive End Expiratory Pressure
PGD	Primary Graft Dysfunction
PMN	Polymorphonuclear leukocyte
p.o.	per os (taken by mouth)
PVR	Pulmonary Vascular Resistance
SAE	Serious Adverse Event
s.c.	Subcutaneous
SOP	Standard Operating Procedure
SpO_2	Peripheral Oxygen Saturation
SVR	Systemic Vascular Resistance
t½	Elimination half life
µg	Microgram
UNOS	United Network for Organ Sharing

1. STUDY SYNOPSIS AND OVERALL DESIGN

Study title

A phase 2, multi-center, randomized, double-blind, placebo-controlled, parallel-group study of repertaxin in the prevention of primary graft dysfunction after lung transplantation.

Study Number REP0104

Study period Actual starting date (first-patient-in): 1 May 2005

Projected completion of patient accrual (last-patient-in): September 2006

Projected study end date (last-patient-last-visit): September 2007

Study design

The study will be a phase 2, multi-center, randomized, double-blind, placebo-controlled, parallel-group (two arms) study. It will involve 100 lung transplant recipients.

Objectives/endpoints

The objective of this clinical trial is to evaluate whether CXCL8 inhibition with repertaxin leads to reduced severity of PGD. The safety of repertaxin in the specific clinical setting will be also evaluated.

Primary efficacy endpoint will be: PaO_2/FiO_2 ratio measured on ICU admission and at 24 hours after ICU admission.

Secondary efficacy endpoints will be: the time profile of PaO_2/FiO_2 ratio (ICU admission, then q24 hours up to extubation or up to 72 hours; additional data obtained as per center standard of care will be collected in patients with clinical diagnosis of PGD up to the clinical recovery of the condition); PGD score (ICU admission, 24 hours, 48 hours, and 72 hours after ICU admission; in patients with clinical diagnosis of PGD additional scoring will be calculated up to the clinical recovery of the condition from available data); time to freedom from mechanical ventilation; duration of ICU stay; ICU mortality; mortality in the first 30 days post-transplant; FEV_1 and FVC at month 1, 6, and 12 post-transplant; BOS score at month 6 and 12 post-transplant; cumulative acute rejection episodes at month 6 and 12 post-transplant; patient survival rate evaluated at month 6 and 12 post-transplant. Any additional information from BAL/biopsies performed according to center standard of care in patients with clinical diagnosis of PGD will be collected up to the clinical recovery of the condition.

PMN count in BAL specimen obtained during mechanical ventilation, in the interval between 12 and 24 hours after reperfusion will be evaluated as a "mechanism of action-targeted" endpoint. Also, plasma levels of repertaxin and its major metabolite, DF2243Y, will be evaluated at steady state conditions (24 ± 6 hours and 48 hours from the start of infusion) and then in the interval 2-6 hours after the end of drug infusion.

Safety endpoints will be: AE recording; standard laboratory tests performed at screening and at hospital discharge; vital signs (BP & HR) evaluated at screening, at ICU admission and then q12 hours up to ICU discharge or up to 5 days after ICU admission; cardiopulmonary physiologic measurements (CO, PA pressure, SVR, PVR, PCWP, CVP), when PA catheter is

present, at ICU admission and then q12 hours up to ICU discharge or up to 5 days after ICU admission. Additional cardiopulmonary data obtained as per standard of care will be collected in patients with clinical diagnosis of PGD up to the clinical recovery of the condition.

Number of patients One-hundred patients undergoing single or bilateral lung transplant.

Inclusion/exclusion criteria

Patients accepted and listed for transplantation (planned isolated lung transplant from a non-living donor with brain death), ages 18-65 years, body weight 30-95 kg, given written informed consent will be included. Patient must have normal renal function (as per calculated creatinine clearance $\geq$ 60 mL/min).

Patients will be excluded if they are: recipients of an intended multiple organ transplant, including heart-lung and liver-lung transplantation; recipients of a lung from a living lobar donor or a lung from a non-heart beating donor; recipients of a re-do lung transplantation. Patients requiring mechanical ventilation at the time of transplant, or with extra-respiratory tract site of infection, or with hepatic dysfunction at the time of transplant, or with hypersensitivity to ibuprofen or to more than one NSAID, or to medications belonging to the class of sulfonamides will be excluded as well. Also, patients planned to receive Orthoclone OKT3 or Campath induction immunosuppression or planned to receive sirolimus in the first three months after transplantation, or simultaneously participating in any other studies involving a study drug to be administered concomitantly with the Investigational Product and/or a study drug intended to prevent ischemia/reperfusion injury will be excluded. Pregnant or breast feeding women will be excluded.

Investigational drug

Repertaxin: 33 mg/mL aqueous injectable solution of repertaxin (per single 10 mL ampoule).

Placebo: 9 mg/mL aqueous injectable solution of sodium chloride (NaCl) (per single 10 mL ampoule)

For each 24 hour administration period, a dosing solution will be prepared from the contents of 23 ampoules (230 mL), diluted with 370 mL of 0.9 % sterile saline.

The study drugs will be administered as a continuous i.v. infusion into a (high flow) central vein, by an infusion pump, starting approximately 2 hours before reperfusion of the (first) transplanted lung occurs. An initial 'loading dose' of repertaxin of 4.488 mg/kg body weight/hour will be administered over 30 min followed by a maintenance dose of 2.772 mg/kg body weight/hour lasting 47.5 hours. Placebo will be volume matched saline. Total infusion volume will not exceed 500 mL/24 hours.

Statistics

Data for the double-blind (main) phase of the study (up to 30 days post-treatment) will be presented in the clinical study report. Data collected during the open phase of the study (from 1 month up to 12 months) will be presented in an addendum to the clinical study report.

Appropriate descriptive statistics will be produced, according to the variable.

The primary endpoint will be analyzed by ANOVA and separate comparisons at two time points (0 hours on ICU admission and 24 hours after ICU admission).

Appropriate descriptive statistics will be produced for all secondary endpoints. Inferential tests will be applied only to PaO_2/FiO_2 ratio, PGD Score, time to freedom from mechanical ventilation and duration of ICU stay.

Safety data will be presented with the appropriate descriptive statistics.

2. BACKGROUND INFORMATION

Repertaxin (DF1681Y) is a specific inhibitor of CXC ligand 8 [CXCL8; formerly interleukin (IL)-8] biological activity, stemming from a program of drug design of molecules intended to modulate chemokine action.

Repertaxin is the first low molecular weight blocker of CXCL8 biological activity in clinical development.

Dompé is currently committed to evaluating the clinical use of repertaxin in the prevention of Delayed Graft Function (DGF) after solid organ transplantations, a clinical condition that can be considered a paradigm of ischemia/reperfusion injury.

Repertaxin received the orphan drug designation by the European Committee of Orphan Medicinal Products in September 2001 and by the Food and Drug Administration (FDA) in January 2003 for such clinical condition. In the 4th quarter of 2003, Dompé has obtained protocol assistance by the European Medicines Evaluation Agency (EMEA) for the development of repertaxin, and a pre-Investigation New Drug (IND) meeting was held with the Food and Drug Administration (FDA) to discuss IND submission.

Relevant pre-clinical, toxicological and phase 1 clinical data are summarized below. Please also refer to the Investigator's Brochure for more detailed information.

As mentioned in the Investigator's Brochure, all previous non clinical and clinical (phase 1) studies were performed with the lysine salt of repertaxin. Repertaxin (free acid) formulated in a lysine buffer will be used for this phase 2 Clinical Trial. In order to allow a readily-accessed information and easy understanding of the study protocol, any doses of the experimental drug mentioned in this protocol are reported as repertaxin.

2.1. RELEVANT PRE-CLINICAL RESULTS

Repertaxin is *in vitro* a potent and specific inhibitor of CXCL8 biological activity. *In vitro* chemotaxis experiments have shown that repertaxin inhibits CXCL8-induced chemotaxis of human polymorphonuclear leukocytes (PMN) in the low nanomolar range. Studies to elucidate the mechanism of action have shown that repertaxin is a non-competitive allosteric inhibitor of the CXCL8 receptors CXCR1 and CXCR2. Interaction of repertaxin with CXCL8 receptors inhibits the intracellular signal transduction events activated by binding of CXCL8 to CXCR1 and CXCR2 [*Bertini, 2004; Souza, 2004*].

In vivo, treatment of rats with repertaxin lysine salt prevented PMN infiltration into the reperfused transplanted kidney and reduced kidney damage, as assessed by an increase in serum creatinine levels. Moreover, repertaxin prevented PMN infiltration and tissue damage in animal models of ischemia/reperfusion injury of liver, brain, intestine, heart and spinal cord. In these models, repertaxin inhibition of PMN recruitment ranged from 40 to 90%, and inhibition of functional damage ranged from 50 to 80%. Efficacy was seen in all models at repertaxin dose of 9.90 mg/kg.

More recently, repertaxin lysine salt has been evaluated in a rat model of lung transplantation. Doses of repertaxin of 9.90 and 19.80 mg/kg given i.v. 15 min before and s.c. 2 and 4 hours

after the reperfusion, reduced PMN infiltration, along with organ damage (lung edema) and functional impairment (decreased PaO_2) induced by ischemia/reperfusion.

The pharmacokinetics of repertaxin was evaluated after i.v. or p.o. single dose in rats, after i.v. single dose in dogs and after i.v. repeated bolus or i.v. continuous infusion as part of toxicity studies in both species. In rats, repertaxin is rapidly eliminated with a half-life ($t_{1/2}$) of between 0.5 and 3 hours. It is eliminated mainly by metabolism with negligible amounts of parent compound excreted in the urine. Pharmacokinetics were linear over a wide range of doses in rats. In dogs, a different pharmacokinetic profile was observed, i.e. repertaxin was less rapidly eliminated ($t_{1/2}$ 12-28 hours) both after single bolus i.v. administration and i.v. continuous infusion.

Metabolic studies indicated the presence of 8 and 10 metabolites in rats and dogs, respectively. Ibuprofen is an expected minor metabolite.

Repertaxin inhibited *in vitro* the isoenzymes CYP3A4 and, to a minor extent, CYP2C19. A phase I metabolism study of [^{14}C]-repertaxin indicated that repertaxin is catalysed by CYP2C9 and to a lesser extent by CYP2C19 with the production of two major metabolites.

In vitro protein binding of [^{14}C]-repertaxin showed that repertaxin is highly bound (approximately 99%) to plasma proteins in rats, dogs, rabbits, cynomolgus monkeys and humans. Albumin is likely to be the major binding protein in plasma in all species.

2.2. A SUMMARY OF TOXICOLOGY DATA

Repertaxin was tested for toxicity in rodent and non-rodent animal species after single and repeated i.v. doses. The repeated dose administration studies were conducted by i.v. continuous infusion, according to the foreseen human administration route.

The general toxicological profile of i.v. repertaxin, in the studies conducted to date, is characterized by a low toxicity after single or repeated dose administrations in rats (LD_{50} = 229.68 mg/kg i.v.; 660.00 mg/kg/day as No Observed Adverse Effect Level from 4 weeks studies) and mice (401.94 mg/kg i.v.). Continuous i.v. administration to dogs for 2 weeks resulted in a safe dose of 39.60 mg/kg/day.

Continuous i.v. infusion of repertaxin to the male and female rat at dose levels of up to 660.00 mg/kg/day did not have any significant adverse effects on mating performance and fertility.

Repertaxin poses no genotoxic hazard for humans.

Repertaxin lysine salt, at doses in excess of those intended to be used in humans, has a safe pharmacology profile in the renal, cardiovascular and respiratory systems of rats and dogs.

The local tolerability of repertaxin lysine salt was assayed in the rabbit ear lateral vein. The compound was well tolerated in concentrations up to 4.95 mg/mL (1 mL/kg) infused over a minute.

In order to provide evidence of the safety of DF2243Y, the main metabolite of repertaxin excreted in urine in humans, safety pharmacology and toxicity studies have been implemented

at doses 2 to 3 times higher that those reached in man, as may occur during the treatment of patients receiving kidney transplantation.

2.3. A SUMMARY OF PHASE 1 DATA

Four phase 1 pharmacokinetics/safety studies have been implemented: three healthy volunteers studies have been completed, and one study is ongoing in patients with chronic renal disease.

In the first study CCI, single doses of repertaxin of 0.66 to 10.56 mg/kg were administered by 30-min i.v. infusion. Each dose group consisted of 4 subjects treated with repertaxin and 2 with placebo. The compound was well tolerated at all doses, with minor and unspecific adverse events that were not dose-related. Repertaxin peak plasma concentrations were achieved at approximately 30 min post-dose. Linear kinetics were observed over the dose range of 0.66-5.28 mg/kg. Repertaxin was eliminated from plasma with a terminal half-life ranging from 0.94 to 1.28 hours. Total body clearance and volume of distribution appeared to be independent of dose. Unchanged repertaxin was unmeasurable in urine and a very small amount was excreted in the feces over 72 hours.

In a second study CCI repertaxin was administered as 48-hour i.v. infusions of doses targeted to achieve repertaxin plasma steady state concentrations (Css) of 10, 20 and 30 μg/mL. Three dose levels were administered: 2.05 mg/kg/h x 30 min followed by 0.66 mg/kg/h x 47.5 h; 2.51 mg/kg/h x 30 min followed by 1.32 mg/kg/h x 47.5 h; and 4.49 mg/kg/h x 30 min followed by 2.77 mg/kg/h x 47.5 h. Twelve subjects (9 active, 3 placebo) were dosed in each dose level. These treatment schedules were chosen in order to closely mimic the possible dose regimen to be used in efficacy trials. Plasma levels of repertaxin, and its metabolites ibuprofen, DF2243Y and DF2188Y were determined. Repertaxin free fraction appeared to increase with total concentration. Elimination from plasma was rapid with terminal half-life of approximately 1.2-1.5 hours. The pharmacokinetics of repertaxin did not appear to be linear, with total clearance and volume of distribution increasing with dose, while maximum plasma concentration (C_{max}) and area under the curve (AUC) appeared to be less than dose proportional. Repertaxin was metabolized and excreted into urine as ibuprofen (about 1% of the dose), DF2243Y (30-40% of the dose), DF 2188Y (18-25% of the dose) and methanesulfonamide (10-12% of the dose). Renal clearance for repertaxin and ibuprofen was dose-dependent, but not for DF2243Y and DF2188Y. The total recovery of metabolites in urine accounted for 60-75% of the administered dose. Repertaxin and ibuprofen were detectable in the feces over 60 hours at very low amounts and only in few subjects. The metabolite DF2188Y had a terminal half-life similar to repertaxin, while terminal half-lives for ibuprofen and DF2243Y were longer than that for repertaxin, suggesting elimination-rate limited pharmacokinetics for these two metabolites. Overall, the pharmacokinetic profiles of repertaxin and its major metabolites are similar in rats and humans. Repertaxin was well tolerated again, resulting in adverse events that were minor and not dose related. The local reactions observed have been reduced by administering a more dilute solution at a higher infusion rate. The study showed that, in healthy volunteers, the proposed dose regimen that gives rise to target concentrations expected to be clinically effective is feasible, reproducible and safe.

A third study CCI was performed to verify whether repertaxin, at plasma concentrations equal to or exceeding that thought to be required for therapeutic efficacy,

could affect drug metabolism through CYP3A4 and CYP2C9 in a clinically significant manner. No clinically significant pharmacokinetic interaction was observed between repertaxin and midazolam or tolbutamide, probe substrates for CYP3A4 and CYP2C9 respectively. Repertaxin free fraction appeared to increase by at least 50% over the infusion period, after midazolam/tolbutamide co-administration, while total repertaxin concentrations remained stable.

The fourth study design CCI was implemented to obtain initial information on the safety and pharmacokinetics of repertaxin in subjects with renal impairment by exposing a very few subjects with the most severe degree of renal impairment, i.e. patients with end stage renal disease (ESRD) undergoing hemodialysis (stage A) to a limited dose of the compound. After determining appropriate dosing for renal failure, a more complete evaluation of the safety and pharmacokinetics of repertaxin was obtained by administering the appropriate dose to a representative number of subjects with various degrees of renal impairment and to male and female healthy volunteers (stage B). A dose of 1.32 mg/kg/h for 6 hours by i.v. continuous infusion is being administered in both ESRD patients and healthy volunteers. Results obtained indicate that the pharmacokinetics profile of repertaxin is not influenced, as expected, by the degree of renal function. On the other hand, renal function had a profound effect on plasma concentrations of the two major metabolites, which were found to increase over time along with the increase of the elimination half-life. Based upon the limited number of subjects there appears to be no apparent gender differences in the pharmacokinetics profile of repertaxin and its metabolites. Repertaxin was well tolerated also in ESRD patients. Very few adverse events were reported, the majority of which was mild in intensity and unlikely due to repertaxin.

2.4. DISEASE REVIEW AND STUDY RATIONALE

Lung transplantation has become a standard therapy for patients with end-stage lung disease. Within last decades, donor management, organ preservation, immunosuppressive regimens and control of infectious complications have been substantially improved. In addition, the operative techniques of transplantation procedures have been developed to an international standard of high quality [*Lau & Patterson, 2003*].

However, despite these refinements, significant reperfusion injury occurs in up to 10-20% of lung transplant recipients as the consequence of unavoidable processes of procurement, preservation and restoring blood flow. This clinical condition, recently termed primary graft dysfunction (PGD), remains an important problem after lung transplantation [*Christie, 1998; King, 2000*], and still represents the single biggest cause of early morbidity and mortality for lung recipients [*Fischer, 2001; Thabut, 2002*]. In addition, there is some evidence to suggest a relationship between reperfusion injury, acute rejection, and the subsequent development of chronic graft dysfunction [*Waddell, 1996; Fiser, 2002*].

Prediction of PGD is made difficult by the complexity of the interactions between the donor lung and the recipient [*Sommers, 1996; McRae, 2000*].

In post-ischemia reperfusion, restoration of the blood supply (reperfusion) after prolonged tissue ischemia is associated with an inflammatory reaction characterized by massive polymorphonuclear neutrophil infiltration into the reperfused tissue [*de Perrot 2003*]. The infiltrating inflammatory cells can perpetuate the initial inflammatory reaction and induce further injuries.

CXCL8 is a member of a class of chemokines involved in leukocyte recruitment and activation in tissues [*Baggiolini, 1994*]; this chemokine is believed to play a key role in the recruitment and activation of polymorphonuclear neutrophils in post-ischemia reperfusion injury [*Welbourn, 1991; Lefer, 1991; Matsumoto, 1997*]. The importance of CXCL8 in lung tissue during the ischemic time and after reperfusion has been clearly demonstrated. Reperfusion of ischemic lung in a rabbit model of lung reperfusion injury caused neutrophil infiltration and tissue damage, as well as a local production of CXCL8. The administration of a neutralizing monoclonal antibody against CXCL8 prevented neutrophil infiltration and tissue injury, providing a causal role of locally-produced CXCL8 in this model [*Sekido, 1993*]. The release of CXCL8 early after reperfusion could initiate neutrophil recruitment and thus induce secondary lung injury [*de Perrot, 2002*].

As a result of these findings, the modulation or inhibition of CXCL8 activity is considered a valid target for the development of innovative treatments for a variety of severe clinical conditions, including ischemia/reperfusion injury occurring after solid organ transplantation.

No currently available pharmacological treatments are intended to cure or to prevent the occurrence of ischemia/reperfusion injury after lung transplantation. The current standard of care in preventing this clinical condition focuses on prevention by way of surgical techniques in the procurement, storage and implantation of graft lungs.

The efficacy of repertaxin in preventing polymorphonuclear neutrophil infiltration and tissue damage in rat models of kidney transplantation and lung transplantation, as well as the safety shown in human phase 1 studies, provide the rationale for a clinical study aimed at evaluating the effect of repertaxin in preventing PGD after lung transplantation.

2.4.1. Selection of dose and treatment schedule in the study

"In vitro", repertaxin inhibits CXCL8-induced chemotaxis of human PMN with an IC_{50} of 1 nM, corresponding to about 0.3 ng/mL.

CXCL8 is a key mediator of PMN infiltration (and hence of tissue damage) in post-ischemia/reperfusion injury. In animal models of ischemia/reperfusion, CXCL8 (and corresponding proteins in animals) is upregulated in the reperfused tissue within minutes from reperfusion, peaks after 4-6 hours and drops to undetectable levels within 24-48 hours from reperfusion [*Sekido, 1993; Yamaguchi, 2000; Miura, 2001; Morita, 2001; DeVries, 2003*].

Repertaxin lysine salt was tested in several animal models of ischemia/reperfusion. Repertaxin was administered either by repeated bolus treatments (s.c. and/or i.v.) or by continuous i.v. infusion and was paired with observations of PMN infiltration and assessment of tissue damage (routinely 24 hours after reperfusion). In both circumstances, repertaxin treatment was started before reperfusion occurred. For convenience and ease of treatment of animals, most experiments were carried out by repeated bolus treatments.

In repeated bolus treatments, repertaxin was most efficacious in inhibiting PMN infiltration and reducing tissue damage in the reperfused tissue at doses ranging from 9.90 to 19.80 mg/kg. Furthermore, there was no overt increase in efficacy at doses of 19.80 vs. 9.90 mg/kg. At the dose of 9.90 mg/kg, C_{max} of total plasma repertaxin was 34 µg/mL, corresponding to 68 ng/mL of protein unbound (free) repertaxin.

In continuous i.v. infusion animal experiments, repertaxin was found to be efficacious at total plasma concentrations of 15 and 3.6 μg/mL, corresponding to 140 and 10 ng/mL of protein unbound (free) repertaxin, respectively. Repertaxin was less efficacious at 0.5 μg/mL total repertaxin, corresponding to approx. 1-2 ng/mL of protein unbound drug.

Although repertaxin was efficacious in animals when given either by repeated boluses or by continuous i.v. infusion, the proposed treatment in humans will be by continuous infusion in order to expose target cells (polymorphonuclear neutrophils) to steady state concentrations in the pharmacological range, thus maximizing the pharmacological activity. It should also be emphasized that repertaxin is a reversible inhibitor of CXCR1/2 activation induced by CXCL8 and that repertaxin has a short plasma half life (0.95 to 1.28 hours in humans).

The proposed dose in this clinical study was found safe in previous phase 1 studies and should produce total plasma concentrations of 30 μg/mL, corresponding to 30 ng/mL of protein unbound (free) repertaxin. Thus, the target plasma concentration of repertaxin obtainable in patients with the proposed dose is in the range found efficacious in both *in vitro* studies of CXCL8 inhibition and in animal models of ischemia/reperfusion. The proposed schedule of administration (48 hours) is intended to expose the patient to repertaxin for the lag time when CXCL8 is believed to be upregulated in ischemia/reperfusion [*Sekido, 1993; Yamaguchi, 2000; Miura, 2001; Morita, 2001; DeVries, 2003*].

2.4.2. Alternative treatments

Because there is no other standard of care pharmacologic agent to prevent or treat PGD, patients not willing to participate in the study will not be offered any specific alternative treatment for the prevention of ischemia/reperfusion injury. All patients, regardless of study participation, will receive the standard of optimal care for the transplanted organ (procurement, storage, reperfusion) and the recipient.

Treatment of lung dysfunction, including the use of supportive measures, as well as overall management of lung transplant recipients will be the same, regardless of participation in the study.

2.4.3. Risk - benefit evaluation

2.4.3.1. Treatment-related risks

Results from preclinical studies support the level of drug exposure planned in this study. Furthermore, the same dose regimen that is proposed for this study has been tested in healthy volunteers without observing any significant, systemic adverse events.

The risk of toxicity at the infusion site, reported in a cohort of volunteers administered by i.v. infusion via a peripheral vein, will be minimized by administering the experimental drug via a high flow central vein.

2.4.3.2. Central line

The study drug will be administered as a continuous i.v. infusion over 48 hours with a catheter inserted in a (high flow) central vein. The risks of a central line are not considered

study-related risks since preliminary survey reveals that central line will be routinely placed in all patients undergoing lung transplantation for their clinical care. This clinically required access line will be maintained for a period of time that is longer than that required for the administration of the Investigational Product. Central line care as routinely practiced by the participating sites will minimize the risk of thrombosis and infections.

2.4.3.3. Bronchoalveolar Lavage

Broncoalveolar lavage (BAL) is an invasive procedure used to monitor emerging problems in lung transplant recipients. A BAL specimen will be obtained from all study patients on mechanical ventilation, in the interval between 12 and 24 hours after reperfusion, to explore the repertaxin effect in reducing target cells polymorphonuclear neutrophil trafficking. To minimize the additional risk of the study specific BAL, the procedure will not be performed in patients with any clinical conditions which would be worsened by BAL procedures, according to the Investigator's judgment.

2.4.3.4. Blood sampling

In addition to the routine blood sampling, participation in the study will require three pharmacological blood samplings (18 mL total), to determine plasma concentrations of repertaxin (total and unbound) and its major metabolite, DF2243Y, in the specific clinical setting. Blood sampling will be performed at steady state conditions (24±6 hours and 48 hours from the start of infusion) and then in the interval 2-6 hours after the end of infusion. To maintain the study blind, blood samples will be obtained from all patients recruited in the study.

Moreover, the study will require additional blood samplings at 12, 24, and 36 hours after the beginning of Investigational Product administration, to ensure that renal and hepatic function are compatible with the ongoing investigational treatment.

2.4.3.5. Other study related procedures

No other procedures specifically related to the study are required. The specific timing of some measurements such as blood gas measurements will be guided by the protocol, but such measurements are firmly entrenched as part of standard patient care and would undoubtedly be performed regardless of study participation.

2.4.3.6. Potential benefit

To the patients: It is quite likely that there will be no direct benefit to individual patients participating in the trial. Half the patients will be receiving placebo and will therefore obtain no benefit other than the potential benefit of increased scrutiny and monitoring that comes with being part of a clinical trial. The patients receiving the Investigational Product may possibly benefit with improved early lung function, but that is not certain.

To society: This study may identify a useful medication that will make lung transplantation safer and more effective for future recipients.

3. OVERALL STUDY DESIGN AND PLAN DESCRIPTION

3.1. STUDY DESIGN

The study will be a phase 2, multi-center, randomized, double-blind, placebo-controlled, parallel-group (two arms) study.

It will involve 100 lung transplant recipients, randomly assigned in a 1:1 ratio to receive repertaxin or placebo, by continuous i.v. infusion for a period of 48 hours starting approximately 2 hours before reperfusion of the (first) transplanted lung occurs. The experimental treatment will be additional to the standard treatment of lung transplant recipients.

Recruitment will be competitive among the study sites, until the planned number of patients is enrolled. Competitive recruitment has been chosen to increase the speed of recruitment and to account for any difference in transplant rate among study sites.

Each patient will be involved in the study for 48 hour treatment, for measurements up to hospital discharge, and then for three visits (@ 1, 6, and 12 months post-transplant).

The double-blind will be maintained for the main part of the study only, i.e. up to the 1 month (at least 30 days post-transplant) follow-up visit of the last patient in. After database lock the randomization code will be broken and the results of the blind phase will be analyzed and the study will proceed in an open fashion.

3.2. STUDY TIME TABLE

- Actual starting date (first-patient-in): 1 May 2005
- Projected completion of patient accrual (last-patient-in): September 2006
- Projected study end date (last-patient-last-visit): September 2007

4. OBJECTIVES AND ENDPOINTS

4.1. STUDY OBJECTIVES

The objective of this clinical trial is to evaluate whether CXCL8 inhibition with repertaxin leads to reduced severity of PGD, as the result of improved functional and clinical outcomes in lung transplantation patients. The safety of repertaxin in the specific clinical setting will be also evaluated.

The ability of repertaxin to reduce target cells (PMN) infiltration into the graft will be evaluated to confirm its mechanism of action. Plasma levels of repertaxin and its major metabolite will be measured at steady state conditions to provide population pharmacokinetic profile in this specific clinical setting.

4.2. STUDY ENDPOINTS

4.2.1. Efficacy endpoints

4.2.1.1. Primary efficacy endpoint

- PaO_2/FiO_2 ratio measured at $FiO_2 = 1$ during mechanical ventilation on ICU admission (time 0) and at 24 hours after ICU admission. Patients on $FiO_2 < 1$ will be turned back to $FiO_2 = 1$ for 5 minutes before gas measurement.

4.2.1.2. Secondary efficacy (exploratory) endpoints

- Time profile of PaO_2/FiO_2 ratio measured at $FiO_2 = 1$ during mechanical ventilation at four potential time points: ICU admission (time 0) and then q24 hours, up to extubation or up to 72 hours after ICU admission, whichever occurs earlier. Additional data obtained as per center standard of care will be collected in patients with clinical diagnosis of PGD up to the clinical recovery of the condition.
- PGD score (stratified by single or bilateral transplant), evaluated according to the scoring system described by Christie et al. of the working group on primary lung graft dysfunction in the Scientific Council on Pulmonary Transplantation, International Society for Heart and Lung transplantation (*2005*; see Appendix 1 for methodological details). Specific planned comparison of PGD scores will be made at four time points: ICU admission (time 0), 24 hours, 48 hours, and 72 hours after ICU admission. In patients with clinical diagnosis of PGD additional scoring will be calculated up to the clinical recovery of the condition from available data as per center standard of care.
- Time to freedom from mechanical ventilation, defined as time between admission to the ICU and the initial time of first extubation (breathing off mechanical ventilation without a tube) which is maintained for more than 24 hours.
- Duration of ICU stay.
- ICU mortality, defined as vital status at the ICU discharge.
- Mortality, defined as any death occurring in the first 30 days post-transplant, regardless of hospital discharge.

- FEV_1 and FVC (stratified by single or bilateral transplant) measured at month 1, 6, and 12 post-transplant.
- BOS score (stratified by single or bilateral transplant) evaluated according to Estenne et al. *(2002)*. BOS score will be measured at month 6, and 12 post-transplant.
- Cumulative acute rejection episodes, biopsy proven, evaluated according to Yousem et al. *(1996)* at month 6 and 12 post-transplant.
- Patient survival rate evaluated at month 6 and 12 post-transplant.
- Any additional information from BAL/biopsies performed according to standard of care in patients with clinical diagnosis of PGD will be collected up to the clinical recovery of the condition.

4.2.2. Mechanism of action-targeted endpoints

- PMN count in BAL specimen obtained during mechanical ventilation, in the interval between 12 and 24 hours after reperfusion.

4.2.3. Pharmacokinetic endpoints

- Plasma levels of repertaxin (total and unbound) and its major metabolite, DF2243Y, at steady state conditions (24±6hours and 48 hours from the start of infusion) and then in the interval 2-6 hours after the end of study drug infusion.

4.2.4. Safety endpoints

- AE recording.
- Standard laboratory tests including hematology (hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count), and clinical chemistry (sodium, chloride, potassium, bicarbonate, serum creatinine, blood urea nitrogen, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), prothrombin time or international normalized ratio, partial thromboplastin time). Laboratory tests will be performed at screening and at hospital discharge.
- Vital signs, i.e. blood pressure (BP) and heart rate (HR), assessed at screening, on ICU admission, and then q12 hours up to ICU discharge or up to 5 days after ICU admission, whichever occurs earlier.
- Cardiopulmonary physiologic measurements, including cardiac output (CO), pulmonary artery pressure (PA pressure), systemic vascular resistance (SVR), pulmonary vascular resistance (PVR), pulmonary capillary wedge pressure (PCWP), right atrial pressure (CVP). Assessment will be performed on ICU admission, and then q12 hours when PA catheter is present, up to ICU discharge or up to 5 days after ICU admission, whichever occurs earlier. Additional cardiopulmonary data obtained as per center standard of care will be collected in patients with clinical diagnosis of PGD up to the clinical recovery of the condition.

5. STUDY POPULATION

One hundred patients, receiving lung transplantation, will be included in the study, each one being randomized to receive either repertaxin or placebo.

Patients will be selected from those on the lung transplant waiting list. Each prospective patient will be enrolled provided that (s)he fully meets all of the study Inclusion and none of the Exclusion Criteria described in Sections 5.1. and 5.2.

5.1. INCLUSION CRITERIA

To be eligible for inclusion into this study, each patient must fulfill the following inclusion criteria:

1. Patients accepted and listed for transplantation due to irreversible, progressive disabling, end-stage pulmonary disease.
2. Ages 18-65 years
3. Body weight 30 - 95 kg, inclusive (i.e. up to 95.99 kg).
4. Planned isolated (single and bi-lateral) lung transplant from a non-living donor with brain death. This includes lobar lung transplant involving excision and sizing of a cadaver donor lobe to meet the thoracic dimension of the recipient before being transplanted.
5. Normal renal function at the time of transplant as per calculated creatinine clearance $\geq$ 60 mL/min. Creatinine clearance will be calculated (CLcr) according to the Cockcroft-Gault formula (*1976*; see section 7.1).
6. Patient willing and able to comply with the protocol procedures for the duration of the study, including scheduled follow-up visits and examinations.
7. Patient given written informed consent, prior to any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to their future medical care.

5.2. EXCLUSION CRITERIA

To be eligible for inclusion into this study, each patient must <u>not</u> fulfill any of the following:

1. Recipients of an intended multiple organ transplant, including heart-lung and liver-lung transplantation.
2. Recipients of a lung from a living lobar donor.
3. Recipients of a lung from a non-heart beating donor.
4. Re-do lung transplantation.
5. Recipients requiring mechanical ventilation at the time of transplant.
6. Recipients with extra-respiratory tract site of infection (positive blood culture(s) and/or fever, associated with other signs of systemic sepsis syndrome). The criterion is not meant to exclude bacteremic cystic fibrosis patients with or without fever, unless they present with other signs of sepsis.
7. Recipients with hepatic dysfunction (bilirubin exceeding 3 mg/dL and/or transaminases >3X upper limit of normal) at the time of transplant.

8. Hypersensitivity to:
   a) ibuprofen or to more than one non steroidal anti-inflammatory drug (NSAID).
   b) medications belonging to the class of sulfonamides, such as sulfamethazine, sulfamethoxazole, sulfasalazine, nimesulide or celecoxib.
9. Patients simultaneously participating in any other studies involving a study drug to be administered concomitantly with the Investigational Product and/or a study drug intended to prevent ischemia/reperfusion injury.
10. Planned use of anti-CD3 monoclonal antibody (Orthoclone OKT3) or alemtuzumab (Campath) induction immunosuppression.
11. Planned use of sirolimus in the first three months after transplantation.
12. Pregnant or breast-feeding women. (NB: pregnancy should be avoided in patients or partners during the first month of participation in the study; no other specific warnings are described, considering even stricter general recommendations concerning pregnancy in transplanted patients, the treatment course of the Investigational Product, its pharmacokinetic profile, and the lack of significant adverse effects on mating performance and fertility in animal studies).

5.3. ASSIGNMENT OF PATIENT NUMBER

The randomization number will be assigned in a sequential manner as patients are found to be eligible for entry into the study and are enrolled. It will consist of the site number, and the patient number e.g. 0210, where first 2 digits represent site number, last 2 digits patient number. If a patient is dropped from the study for any reason, the patient's number will not be reassigned.

From the signing of the Patient Informed Consent Document until the allocation of a randomization number, patients will be identified by their initials and date of birth.

6. STUDY MEDICATION

Repertaxin is a new chemical entity with chemical name R(-)-4-Isobutyl-alpha-methylphenylacetyl-methanesulfonamide.

6.1. PRESENTATION, STORAGE, PACKAGING AND LABELING

6.1.1. Presentation of Investigational Products

In this study the Investigational Products will be repertaxin and placebo.

They will be provided as clear glass class I ampoules, each containing 10 mL of the following products:

Repertaxin: 33 mg/mL aqueous injectable solution of repertaxin with the following composition (per single 10 mL unit):

NAME OF INGREDIENT	PER-UNIT FORMULA	FUNCTION OF INGREDIENT	REFERENCE TO QUALITY STANDARDS
Repertaxin (DF 1681Y)	330 mg	Active substance	Manufacturer monograph and specification

[redacted]

Placebo: 9 mg/mL aqueous injectable solution of sodium chloride (NaCl) with the following composition (per single 10 mL unit):

NAMES OF INGREDIENT	PER-UNIT FORMULA	FUNCTION OF INGREDIENT	REFERENCE TO QUALITY STANDARDS
Sodium Chloride	90 mg	Isotonizing agent	European Pharmacopoeia - current edition
Water for injections	qs to 10 mL	Solvent	European Pharmacopoeia - current edition

Certificates of Analysis will be provided together with the Investigational products.

6.1.2. Receipt of drug at the center/Stability during shipment

No Investigational Product will be sent to the Investigator before receiving Institutional Review Board (IRB)/Research Ethics Board (REB) approval.

A temperature probe and data logger will accompany the drug on shipment. It is essential that centers read the graphical record on receipt in order to verify the temperature range reached

during shipment, so that potential stability concerns during shipment can be investigated and appropriate action taken.

6.1.3. Preparation of the Dosing Solution

The dosing solution for infusion will be prepared aseptically at the designated Pharmacy within each center.

For each 24 hour administration period, the contents of 23 ampoules (230 mL) will be diluted with 370 mL of 0.9 % sterile saline. This volume includes that needed for dead-space priming. Thus, repertaxin will be dispensed as a 12.65 mg/mL solution.

The dosing solution will be placed in a 1000 mL sterile empty infusion bag. Bags will be identified as Infusion Bag No. 1 (0-24 hour infusion) and Infusion Bag No. 2 (24 - 48 hour infusion).

Detailed instruction for the preparation of the dosing solution will be reported in the 'Instructions to the Pharmacy' provided with each 'Patient Box' of medication (see below).

Dosing solutions will be prepared and used within 72 hours from preparation.

6.1.4. Storage/Stability of Investigational Products

Investigational Products must be stored not above 30°C (86°F).

All Investigational Products must be stored in a secure location, preferably in a temperature controlled locked room, and may be dispensed only by the Pharmacist or by a member of staff specifically authorized by the Investigator, as appropriate. Any deviations from the recommended storage conditions should be immediately reported to PPD and the use of the drug suspended until authorization for its continued use has been given by Dompé.

6.1.5. Packaging/Labeling of Investigational Products

Investigational Products have been manufactured by CCI [redacted]

Current Repertaxin/Placebo ampoules have been packaged by CCI [redacted]. Any additional future supplies will be packaged by CCI [redacted], (formerly known as CCI [redacted] All study medication is provided as 'Patient Boxes'. Each Patient Box will contain 2 'Treatment Boxes' (Treatment Box No. 1 = 0-24 hour infusion; Treatment Box No. 2 = 24-48 hour infusion) of 25 ampoules each, 23 to be used for the preparation of the dosing solution, 2 to be kept as reserve. Two sterile empty bags (Infusion Bag No. 1 and Infusion Bag No. 2), plus an additional reserve bag, will be included in each Patient Box, along with the 'Instructions to the Pharmacy', a booklet detailing instructions for Investigational Product handling and preparation of the dosing solution.

If unused, the two ampoules in excess must remain in the Treatment Box. If they are used, the reasons for use must be documented.

Dosing solutions will be prepared at the center Pharmacy.

Current labeling of all study materials has been provided by CCI. Any future labeling will be provided by CCI. Labeling is prepared to meet local regulatory requirements.

Details of packaging and labeling are reported in Appendix 2.

6.1.6. Blinding

Investigational Products

Repertaxin and placebo will be packaged in identical containers (boxes, ampoules, bags) displaying the same information on the labels so as to preserve the study blind. Moreover, solutions of repertaxin and placebo, either in the ampoules or in the bag (dosing solutions), will be similar as per appearance and smell.

Nevertheless, study Pharmacists might not be fully blinded, since a transient foamy layer occasionally results from the preparation of the repertaxin dosing solution. Once the foamy layer, if any, bas subsided, the dosing solution of repertaxin will be indistinguishable from that of placebo.

Study blind

After database lock of data recorded in the main part of the study, corresponding to the 1 month follow-up visit of the last patient in, the blind code will be broken and the study will proceed in an open fashion.

6.2. DOSE, ROUTE AND SCHEDULE OF INVESTIGATIONAL PRODUCT ADMINISTRATION

According to the parallel-group design, patients will receive either repertaxin or placebo as determined by a previously generated randomization schedule.

An initial 'loading dose' of repertaxin of 4.488 mg/kg body weight/hour will be administered over 30 minutes followed by a maintenance dose of 2.772 mg/kg body weight/hour lasting 47.5 hours. Placebo will be volume matched saline.

The study drugs will be administered as a continuous i.v. infusion into a (high flow) central vein, by an infusion pump adequate to provide reliable infusion rates (see below), as per treatment schedule. Total infusion volume will not exceed 500 mL/24 hours.

The infusion will start approximately 2 hours before the anticipated time of reperfusion. For the purpose of this trial, reperfusion is defined as "the time of resumption of blood flow after vascular de-clamping of the first allograft". If cardiopulmonary bypass is used, the time of reperfusion is when the patient comes off the bypass, unless partial de-clamping occurs prior to complete stop of cardiopulmonary bypass. The surgeon will identify the time to start study drug infusion. In most instances, this time will be close to the time of the incision that begins the operation.

The loading and maintenance doses will be given using the same dosing solution (repertaxin 12.65 mg/mL), but the pump rate will be altered to provide an infusion rate of approximately

0.35 mL/kg/hour and 0.22 mL/kg/hour, respectively. Actual infusion rate (mL/hour), adjusted to body weight, is tabulated in Appendix 3. Figures in the appendix represent mathematical rounding of original infusion rates derived from the following formula:

$$\text{Infusion rate (mL/hour)} = \frac{\text{dose (mg/kg/hour) x body weight (kg)}}{\text{12.65 mg/mL}}$$

Such a rounding affects actual administered dose/kg/hour by less than 1%.

Technical characteristics of the infusion pump should include infusion rate covering the range from 6.6 to 31.9 mL/hour, in 0.1 mL/hour increments, and capacity in excess of 600 mL/day.

6.3. CRITERIA FOR SCHEDULE ADJUSTMENT/DOSE-MODIFICATION/ DISCONTINUATION OF INVESTIGATIONAL PRODUCT

6.3.1. Criteria for schedule adjustment or dose modification

No schedule adjustment and/or dose modification is foreseen, except for discontinuation of drug as detailed below.

6.3.2. Criteria for discontinuation of Investigational Product

Administration of the Investigational Product should be immediately discontinued in case the patient develops renal or hepatic dysfunction defined as:

renal dysfunction: the occurrence of both CLcr < 60 mL/min and urine output < 0.5 mL/kg/hour on two consecutive sampling time points (12, 24, 36 hours after the beginning of Investigational Product infusion).

hepatic dysfunction: bilirubin exceeding 3 mg/dL and/or transaminases >3X upper limit of normal).

Phase 1 studies in patients with ESRD have shown that renal function has a profound effect on plasma concentrations of a major, marginally active metabolite, carboxy-repertaxin (DF2243Y), which was found to accumulate over time along with the increased elimination half-life. Even if toxicological results to date suggest that DF2243Y does not raise any safety concern, limited experience in humans recommends to discontinue the drug in case of renal impairment as it would not be possible to predict the risk associated with elevated plasma levels. However, transient alterations of serum creatinine or urine output, as defined above, do not indicate a renal dysfunction that would cause impaired excretion of DF2243Y. Because repertaxin undergoes extensive hepatic metabolism, it is also recommended that the infusion is discontinued in case of hepatic dysfunction to avoid an increase of plasma level of repertaxin, exceeding the target and safe concentration.

CLcr, urine output, bilirubin and transaminases will be evaluated at 12, 24 and 36 hours after the beginning of Investigational Product infusion. Samples will be processed immediately and results made available as soon as possible to the Investigator. Urine output will be calculated for each of the 12 hour periods: from start of infusion to 12 hours, 12-24 hours, and 24-36 hours.

Investigational Product will also be immediately discontinued in the event of any other possibly drug related occurrences that the Investigator believes might compromise patient safety.

If the Investigational Product therapy is prematurely discontinued the primary reason for discontinuation must be recorded in the CRF. Patients who discontinue the treatment with the Investigational Product will not be withdrawn from the study by default, but will complete observations as per the protocol, unless otherwise withdrawn at the Investigator's decision.

6.4. ACCOUNTABILITY

All supplies will be maintained under adequate security by the responsible member of the Pharmacy staff.

The Investigator will ensure that study treatment is only administered by designated staff within the center. In particular, for U.S. sites, the Investigator will ensure that study treatment is only administered by those named as sub-investigators on the FDA form 1572 and designated staff.

When Investigational Product is received by the Pharmacist (or designee), he/she will check for accurate delivery and acknowledge receipt by signing and dating the documentation provided by or on behalf of PPD and returning it to PPD. A copy will be retained for the Investigator/Pharmacy file.

The dispensing of the Investigational Product will be carefully recorded on appropriate drug accountability forms (these will be provided by PPD) and an accurate accounting will be available for verification by the CRA at each monitoring visit.

Drug accountability records will include:

- confirmation of delivery of the Investigational Product to the trial site,
- the inventory at the site (ampoules/bags),
- the use by each patient,
- the return to Dompé or alternative disposition of unused product(s),
- accounts of any Investigational Product accidentally or deliberately destroyed,
- accounts of any reserve ampoules used.

They should include dates, quantities, batch numbers, expiration dates (if applicable), and any unique code numbers assigned to the Investigational Product(s) and/or patients. Investigators should maintain records which document adequately that:

- the patients were provided the doses specified by the protocol/amendment(s),
- all Investigational Product provided was fully reconciled.

The PPD will review the drug accountability forms and check all Investigational Product ampoules (both unused and used) prior to making arrangements for their return to Dompé, or authorizing their destruction by the study site.

Investigational Product which has been dispensed to a patient and returned unused will not be re-dispensed to a different patient.

Unused Investigational Product must not be discarded or used for any purpose other than the present study. Any remaining test material at the end of the trial will be returned to Dompé or disposed of, as determined by the Sponsor.

6.4.1. Assessment of compliance

Compliance will be assured by the person(s) within the center in charge of Investigational Product administration.

Immediately before the start of each 24-hours treatment period, the removable label on the Infusion Bag will be detached and attached to the relevant page of the CRF. Actual date and time of infusion start, switch of infusion rate (first 24 hours period only) and end of infusion will be recorded in the CRF, as well as infusion rates in each period.

Compliance with the study product dosing schedule will be verified by a CRA during on-site monitoring visits, as per records in the CRF, versus accountability records.

6.5. CONCOMITANT THERAPY

Any medications required for the patient's welfare are permitted and will be given at the discretion of the Investigator, except for drugs described in paragraphs below.

Administration of all prior and concomitant medications, apart from the agents listed below, from the time of enrolment until 10 days after the end of study drug administration or until hospital discharge (whichever occurs earlier) will be reported in the appropriate section of the CRF. For the intervals between 10 days or hospital discharge up to 12 month visit, only medications used for immunosuppression, prophylaxis of infections, and AE that the Investigator assesses as at least possibly related to the Investigational Product will be reported.

All the details as per the CRF fields (sequential number, drug name, indication, starting dose, doses at month 1, 6, and 12, route of administration, start/stop dates) will be recorded for the medications used for immunosuppression and prophylaxis of infections. Only the sequential number, the drug name, the indication and the start date will be recorded for all the other concomitant medications.

The following agents do not need to be recorded:

Saline and other hydration solutions (including additional electrolytes)
TPN and enteral feeds
Homeopatic medications
Elective vitamins and minerals
Topical agents (apart from inhaled) with no or negligible systemic absorption
Osmotic laxatives and locally acting antacids

6.5.1. Immunosuppressive therapy

Neither guidelines nor formal consensus on "best" or "standard" immunosuppressive strategies after human lung transplantation are currently available. Therefore,

immunosuppression after lung transplantation is derived from the wider experience gained in renal and heart transplant recipients integrated by the results of specific lung trials.

6.5.1.1. Induction

Data from the International Society for Heart and Lung Transplantation (ISHLT) registry indicate that about 45% of lung transplant recipients receive some type of induction therapy, mainly polyclonal antilymphocyte preparations or anti-interleukin (IL)-2-receptor antibodies. The use of Orthoclone OKT3 has been frequently associated to a cytokine release syndrome, which may induce cardiopulmonary instability. Also, experience with anti-CD52 (Campath) in the specific clinical setting is very limited. Therefore, **induction with Orthoclone OKT3 or Campath will not be allowed in the trial.**

Induction therapy will not be mandatory in this trial and the decision, including the choice of the immunosuppressive agent, with the exclusion of Orthoclone OKT3 and Campath, will be based on center's practice.

6.5.1.2. Maintenance

At the present time, maintenance with a triple-drug regimen can be considered the norm. Therefore, this study will follow the common practice currently in use, recommending a triple-drug regimen of either cyclosporin A or tacrolimus, plus steroids (at a moderate dose in the first seven days), plus either azathioprine or mycophenolate mofetil/mycophenolic acid.

The use of sirolimus will be discouraged, at least in the first three months after lung transplantation, as cases of bronchial anastomotic dehiscence, most fatal, have been reported in de novo lung transplant patients when the drug has been used as part of an immunosuppressive regimen. Programs intending to use sirolimus in the first three months should refrain from enrolling such patients in the trial.

Monitoring strategies for dose adjustment as well as strategies for weaning steroids will be as per center's practice.

6.5.1.3. Treatment of acute and chronic rejection

Treatment of acute rejections will be handled as per center practice.

Treatment of established chronic rejection is currently not supported by evidence obtained in adequately designed clinical studies. Any strategy deemed useful by the Investigator will be allowed.

6.5.2. Prophylaxis of opportunistic infections

Prophylaxis of opportunistic infections will be handled as per center practice.

6.5.3. Other treatments

Nitric Oxide, Surfactant, Prostaglandin E1 will be administered as per center practice.

7. STUDY PROCEDURE AND ASSESSMENTS

A schedule for the tests and evaluations to be conducted in this study is found in the flow chart in Appendix 4. A list of acceptable assessment/procedure time windows is detailed in Appendix 6.

7.1. SCREENING AND RANDOMIZATION

Potential study patients will be identified whilst on the center transplant waiting list. On arrival at the center for transplantation, consented patients will undergo confirmatory screening for the study.

Compliance with inclusion/exclusion criteria will be verified vs demographic and clinical information available as per Organ Procurement Agency - e.g. United Network for Organ Sharing (UNOS) - form and standard clinical practice.

Laboratory tests (hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count, sodium, chloride, potassium, bicarbonate, serum creatinine, blood urea nitrogen, total bilirubin, ALT, AST, prothrombin time or international normalized ratio, partial thromboplastin time) will be performed by local laboratories.

Screening also includes measurement of BP, HR, body weight (kg), and testing for renal and hepatic function, as well as for extra-respiratory tract site of infection.

Renal function will be evaluated by CLcr, calculated according to the following formula (*Cockcroft-Gault; 1976*):

$$\text{Male: } CLcr = \frac{[140 - \text{age (years)}] \bullet \text{Weight (kg)}}{\text{Serum Creatinine (mmol / L)} \bullet 815}$$

$$\text{Female: } CLcr = \frac{[140 - \text{age (years)}] \bullet \text{Weight (kg)}}{\text{Serum Creatinine (mmol / L)} \bullet 815} \bullet 0.85$$

Hepatic function will be evaluated by bilirubin (mg/dL) and transaminases (ALT and AST).

Extra-respiratory tract sites of infection will be diagnosed by blood culture(s) and/or fever associated with other signs of systemic sepsis syndrome. Previous results of blood cultures can be used to verify inclusion criteria, provided that the sample has not been obtained earlier than 72 hours prior to randomization.

Eligible patients will be randomized (randomization number assigned) by an Investigator or designee before proceeding to the operating room. The Investigator will forward to the Pharmacy an order to start preparation of Investigational Product dosing solution.

A screening form will be completed for all patients who signed the Patient Informed Consent Document, regardless of sequent entry into the study. Patients will be identified by their

initials and date of birth; in addition their race, sex and reasons for exclusion from the study will be recorded.

If entered into the study, the following recipient data will be recorded in the CRF:

- demographic, i.e. age, sex, race, weight, height,
- immediate pretransplant and transplant clinical information as per Organ Procurement Agency - e.g. UNOS - form,
- results of laboratory tests.

7.2. ORGAN PROCUREMENT

Standard protocols for procuring and preserving donor lungs will be used. Lungs will be resected en bloc following systemic heparinization.

Lungs will be washed by anterograde plus retrograde perfusion via the pulmonary artery with Low Potassium Dextran (Perfadex®) - based solution (with or without additions as per center practice) at 4°C. The lungs will be transported in the partially inflated state immersed in Perfadex® solution at 4°C.

Donor lung data will be recorded in the CRF as per Organ Procurement Agency - e.g. UNOS - form.

7.3. OPERATIVE COURSE

In principle, standard surgery procedures, excluding possible graft reflush with leukocyte depleted blood, will be applied as per center practice. However, it is suggested to adopt whenever possible, the following strategies:

- Application of topical hypothermia during implantation.
- After implantation, blood flow will be progressively, in a controlled way, restored over a period of up to 10 minutes (minimum 2 min).
- Protective ventilation strategy will be adopted during the initial period of reperfusion. It is suggested that the newly implanted lung allograft is gently reinflated with a sustained airway pressure of 20 cmH_2O before reperfusion and then ventilated with FiO_2 of 50%, PEEP =5 cmH_2O, and pressure control ventilation limiting peak airway pressures to 20 to 25 cmH_2O. After reperfusion has occurred, changes of ventilation will be directed by the anesthesiologist according to his/her judgment of the functional status of the implanted lung.

Relevant operative details will be recorded in the CRF, including gross abnormalities of the donor lung(s), duration of cold and warm ischemia, time of reperfusion, use and duration of cardiopulmonary bypass, lung reduction procedures, anastomotic difficulties, hypoxemia, and pulmonary edema, use of nitroglycerin/nitric oxide/inhaled prostacyclin/surfactant.

7.4. ADMINISTRATION OF INVESTIGATIONAL PRODUCT IN THE OPERATING ROOM

The infusion Bag No. 1, containing the dosing solution matching the patient randomization number, should be available in the operating room prior to anesthesia induction.

Infusion of the Investigational Product will start approximately 2 hours before the anticipated time of reperfusion of first allograft. The surgeon will identify the time to start study drug infusion.

The infusion pump will be set to provide the infusion rate corresponding to the loading dose. The loading dose will be infused for 30 min. Thereafter, the infusion rate will be reduced to provide the maintenance dose. Infusion rate (mL/hour) corresponding to each unit increment (kg) of body weight, is reported in Appendix 3.

Immediately before the start of infusion, the removable label on the Infusion Bag will be detached and attached to the relevant page of the CRF. Starting time of drug administration, time of switch to maintenance dose and infusion rates will be recorded in the CRF.

7.5. ADMISSION TO ICU

7.5.1. Arrival to the ICU

Date and time of ICU admission will be recorded in the CRF.

All patients will be mechanically ventilated through a oral endotracheal tube. The ventilator mode and rate will be set as per center practice, to ensure best standard of patient care.

Upon arrival to the ICU, the patient will be hemodynamically stabilized and placed on FiO_2 =1.0 and PEEP =5 cmH_2O (or greater, if clinically necessary, to achieve SpO_2 > 92% and $PaO_2 \geq 75mmHg$).

The following will be assessed as per center practice (unless otherwise specified) on arrival to the ICU and recorded in the CRF, apart from SVR and PVR which will be calculated during data-management:

- PaO_2/FiO_2 ratio (FiO_2 =1; PEEP =5).
- Chest X-rays (see Appendix 1 for methodological details).
- BP, HR.
- CO, PA pressure, SVR, PVR, PCWP, CVP.

7.5.2. Assessment in the ICU

The following will be assessed during ICU stay and recorded in the CRF, apart from SVR and PVR which will be calculated during data-management. Measurement will be performed as per center practice, unless otherwise specified. For the purpose of this study, clinically diagnosed PGD is defined as:

- An episode of decreased PaO_2/FiO_2 ratio and chest X-ray results consistent with a PGD score of grade 3, which is not the result of some other secondary cause of graft dysfunction [*Christie, 2005*]. When diagnosed, the condition is considered clinically recovered for a PGD score of ≤ 1.

- PaO_2/FiO_2 ratio. PaO_2/FiO_2 ratio will be measured at $FiO_2 = 1$ q24 hours, up to extubation or up to 72 hours after ICU admission, whichever occurs earlier. Patients on $FiO_2 < 1$ will be turned back to $FiO_2 = 1$ for 5 minutes before gas measurement. Additional PaO_2/FiO_2 ratio values obtained as per center standard of care in patients with clinical diagnosis of PGD will be recorded in the CRF up to the clinical recovery of the condition.

- Chest X-rays. A chest X-rays will be obtained 24 hours, 48 hours, and 72 hours after ICU admission (see Appendix 1 for details).

- BP, HR. Vital signs will be measured q12 hours up to ICU discharge or up to 5 days after ICU admission, whichever occurs earlier.

- CO, PA pressure, SVR, PVR, PCWP, CVP. Cardiopulmonary physiologic measurements will be assessed q12 hours when a PA catheter is present, up to ICU discharge or up to 5 days after ICU admission, whichever occurs earlier. Additional measurements obtained as per standard of care in patients with clinical diagnosis of PGD will be recorded in the CRF up to the clinical recovery of the condition.

For all measurements above, the actual date and time of assessment will be recorded in the CRF.

- A BAL will be performed during mechanical ventilation, in the interval between 12 and 24 hours after reperfusion, unless the patient is suffering from any clinical conditions which would be worsened by BAL procedures, in the opinion of the Investigator (see Appendix 1 for methodological details). Date and time of sampling will be recorded in the CRF along with location of BAL (lung, site) number of aliquots injected, amount recovered and summary of the most recent chest X-rays report. Specific reasons (hemodynamic instability, severe hypoxemia, etc.) for the decision to omit the BAL should be recorded in the CRF.

Renal (CLcr, urine output) and hepatic (bilirubin, transaminases) function will be tested at 12, 24, and 36 hours after the beginning of Investigation Product infusion.

Other clinical findings, including bronchoscopic and/or BAL findings, reports for all routine chest X-rays and thoracic CT scans, and results of routine biopsy will be copied into the CRF from the medical charts. AEs will be also recorded.

7.5.3. Weaning mechanical ventilation

Weaning procedures will be as per center practice.

Every morning (@08:00) that a patient has not started weaning procedures, reasons for not weaning are recorded in the CRF.

Date and time of first extubation which is maintained for more than 24 hours are recorded in the CRF.

7.6. ICU DISCHARGE - HOSPITAL STAY

Date and time of ICU discharge will be recorded in the CRF, as well as vital status at ICU discharge. Every morning (@08:00) that a patient has not been discharged from the ICU, reasons for not discharging are recorded in the CRF.

Renal (CLcr, urine output) and hepatic (bilirubin, transaminases) function will be tested at 12, 24, and 36 hours after the beginning of Investigation Product infusion.

Before hospital discharge, laboratory tests will be performed. Results will be recorded in the CRF.

Length of hospital stay, vital status at hospital discharge and AEs will be recorded.

7.7. DRUG ADMINISTRATION IN THE ICU/HOSPITAL AND BLOOD SAMPLING FOR PHARMACOKINETIC ASSAY

Drug administration (maintenance dose) will continue during ICU/hospital stay.

Twenty-four hours after the start of the infusion, Infusion Bag No. 1 will be replaced by Infusion Bag No. 2. The removable label on Bag No. 2 will be detached and attached to the relevant page of the CRF. A blood sample will be obtained for pharmacokinetic assay @ 24±6 hours.

Infusion of Investigational Product will continue up to 48 hours. Immediately before the infusion is terminated, a blood sample will be obtained for pharmacokinetic assay.

An additional blood sample will be obtained in the interval 2-6 hours after the end of infusion.

Blood sampling for pharmacokinetic assay will be via an indwelling intravenous (or intra-arterial, if already in place) cannula or by direct venipuncture. On each time point, a 6 mL sample will be taken into lithium heparin monovettes. Details of sample handling are reported in Appendix 1.

Time of bag replacement, end of infusion, and blood sampling will be recorded in the CRF.

7.8. MONTH 1 POST-TRANSPLANT

Patient will attend the center for study assessment at month 1 (at least 30 days post transplant). The following measurements will be performed as per center practice and recorded in the CRF:

- FEV_1, FVC. Actual date of assessment will be recorded in the CRF.

Any AEs that occurred during the interval i.e. since 10 days after the end of study drug administration or since hospital discharge (if applicable), and that the Investigator assesses as at least possibly related to the Investigational Product, will be recorded at this visit, as well as patient vital status.

7.9. MONTHS 6 AND 12 POST-TRANSPLANT

Patient will attend the center at months 6 and 12 +/- 2 weeks post-transplant. The following measurements will be performed as per center practice (unless otherwise specified) and recorded in the CRF:

- FEV_1, FVC, BOS score will be assessed at each visit. BOS score will be evaluated according to Estenne et al. *(2002)*.

- Acute, biopsy proven rejection episodes diagnosed according to Yousem et al. *(1996)*. Cumulative assessment at each visit.

For all measurements above, actual date of assessment will be recorded in the CRF. Diagnosis and treatment of acute rejection, if any, will be also recorded in the CRF.

Patient vital status, as well as any AEs occurring during the intervals between study visits that the Investigator assesses as at least possibly related to the Investigational Product will be recorded at each visit.

7.10. EARLY PATIENT WITHDRAWAL

7.10.1. Withdrawal criteria

Patients will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and are not obliged to state their reasons. Any withdrawals must be fully documented in the CRF and should be followed up by the Investigator.

Additionally, the Investigator may withdraw a patient at any time if (s)he considers this to be in the patient's best interest. Also, a patient might be withdrawn from the study, at the Investigator's judgment, in case of protocol violations, including (but not limited to) non-compliance with study procedures, patient lost to follow-up or administrative reasons.

Patients **must** be annulled from the study for the following reasons:

- Patient was randomized, but did not receive the Investigational Product,
- Patient was randomized, but did not proceed to lung transplantation.

If a patient fails to return to the center for a scheduled visit, attempts should be made to contact the patient to ensure that the reason for not returning is not an AE that may at least possibly be related to Investigational Product. Likewise if a patient declares his/her wish to discontinue from the study e.g. for personal reasons, an attempt should be made to establish that the true reason is not an AE (bearing in mind the patient is not obliged to state his/her reasons).

7.10.2. Replacement policy

Only patients withdrawn due to one of the following reasons will be replaced:

- Patient was randomized, but did not receive any Investigational Product,

- Patient was randomized, but did not proceed to lung transplantation.

Should a patient be replaced, his/her number will not be reallocated. A new patient will be enrolled and assigned the next number available.

8. ADVERSE EVENTS

8.1. DEFINITIONS

8.1.1. Definition of an Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product [*Clinical safety data management: Definitions and Standards for Expedited Reporting*].

8.1.2. Definition of a Serious Adverse Event

A Serious Adverse Event (SAE) is defined [*Clinical safety data management: Definitions and Standards for Expedited Reporting*] as any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening (i.e. 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 anomaly/birth defect,
- is an important medical event that based upon appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.2. EMERGENCY PROCEDURES

All AEs should be followed-up to determine outcome of the reaction. The Investigator should follow up the event until resolution or stabilization of the condition. It is the Investigator's responsibility to assure that the subjects experiencing AEs receive definite treatment for any AE, if required.

The treatment allocation for each patient will be provided in individual sealed envelopes. The location of these envelopes must be communicated to relevant study site staff and documented in the Investigator file. An envelope should only be opened in case of emergency where knowledge of the double-blind treatment may influence the further care of the patient. All such envelopes (both opened and unopened) will be collected by PPD as each patient completes the study. If an envelope is opened for any reason, the Investigator will notify PPD immediately and a record will be kept of when it was opened, by whom and why.

8.3. RECORDING

AE data should be obtained through observation of the patient, from any information volunteered by the patient, or through patient questioning.

All AEs encountered during the clinical study, including SAEs will be reported in the appropriate section of the CRF. It is important that this includes the duration of the AE (onset/resolution dates), the relationship to the drug, the severity, and relevant concomitant treatments dispensed (or other action taken) (see Sections 8.3.2., 8.3.3., and 8.3.4. below).

8.3.1. AE reporting period

All AEs which occur during the treatment period (i.e. the first to last day of Investigational Product administration) and up to 10 days after the end of Investigational Product administration or up to hospital discharge, whichever occurs earlier, will be recorded in the CRF.

In addition, any known untoward event that occurs subsequent to the AE reporting period that the Investigator assesses as at least possibly related to the Investigational Product should also be reported as an AE.

8.3.2. Relationship of AEs to the Investigational Product

The Investigator will assess the possible relationship between the AE and the investigational medication, according to the criteria in Table 1 below:

Table 1: Relationship of the Adverse Event to the Investigational Product

None (Intercurrent Event)	An event that is not and cannot be related to the Investigational Product, e.g. patient is a passenger in a road traffic accident
Unlikely (remote)	Relationship is not likely e.g. a clinical event including laboratory test abnormality with temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals or underlying disease provide plausible explanations
Possible	Relationship may exist, but could have been produced by the patient's condition or treatment or other cause
Probable	Relationship is likely, the AE abates upon discontinuation of Investigational Product and cannot be due to the patient's condition
Highly Probable	Strong relationship, the event abates upon discontinuation of Investigational Product and, if applicable, re-appears upon repeat exposure

An **Adverse Drug Reaction (ADR)** is defined as an adverse experience which is a reasonably likely to have been caused by the drug. Any AE reported in the study having a possible, probable or highly probable relationship to study drug will be considered as an ADR.

8.3.3. Severity of AEs

The Investigator will grade the severity of any AE using the definitions in Table 2. For each episode, the highest severity grade attained should be reported.

Table 2: Severity of the Adverse Event

Mild	Grade 1 - Does not interfere with patient's usual function (awareness of symptoms or signs, but easily tolerated [acceptable]).
Moderate	Grade 2 - Interferes to some extent with patient's usual function (enough discomfort to interfere with usual activity [disturbing]).
Severe	Grade 3 - Interferes significantly with patient's usual function (incapacity to work or to do usual activities [unacceptable])
Life Threatening	Grade 4 - Results in risk of death, organ damage, or permanent disability [unacceptable]

8.3.4. Frequency of AEs

The frequency of AEs should be described as per Table 3.

Table 3: Frequency of the Adverse Event

Once	The AE occurred only once and resolved in < 24 hours
Occasionally	The AE occurred sporadically or episodically between the onset and resolution dates (and times)
Continuously	The AE was present for the entire time between onset and resolution dates (and times) and was > 24 hours duration

8.4. SERIOUS ADVERSE EVENT REPORTING

8.4.1. Reporting Procedure for Investigators to PPD

The Investigator must report all SAEs, regardless of presumed causal relationship, to the PPD Pharmacovigilance Department, by fax within 24 hours of learning of the event. Details of the relevant fax number for SAE reporting are provided in the section "Contact Information".

Information on SAEs will be recorded on a specific Non-Carbon Repeat SAE form. Blank copies will be included in the Investigator's Site File.

Follow-up reports (as many as required) should be completed and faxed following the same procedure above.

A final report is required in any case once the condition is resolved or stabilized and no more information about the event is expected. The final report should be completed and faxed following the same procedure above.

PPD will report to Dompé all information received by the Investigators, by fax within 24 hours of knowledge.

8.4.2. Reporting Procedure for Investigators to IRB/REB

In addition to reporting the SAE to PPD , the Investigator must also comply with the requirements related to the reporting of SAEs to the IRB/REB which approved the study.

The requirements of IRB/REB varies from one state and indeed one country to another, however as a minimum requirement all Investigators must promptly report all serious unexpected* ADRs, life-threatening problems or deaths to their IRB/REB.

PPD will inform Investigators of all serious unexpected ADRs, which are reported to PPD from other Investigators. These SAEs should also be reported promptly to the IRB/REB in compliance with the local regulations. Copies of all correspondence relating to reporting of any SAEs to the IRB/REB should be maintained in the Investigator's Files and provided to PPD .

* An **unexpected ADR** is defined as any adverse drug experience, the nature or severity of which is not consistent with the current Investigator's Brochure.

8.4.3. Reporting Procedure for Regulatory Authorities

This study is intended to take place at centers in the USA and Canada and therefore should satisfy both FDA and Health Canada SAE reporting requirements.

During the course of the clinical trial, Dompé (or designee) shall inform FDA/Health Canada of any serious unexpected ADR as soon as possible and in no event later than:

(*a*) seven calendar days after becoming aware of the information if it is fatal or life threatening; and

(*b*) fifteen calendar days after becoming aware of the information if it is neither fatal nor life threatening.

Dompé (or designee) shall, within eight days after having informed Health Canada under paragraph (*a*), submit a complete report in respect of that information that includes an assessment of the importance and implication of any findings made.

Furthermore Dompé shall promptly investigate all safety information received by it and follow up information to a safety report shall be submitted to the appropriate authorities as soon as the relevant information is available.

If the results of an investigation show that an adverse drug reaction not initially determined to be reportable is reclassified as reportable, Dompé (or designee) shall report such reaction in a written safety report as soon as possible, but in no event later than 15 calendar days after the determination is made.

8.5. DATA MONITORING COMMITTEE

An independent Data Monitoring Committee (DMC) will be established to ensure patient safety, to consider patient risks against the potential for meeting trial objectives, and to provide recommendations to Dompé with respect to the conduct and analysis of the trial. The DMC will operate independently of Dompé, and its members will not have connections to the Sponsor with the exception of the compensation to DMC members related to DMC activities.

The DMC will comprise three voting members. They will be a multidisciplinary group that will include:

- A surgeon with extensive experience in lung transplantation,
- A physician with relevant experience in pulmonary and critical care medicine (e.g. a. Medical Director of a Lung Transplant Program),
- A biostatistician.

The DMC will perform the following major functions:

- The DMC will be responsible for the ongoing review of safety data throughout the trial. Primary among the safety data that will be reviewed are serious AEs, ADRs and relevant available clinical laboratory results.

- The DMC may review acute and additional safety data, on a patient-by-patient basis, at their discretion. This review would be warranted if significant unexpected ADRs are observed.

- The DMC may request additional safety data if, in the view of the Committee, these additional data are needed in order to make an informed, responsible decision about the conduct of the study.

- The DMC will advise Dompé regarding safety of patients and continuing validity and scientific merit of the trial. The DMC will also monitor the number of deaths for both arms of the study to ensure that mortality is consistent with historical data.

All details of the conduct and responsibilities of the study DMC will be described in the 'DMC Charter' which will be finalized prior to the start of patient enrolment.

9. STATISTICAL ISSUES

9.1. SAMPLE SIZE

The sample size has been estimated based on PaO_2/FiO_2 ratios immediately and at 24 hours from the results of 700 lung transplant operations at the Washington University. The database reports means and standard deviations for this endpoint of 329±137 mmHg at the initial ICU assessment and 315±117 mmHg at 24 hours.

A sample size of 50 in each group will have a 81% power to detect a difference in means of 70 mmHg (the difference between a placebo mean of 315 and a treated mean of 385, a 22% improvement) assuming that the common standard deviation is 137 using a two group t-test with a 0.05 one-sided significance level. Alternatively, a sample size of 50 in each group will have a 56% power to detect a difference in means of 50 mmHg (the difference between a placebo mean of 315 and a treated mean of 365, a 15% improvement) assuming that the common standard deviation is 137 using a two group t-test with a 0.05 one-sided significance level.

There is no currently available data to provide a knowledgeable estimate of the effect size in this study. If the effect size is smaller than 15%, the power of this study will be reduced and the chance of a Type II error is increased. Under such circumstances, this study would still provide valuable data regarding the effect size in the primary outcome variable as well as the results in all of the secondary, exploratory endpoints and subgroup analysis.

9.2. RANDOMIZATION

Patient will be randomized in a 1:1 fashion to either repertaxin or placebo. Treatment will be balanced within centers.

A master randomization list will be prepared, randomizing an excess of patients to allow flexible/competitive recruitment within each center. The randomization list will be prepared by PPD according to the current version of the Standard Operating procedure (SOP).

The randomization code will be broken at completion of the main part of the study, i.e. when the last patient in has completed his/her 30 days follow-up visit, and once the database has been locked.

9.3. ANALYSIS POPULATION

The Safety population will consist of all patients who received any study medication, and will be based on the treatment actually received. The Safety population will be used to present the demographic and baseline data, and all safety data.

The Intent to Treat (ITT) population will consist of all patients who received any study medication and the transplant, and will be based on the treatment randomized, regardless of the treatment actually received.

The Per-Protocol (PP) population will consist of all patients in the ITT population who did not have any major protocol violations, defined to be:

- Patient who started study drug infusion after reperfusion, i.e. after vascular de-clamping of the first allograft,
- Patients who did not receive the study medication for at least 24 hours,
- Patients without data for PaO_2/FiO_2 ratio at 0 and 24 hours, while on mechanical ventilation,
- Patients who receive Orthoclone OKT3 or Campath induction immunosuppression,

The primary and secondary efficacy analyses will be presented for the ITT population, primarily, and confirmed by the PP population.

9.4. STATISTICAL METHODOLOGY

Appropriate descriptive statistics will be produced, according to the variable. For continuous data the mean, standard deviation, median and range (minimum and maximum) will be presented. For categorical data, frequencies and percentages will be presented. If appropriate, confidence intervals around the mean or the proportions will be presented.

All patient data collected on the CRF will be listed by patient, treatment group and center.

The data for the double-blind phase of the study (up to 30 days post-treatment) will be presented in the clinical study report. Data collected during the open phase of the study (from 1 month up to 12 months) will be presented in an addendum to the clinical study report.

Unless otherwise specified, the significance level used for statistical testing will be 5% and two-sided tests will be used.

9.4.1. Demographic and baseline characteristics

Demographic and baseline characteristics will be summarized for all patients in the Safety population, by treatment group.

9.4.2. Primary efficacy analysis

The PaO_2/FiO_2 ratio will be analyzed separately, at the two time points: 0 hours (ICU admission) and 24 hours after ICU admission. Analysis of variance will be used, including terms for treatment and center. The importance of the treatment by center interaction will be investigated but if this is not significant at the 10% level, this term will be excluded from the final model. If a center by treatment interaction is detected, alternative methods of presentation will be explored. Treatment effect will be primarily compared using a one-sided 0.05 level test and then re-tested for consistency with a two-sided 0.05 level test. Type of transplant (single or bilateral) will then be included in the model of the analysis to explore any possible, even unanticipated, differences due to the specific procedure.

Primarily the data will be analyzed excluding missing data. As confirmatory analysis, the ANOVAs will be performed substituting any missing values by the last observation carried forward (LOCF) method, in patients extubated before 24 hours. If the data are missing due to

patient death, the PaO_2/FiO_2 ratio will be given a zero value. If the data are missing due to lack of recording while on mechanical ventilation, these data will not be substituted.

9.4.3. Secondary efficacy analysis

The PaO_2/FiO_2 ratio will be analyzed, as described for the primary efficacy endpoint, at 48 hours and 72 hours after ICU admission. Analysis of variance will be used, including terms for treatment and center. The importance of the treatment by center interaction will be investigated but if this is not significant at the 10% level, this term will be excluded from the final model. If a center by treatment interaction is detected, alternative methods of presentation will be explored. Type of transplant (single or bilateral) will then be included in the model of the analysis to explore any possible, even unanticipated, differences due to the specific procedure.

Analysis of PGD score will be performed separately at the four time points (0, 24, 48, 72 hours) to test for differences between treatments. Two analyses will be performed at each time-point, using Cochran-Mantel Haenszel statistics, with the first analysis using type of transplant as a stratification factor, and the second using center as a stratification factor.

The time to freedom from mechanical ventilation and the duration of ICU stay will be presented using Kaplan-Meier plots, and will be analyzed using the Median test, to test for differences between treatments. Time to freedom from mechanical ventilation and the duration of ICU stay will also be presented by type of transplant, and the Median test will be performed on each type of transplant, if there are sufficient patient numbers in both categories.

All other secondary endpoints will be presented using appropriate descriptive statistics, and no formal statistical testing will be performed.

ICU mortality and the number of patients who die within the first 30 days post transplant will be tabulated. FEV_1 and FVC at 1 month post-transplant will be presented by type of transplant.

FEV_1 and FVC, BOS score, cumulative acute rejection episodes and patient survival at 6 and 12 months post-transplant will be presented in an addendum to the clinical study report, using descriptive statistics. BOS score, cumulative acute rejection episodes and patient survival will also be presented by type of transplant.

9.4.4. Analysis of the endpoint targeted to the mechanism of action

PMN count in BAL specimen will be presented using descriptive statistics.

9.4.5. Analysis of pharmacokinetic endpoints

Plasma levels of repertaxin (total and unbound) and its major metabolite, DF2243Y, are recorded 24±6 hours and 48 hours from the start of infusion, and then in the interval 2-6 hours after the end of infusion. Data will be summarised using descriptive statistics.

9.4.6. Safety analysis

Safety variables will be presented for the Safety population.

AEs will be presented, by treatment group, in terms of the incidence, severity and relationship to the study drug, overall and by body system and preferred term. SAEs will be presented in the same way.

Laboratory tests are performed at screening and at hospital discharge and shift tables will be used to present shifts from within/outside the normal range between screening and discharge.

Vital signs at each time point, the change in vital signs from screening to each time point, and cardiopulmonary physiologic measurements at each time point will be presented using descriptive statistics.

10. ETHICAL CONSIDERATIONS

10.1. INSTITUTIONAL REVIEW BOARD/RESEARCH ETHICS BOARD

It is the responsibility of the Investigator to obtain approval of the trial protocol/amendments from the Institutional Review Board/Research Ethics Board (IRB/REB).

Prior to the initiation of the study, the Investigator will submit to the appropriate IRB/REB for approval the followings:

- the study protocol,
- the Patient Informed Consent Document and any other written documents to be provided to the patient,
- the current version of the Investigator's Brochure,
- Investigator's current curriculum vitae,
- patient recruitment procedures e.g. advertisements,
- any other requested document(s),

A copy of the IRB/REB approval will be sent to PPD along with **all** other correspondence with the IRB/REB, including the submission documents. The Investigator should file all correspondence with the IRB/REB in the Investigator Site File. PPD will promptly send to Dompé a copy of the IRB/REB approval.

The study will not be started until full written approval has been obtained from the appropriate IRB/REB. The letter of approval should be dated, and should specify the type (e.g. protocol number) and the date of the documents which were reviewed and approved.

The Investigator will submit any future amendment to the protocol to the IRB/REB which granted the original approval (and other local authorities, according to local regulations). Any amendment will be implemented only when full approval has been obtained from the appropriate IRB/REB, except for those amendments which involve only logistical or administrative aspects of the study.

The Investigator will send to the IRB/REB any updated Investigator's Brochure.

A dated list of the voting members of the IRB/REB who were present when the protocol/amendment was reviewed and approved, including their titles/occupations and institutional affiliations should be provided where possible by the Investigator to PPD prior to study initiation. The Investigator will make all attempts to obtain a statement from the IRB/REB that it is constituted and operates in accordance with the ICH-GCP and any local regulations.

The Investigator will submit required progress reports to the IRB/REB which approved the protocol at least annually, as well as report any serious unexpected ADRs, life-threatening problems or deaths. The Investigator will also inform the IRB/REB of reports of serious unexpected ADRs (provided to him/her by PPD) occurred at other sites participating to this clinical trial and/or in other clinical studies conducted with repertaxin.

The Investigator must inform the IRB/REB of the termination of the study.

10.2. INFORMED CONSENT

No study-related procedures (including non-invasive and diagnostic procedures) will be undertaken prior to completion of the consenting process. Each potentially eligible patient will be informed of the study's objectives and overall requirements. The Investigator will explain the study fully to him/her using the Patient Informed Consent Document. If the patient is willing to participate in the study, (s)he will be requested to give written informed consent after being given sufficient time to consider his/her participation and the opportunity to ask for further details. The Patient Informed Consent Document will be signed and personally dated by **both** the patient and the Investigator.

A copy of the signed form will be provided to the patient and the original signed Patient Informed Consent Document will be retained and filed in the Investigator Site File.

Although nursing staff may be involved in describing the trial to a patient, the Investigator must participate in discussions with the patient **and sign** and personally date the Patient Informed Consent Document.

Individual (i.e. site specific) Patient Informed Consent Forms will be based on a master document provided by PPD and must be approved by PPD prior to submission to the IRB/REB. Any changes requested by the IRB/REB must be approved by PPD prior to the documents being used. A copy of the final, IRB/REB-approved Patient Informed Consent Document must be submitted to PPD prior to initiation of this study.

Where applicable, patients next on the waiting list (max 25 patients) will be pre-consented, i.e. consent is provided before the patient is called into the hospital for transplantation.

10.3. CONFIDENTIALITY

The Investigator must ensure that the subjects' anonymity will be maintained. On the CRFs or other documents submitted to PPD patients should NOT be identified by their names, but by the assigned patient number if randomized plus their initials and date of birth (if not randomized).

If patient names are included on copies of documents submitted to PPD the names (except for initials) will be obliterated or masked and the assigned patient number added to the document.

The Investigator should keep a separate log (Patient Master List) of patient's codes, names, addresses, telephone number and hospital number (if applicable). Documents that are collected but not required for submission to PPD (e.g. signed Patient Informed Consent Forms) should be maintained by the Investigator in strict confidence.

10.4. COMPENSATION FOR MEDICINE-INDUCED INJURY AND INDEMNIFICATION

Before the trial formally starts, Dompé will take out an insurance contract covering the amount requested by the respective national/federal laws for patients participating in clinical trials. The insurance contract will also cover the Investigator/Institution against claims arising from the trial, except for claims that arise from malpractice and/or negligence, including violations of the study protocol.

Should any patients suffer adverse effects as a direct consequence of the Investigational Product administration in accordance with the protocol, Dompé will reimburse for hospital medical costs required for diagnosis and treatment. Medical treatment shall be provided primarily through the study center.

Dompé will not provide for the costs of medical care unrelated to the study.

In case of questions about medical care, cost for medical care or insurance, patients can talk to their Investigator. Contact details will be given in the Patient Informed Consent Document.

11. DATA HANDLING AND RECORD KEEPING

11.1. CASE REPORT FORMS COMPLETION

CRFs will be supplied by PPD. CRFs are the sole property of PPD and should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from PPD.

A CRF is required and should be completed for each included patient. The Investigator will be responsible for the accuracy of the data entered in the CRFs. All entries must be written in ***ENGLISH*** in black ink. Source documents should be available to support all the data recorded in the CRF. Location of source data, including those for which the CRF might be accepted as being the sole source document, will be specified and listed at the center Initiation Visit.

The CRF must be available for review/collection to designated PPD representatives at each scheduled monitoring visit.

11.2. DATA MANAGEMENT

Data management of the CRFs will be performed by PPD and will be split into two distinct phases.

For the blinded (main) part of the study the CRF pages for all patients up to the month 1 visit will be data-entered (double data entry) into the study database, and the data will be verified for missing data, inconsistencies, and for any necessary medical clarifications.

Queries arising from these checks will be sent to the Investigator for response and signature. All possible attempts should be made by the Investigator to complete and return the signed responses as instructed to PPD within the requested timeframes.

Once all data queries pertaining to the blinded part of the study have been resolved, the study will be declared to be "clean", and the study data will be locked ready for analysis. Following the data lock the study blind will be broken and each patient's medicinal assignment (i.e. repertaxin or placebo) will be revealed to the appropriate Investigator.

The CRF pages for both follow up visits i.e. months 6 and 12 will be entered into a different location of the same database using the same procedure as described above. Furthermore when the last patient's month 12 visit has been "cleaned" the database will be locked ready for the final analysis.

After the database lock has been achieved, the Investigator may archive the copies of the CRFs retained at the center. The original CRFs collected by PPD will be archived by Dompé.

All data management will be conducted in accordance with good clinical, scientific and data management principles and in compliance with current PPD SOP.

11.3. DOCUMENTATION REQUIRED PRIOR TO INITIATION

In addition to the documents mentioned in Sections 10.1 and 12.1, the following will be required from the Investigator prior to the initiation visit:

- Current, signed and dated Curriculum Vitae of Principal Investigator and any Sub-Investigators/co-workers,
- Normal ranges of all laboratory tests to be performed at the study site and a recent certification or accreditation of established quality control (or other documentation of established quality control or external quality assessment or other validation),
- A signed original of the final protocol and any amendments,
- Indemnity / certificate of Insurance,
- A signed original of the study Financial Agreement/Clinical Study Agreement with Dompé, including Pharmacy, radiology etc (i.e. all study specific costs),
- Form 1572 (USA and Canada), Qualified Investigator Undertaking, Research Ethics Board Attestation, Clinical Trial Site Information Form (Canada only),
- Financial disclosure form 3455 from all persons listed on the 1572,
- Data protection form (HIPAA in USA) signed by the Investigator (giving the Investigator's approval and the patient's consent for personal data to be kept on file at PPD),
- List of delegated responsibility (Study Team Signature List / Delegation of Responsibilities form).

11.4. DOCUMENTATION REQUIRED DURING THE STUDY

The following will be required from the Investigator during the study:

- Current, signed and dated Curriculum Vitae of Principal Investigator and any Co-Investigators/co-workers who are delegated protocol related responsibilities after study initiation,
- Updates of normal ranges (including the dates from which they become effective) of all laboratory tests to be performed at the study site and updates in certification or accreditation of established quality control (or other documentation of established quality control or external quality assessment or other validation),
- A signed original of any protocol amendments,
- Copies of any new approvals from or correspondence with the IRB/REB,
- Renewals of certificates of Insurance, as applicable per country regulations,
- Updated form 1572 (USA and Canada), Qualified Investigator Undertaking, Research Ethics Board Attestation, Clinical Trial Site Information Form (Canada only),
- Financial disclosure form 3455 from all **new** persons listed on the 1572,
- Updated list of delegated responsibility (Study Team Signature List / Delegation of Responsibilities form).

11.5. ESSENTIAL DOCUMENT RETENTION

The Investigator will retain copies of all the essential documents (as defined by ICH-GCP) until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the Investigational Product. These documents should be retained for a longer period however if required by the applicable regulatory requirements. The Investigator should take measures to prevent accidental or premature destruction of these documents.

The essential documents include at least: the signed protocol, copies of the completed CRFs, signed Patient Informed Consent Forms from all patients who consented, hospital records, and other source documents, IRB/REB approval and all related correspondence, including approved documents, and all other documentation included in the Investigator Site File and Pharmacy/Dispensing File.

The Investigator will inform PPD of the storage location of these essential documents and must contact PPD before disposing of any. If the Investigator wishes to assign the files to someone else or to remove them to another location, the PPD Medical Director should be consulted about this change.

Dompé will inform the Investigator in writing when these documents no longer need to be retained.

12. STUDY MANAGEMENT

The study will be performed in accordance with the protocol, the Declaration of Helsinki (Appendix 5), and ICH Harmonised Tripartite Guideline for Good Clinical Practice (*ICH-GCP*) and any local regulations.

12.1. REGULATORY BODY APPROVAL

The study will not be started until full written approval from the relevant regulatory body (FDA and Health Canada) has been received by Dompé. PPD will then provide the Investigator with a copy of appropriate document on behalf of Dompé.

PPD undertakes to obtain (on behalf of Dompé) the necessary approval from the Health Canada prior to initiation of the study. The responsibility for gaining FDA approval lies with an independent regulatory consultant employed by Dompé.

12.2. STAFF INFORMATION & RESPONSIBILITIES

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, including detailed knowledge of and training in all procedures to be followed.

The Investigator will provide a list of delegated responsibility to PPD detailing the various study tasks to be performed by each member of his/her study staff. Each staff member should sign in agreement to their performing each of the tasks delegated to them on the list.

12.3. MONITORING

Monitoring will be carried out by PPD, according to the current version of PPD SOP.

Prior to study start, the Investigator will be informed of the anticipated frequency of the monitoring visits. He/she will also receive a notification prior to each monitoring visit during the course of the study. It is expected that the Investigator and/or his/her sub-Investigator(s) and other appropriate staff will be available on the day of the visit to discuss study conduct and to cooperate with the monitor to ensure that any problems detected during the course of these monitoring visits are resolved.

The purpose of the monitoring visit is to verify that the rights and the wellbeing of the patient are protected, that the reported data are accurate, complete and verifiable from source documents and that the conduct of the trial complies with the currently approved protocol and any amendments, with ICH GCP, and with regulatory requirements.

The CRA will complete a standard report following each Monitoring Visit and provide this report to the Project Leader for review within a specified timeframe.

12.3.1. Access to records

The Investigator will allow designated PPD representatives, designated Dompé representatives, and regulatory bodies to have direct access to the source documents to verify the data reported in the CRFs. Source documents are the originals of any documents used by the Investigator or hospital/institution that allow verification of the existence of the patient and substantiate the integrity of the data collected during the trial. Source documents should be available to support all the data recorded in the CRF. Location of source data, including those for which the CRF might be accepted as being the sole source document, will be specified and listed at the center Initiation Visit.

12.4. AUDIT AND INSPECTION

Audit activities will be performed by PPD Quality Assurance group, except for audit of Protocol/Amendments, Patient Informed Consent Document, and CRF, which will be performed by the Dompé Quality Assurance Unit.

On one or more occasions the study site may be audited by PPD. The Investigator will be informed in advance of such a visit. All audits will be performed by PPD personnel according to the current version of PPD SOP.

Additionally the study site may be inspected by Dompé representatives or a regulatory agency on one or more occasions.

12.5. PROTOCOL DEVIATIONS/AMENDMENTS

Changes to the Protocol will be implemented only when written amendments have been signed by all individuals who signed the protocol.

Any amendment will be sent to the appropriate IRB/REB. No deviations from or changes to the protocol will be implemented without documented approval of an amendment from the IRB/REB which granted the original approval, except where necessary to eliminate an immediate hazard(s) to trial patient, or when the change(s) involves only logistical or administrative aspects of the trial. The deviations from or changes to the protocol implemented to eliminate an immediate hazard to the trial patient and the proposed amendment, if appropriate, should be submitted to the IRB/REB for review and approval as soon as possible.

Any other deviation from the protocol that has not been approved by PPD and the IRB/REB could result in a discontinuation from the study at the center involved.

Any written amendment will be sent to all recipients of the protocol and to the regulatory authorities (FDA/Health Canada).

12.6. DISCONTINUATION OF THE STUDY

Dompé reserves the right to stop the study at any time on the basis of new information regarding safety or efficacy, or if study progress is unsatisfactory, or for other valid administrative reasons. After such a decision is made, the Investigator must inform all relevant persons e.g. study staff, potential patients etc. within 2 weeks. All delivered study materials must be collected and all CRFs completed to the extent possible.

Study discontinuation will be notified to FDA/Health Canada within 5 days from decision.

12.7. PUBLICATIONS

Any manuscript, abstract or other publication or presentation of results or information arising in connection with the study must be prepared in conjunction with Dompé and must be submitted to the Dompé Medical Director for review and comment at least 45 days prior to submission for publication or presentation.

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14. APPENDICES

14.1. APPENDIX 1 - METHODOLOGICAL DETAILS

14.1.1. PGD score - ISHLT system for grading of severity of primary graft dysfunction [*Christie et al, 2004*]

Grade 0	$PaO_2/FiO_2 \geq 300$ mmHg; no radiographic infiltrates
Grade 1	$PaO_2/FiO_2 \geq 300$ mmHg + radiographic infiltrates consistent with pulmonary oedema
Grade 2	200 mmHg $\leq PaO_2/FiO_2 < 300$ mmHg + radiographic infiltrates consistent with pulmonary oedema
Grade 3	$PaO_2/FiO_2 < 200$ mmHg + radiographic infiltrates consistent with pulmonary oedema

Any patient with no infiltrate on chest X-rays is automatically grade 0, regardless of PaO_2/FiO_2 value. If the patient is on nasal cannula for oxygen or $FiO_2 < 0.3$, the patient is graded as 0 or 1, based on chest X-rays. Any patient on extra corporeal membrane oxygenation is automatically grade 3. Any subject mechanically ventilated with FiO_2 greater than 0.5 or requiring nitric oxide beyond 48 hours from the time of transplant should be considered grade 3.

If multiple blood gas values are available, the worst PaO_2/FiO_2 ratio will be used for the purpose of this grading scheme.

PGD score will be evaluated at the following time points:

- Time-0 (T0): defined as within 6 hours of final lung reperfusion. The first blood gas in ICU is ideal for this. Ideally, measurement should be done on FiO_2 1.0 and PEEP =5 while still on mechanical ventilation.
- T24, T48, T72: Later times being measured at potentially multiple time points after 24 hours, up to 72 hours. Time will be measured following T0$\pm$6 hours.

Chest X-rays will graded by an Investigator, and then validated by a (blinded) transplant radiologist.

In patients with clinical diagnosis of PGD additional time points will be considered up to the clinical recovery of the condition as per available data according to center standard of care.

14.1.2. Bronchoalveolar lavage methods

BAL specimen

BAL sample will be taken from the allograft (first implanted allograft, in case of bi-lateral lung transplantation).

The distal bronchoscope will be wedged in a subsegmental bronchus of either the right middle or left lingular lobe. If a prominent, focal infiltrate is present on chest X-rays, BAL will be performed in the area of maximal radiographic abnormality.

Approximately 50 mL aliquots of sterile, pre-warmed, non-pyrogenic, isotonic sodium chloride solution will be instilled through the bronchoscope and immediately recovered by gentle suction (60 to 80 mmHg). This "back and forth" is repeated for a maximum of 4 washes.

The recovered fluid will be pooled and mixed thoroughly. Total recovered volume will be measured. BAL fluid will be transferred to the center laboratory on ice, but it can be transported at room temperature if processing will be undertaken within 30 minutes.

BAL processing

The recovered cell suspension will be filtered through surgical cotton gauze to remove excess mucus. To determine the total cell yield, a small aliquot of the fluid will be used for counting the cells on a hemacytometer. Red blood cells are not included in the total cell yield.

The BAL fluid will be then placed in siliconized glass or non-cell-adherent plastic centrifuge tubes and will be centrifuged at 200 x g for 5 minutes. The supernatant will be discharged, taking care not to disturb the cell pellet. The cells pellet will be resuspended in and washed twice with Hanks' balanced salt solution (or another medium such as phosphate-buffered salt solution) without Ca^{2+} and Mg^{2+}.

To obtain differential cell counts the cell suspension (approximately $1x10^5$ cells) will be cytocentrifuged at 800 g for 10 minutes. The slides will be stained as per center practice (May-Grunwald-Giemsa or Diff-Quick stain is suggested) and air dried at room temperature for 1 hour. Stained spot will be fixed (Entellan fixative is suggested), and slides covered by a microscope cover glass and stored until shipment to Dompé pha.r.ma s.p.a. (see below). Detailed packaging and labeling instruction and shipment procedures will be communicated at each center at study initiation.

Cell differentials will be performed by Dompé pha.r.ma s.p.a. - Preclinical Pharmacology Department. Two hundreds cells will be counted in each slide.

14.1.3. Handling of samples for pharmacokinetic assay

Samples will be centrifuged ideally within 15 minutes at 2500g (3350 rpm) at 4°C for 10 minutes. If that is not possible then samples can be stored at room temperature for not more than 2 hours or at 4°C for not more than 24 hours before centrifugation. At least 3 mL of plasma will be obtained and divided equally into 2 aliquots, one to be used for total repertaxin and DF2243Y, the other for the repertaxin unbound fraction. Plasma samples will be stored at approximately -80°C into stopped glass tubes, until shipment to the centralized analytical laboratory for assay.

Samples will be shipped to the centralized laboratory in appropriate package in dry ice (solid CO_2) to maintain frozen conditions. Detailed packaging and labeling instruction and shipment procedures will be communicated at each center at study initiation. Analysis will be performed by a centralized laboratory according to standardized and validated methods as per Good Laboratory Practices.

PPD, has been identified as the centralized laboratory where pharmacokinetic analysis is performed.

14.2. APPENDIX 2 - PACKAGING AND LABELING DETAILS

A "Patient Box" will be prepared for each patient. Each Patient Box will contain:

- Two Treatment Boxes, each with 25 x 10 mL ampoules - either containing repertaxin or placebo.
- Two (plus one reserve) sterile empty infusion bags
- Instructions to the Pharmacy

All items will have labels (English and French language for all, except ampoules); specimens of these are provided below. Only the label of the infusion bag will be a "double - tear off" label. The Investigational Product already packaged to the date of this protocol revision, whether sent to the site or stored at Aptuit, WILL NOT BE RE-LABELED. Labels of any additional future supplies will have the same format/information, apart from the name of the sponsor that will read "Dompé pha.r.ma s.p.a.".

NOTE:
- Patient No. XXYY where XX = Study site
  YY = Patient sequential number within the site
  (The first patient at Study Site No. 1 will have the number 0101).
- Infusion Bag - "Preparation" date and time, as well as "Use by" date and time will be entered by the Pharmacist. Use by date/time will be calculated as date/time of preparation + 72 hours (expiry date/time of the dosing solution).
- Patient Box and Infusion Bag - Patient initials will be entered by the Pharmacist.

Specimen Label for exterior of each Patient Box

STUDY (*ESSAI*) REP0104 Sponsor (*Promoteur*): Dompé s.p.a.; Via Campo di Pile, L'Aquila – Italy

PATIENT BOX (*BOITE pour PATIENT*)

PATIENT No. XXYY / ***PATIENT N°*** **PATIENT INITIALS** |__|__|__| [to be entered by the Pharmacist] / ***INITIALES DU PATIENT*** *[à compléter par le Pharmacien]*

CONTAINS: TWO TREATMENT BOXES [No. 1 (0-24 h); No. 2 (24-48 h)], TWO STERILE EMPTY INFUSION BAGS (PLUS ONE RESERVE BAG), INSTRUCTIONS TO THE PHARMACY
CONTENU: *DEUX BOITES DE TRAITEMENT [N° 1 (0-24 h); N° 2 (24-48 h)], DEUX POCHES DE PERFUSION VIDES ET STERILES (PLUS UNE POCHE DE RESERVE), LES INSTRUCTIONS POUR LA PHARMACIE*

INVESTIGATIONAL PRODUCTS: REPERTAXIN OR PLACEBO INJECTABLE SOLUTION
PRODUITS DE RECHERCHE: SOLUTION INJECTABLE DE REPERTAXIN OU DE PLACEBO

BLINDED BATCH No. / *N° DE LOT CODIFIE*

EXPIRY DATE mm/yyyy / *DATE DE PEREMPTION mm/aaaa*

DO NOT STORE AT >30°C (86°F) / ***NE PAS ENTREPOSER A >30°C (86°F)***

For questions - CRO contact = / *Pour toute question contacter la ORC=* PPD

DIRECTIONS: Use ampoules in Treatment Box No. 1 and Treatment Box No. 2 to prepare the dosing solution of Investigational Product for the 0-24 hours and 24-48 hours periods, respectively.
INSTRUCTIONS: *Utiliser respectivement les ampoules de la Boîte de Traitement N° 1 et de la Boîte de Traitement N° 2 pour préparer les doses de solutions du Produits de Recherche durant les périodes de 0-24 heures et 24-48 heures.*

CAUTION: New Drug-Limited by Federal law to investigational use. Investigational drug to be used by a qualified investigator only.
ATTENTION: *Nouvelle substance - Limitée à la recherche par la loi Fédérale. Produit de recherche, ne doit être utilisé que par un investigateur qualifié.*

Specimen Label for Treatment Box No. 1 [time period 0-24 hours]

STUDY (*ESSAI*) REP0104 Sponsor (*Promoteur*): Dompé s.p.a.; Via Campo di Pile, L'Aquila – Italy

TREATMENT BOX No. 1 (0-24 hours)
BOITE DE TRAITEMENT N° 1 (0-24 heures)

PATIENT No. XXYY
PATIENT N°

CONTAINS: 25 AMPOULES OF 10 mL REPERTAXIN (33 mg/mL) OR PLACEBO FOR INJECTABLE SOLUTION
CONTENU: *25 AMPOULES DE 10 mL DE SOLUTION INJECTABLE DE REPERTAXIN (33 mg/mL) OU DE PLACEBO*

BLINDED BATCH No.
N° DE LOT CODIFIE

EXPIRY DATE mm/yyyy
DATE DE PEREMPTION mm/aaaa

DO NOT STORE AT >30°C (86°F)
NE PAS ENTREPOSER A >30°C (86°F)

For questions - CRO contact =
Pour toute question contacter la ORC=

PPD

DIRECTIONS: Transfer into infusion BAG No. 1 the contents of **23 AMPOULES** + 370 mL of 0.9% NaCl, according to procedures detailed in the "Instructions to the Pharmacy". Report preparation date and time, and use by date and time (72 hours from preparation) on the bag label.
NOTE: This box contains **2 AMPOULES** in excess that are to be used as **RESERVE ONLY**.

INSTRUCTIONS: *Transférer le contenu de 23 ampoules dans la Poche de perfusion N° 1 + 370 ml de NaCl à 0.9%, comme indiqué dans la procédure détaillée « Instructions pour la Pharmacie ». Ecrire la date et l'heure de préparation ainsi que la date et l'heure de péremption (72 heures à partir de la préparation) sur l'étiquette de la poche.*
*NOTE: Cette boîte contient **2 AMPOULES** supplémentaires à n'utiliser que **COMME RESERVE**.*

CAUTION: New Drug-Limited by Federal law to investigational use. Investigational drug to be used by a qualified investigator only.
ATTENTION: *Nouvelle substance - Limitée à la recherche par la loi Fédérale. Produit de recherche, ne doit être utilisé que par un investigateur qualifié.*

Specimen Label for Treatment Box No. 2 [time period 24-48 hours]

STUDY (*ESSAI*) REP0104 Sponsor (*Promoteur*): Dompé s.p.a.; Via Campo di Pile, L'Aquila – Italy

TREATMENT BOX No. 2 (24-48 hours)
BOITE DE TRAITEMENT N° 2 (24-48 heures)

PATIENT No. XXYY
PATIENT N°

CONTAINS: 25 AMPOULES OF 10 mL REPERTAXIN (33 mg/mL) OR PLACEBO FOR INJECTABLE SOLUTION
CONTENU: *25 AMPOULES DE 10 mL DE SOLUTION INJECTABLE DE REPERTAXIN (33 mg/mL) OU DE PLACEBO*

BLINDED BATCH No.
N° DE LOT CODIFIE

EXPIRY DATE mm/yyyy
DATE DE PEREMPTION mm/aaaa

DO NOT STORE AT >30°C (86°F)
NE PAS ENTREPOSER A >30°C (86°F)

For questions - CRO contact =
Pour toute question contacter la ORC=

PPD

DIRECTIONS: Transfer into infusion BAG No. 2 the contents of **23 AMPOULES** + 370 mL of 0.9% NaCl, according to procedures detailed in the "Instructions to the Pharmacy". Report preparation date and time, and use by date and time (72 hours from preparation) on the bag label.
NOTE: This box contains **2 AMPOULES** in excess that are to be used as **RESERVE ONLY**.

INSTRUCTIONS: *Transférer le contenu de 23 ampoules dans la Poche de perfusion N° 2 + 370 ml de NaCl à 0.9%, comme indiqué dans la procédure détaillée « Instructions pour la Pharmacie ». Ecrire la date et l'heure de préparation ainsi que la date et l'heure de péremption (72 heures à partir de la préparation) sur l'étiquette de la poche.*
*NOTE: Cette boîte contient **2 AMPOULES** supplémentaires à n'utiliser que **COMME RESERVE**.*

CAUTION: New Drug-Limited by Federal law to investigational use. Investigational drug to be used by a qualified investigator only.
ATTENTION: *Nouvelle substance - Limitée à la recherche par la loi Fédérale. Produit de recherche, ne doit être utilisé que par un investigateur qualifié.*

Specimen double tear off Label for Infusion Bag No. 1 [time period 0-24 hours]

STUDY (*ESSAI*) REP0104 Sponsor (*Promoteur*): Dompé s.p.a.; Via Campo di Pile, L'Aquila – Italy

INFUSION BAG No. 1 (0-24 hours) - *POCHE DE PERFUSION N° 1 (0-24 heures)*

PATIENT No. XXYY / ***PATIENT N°*** **PATIENT INITIALS** |__|__|__| [to be entered by the Pharmacist] / ***INITIALES DU PATIENT*** *[à compléter par le Pharmacien]*

CONTAINS: 600 mL OF REPERTAXIN (12.65 mg/mL) OR PLACEBO INJECTABLE DOSING SOLUTION
CONTENU: *600 mL DE SOLUTION INJECTABLE DE REPERTAXIN (12.65 mg/mL) OU DE PLACEBO*

PREPARED ON / ***PREPAREE LE*** |__|__|/|__|__|/|__|__|__|__| dd (jj) / mm / yyyy (aaaa) |__|__| : |__|__| h : min [to be entered by the Pharmacist] *[à compléter par le Pharmacien]*

DO NOT STORE AT >30°C (86°F)
NE PAS ENTREPOSER A >30°C (86°F)

USE BY / ***UTILISER AVANT LE*** |__|__|/|__|__|/|__|__|__|__| dd (jj) / mm / yyyy (aaaa) |__|__| : |__|__| h : min [to be entered by the Pharmacist] *[à compléter par le Pharmacien]*

For questions - CRO contact = PPD
Pour toute question contacter la ORC=

DIRECTIONS: Administer as a continuous i.v. infusion into a (high flow) central vein, by an infusion pump. Infusion will start approximately 2 hours before reperfusion. Pump rate for the loading (0-30 min infusion) and maintenance (30 min – 24 hours infusion) dose will be adjusted according to patient body weight as per Appendix 3 of Study Protocol.
INSTRUCTIONS: *Administrer à l'aide d'une pompe à infusion en infusion continue i.v. dans une veine centrale (haut debit). L'infusion doit commencer environ 2 heures avant la reperfusion. Le flux de la pompe pour la dose de charge (infusion 0-30 min) et d'équilibre (infusion 30 min – 24 heures) devra être ajusté en fonction du poids du patient comme indiqué dans l'Appendice 3 du Protocole d'Essai.*

CAUTION: New Drug-Limited by Federal law to investigational use. Investigational drug to be used by a qualified investigator only.
ATTENTION: *Nouvelle substance - Limitée à la recherche par la loi Fédérale. Produit de recherche, ne doit être utilisé que par un investigateur qualifié.*

Specimen double tear off Label for Infusion Bag No. 2 [time period 24-48 hours]

STUDY (*ESSAI*) REP0104 Sponsor (*Promoteur*): Dompé s.p.a.; Via Campo di Pile, L'Aquila – Italy

INFUSION BAG No. 2 (24-48 hours) - *POCHE DE PERFUSION N° 2 (24-48 heures)*

PATIENT No. XXYY / ***PATIENT N°*** **PATIENT INITIALS** |__|__|__| [to be entered by the Pharmacist] / ***INITIALES DU PATIENT*** *[à compléter par le Pharmacien]*

CONTAINS: 600 mL OF REPERTAXIN (12.65 mg/mL) OR PLACEBO INJECTABLE DOSING SOLUTION
CONTENU: *600 mL DE SOLUTION INJECTABLE DE REPERTAXIN (12.65 mg/mL) OU DE PLACEBO*

PREPARED ON / ***PREPAREE LE*** |__|__|/|__|__|/|__|__|__|__| dd (jj) / mm / yyyy (aaaa) |__|__| : |__|__| h : min [to be entered by the Pharmacist] *[à compléter par le Pharmacien]*

DO NOT STORE AT >30°C (86°F)
NE PAS ENTREPOSER A >30°C (86°F)

USE BY / ***UTILISER AVANT LE*** |__|__|/|__|__|/|__|__|__|__| dd (jj) / mm / yyyy (aaaa) |__|__| : |__|__| h : min [to be entered by the Pharmacist] *[à compléter par le Pharmacien]*

For questions - CRO contact = PPD
Pour toute question contacter la ORC=

DIRECTIONS: Administer as a continuous i.v. infusion into a (high flow) central vein, by an infusion pump. Pump rate for the maintenance dose (24-48 hours infusion) will be adjusted according to patient body weight as per Appendix 3 of Study Protocol.

INSTRUCTIONS: *Administrer à l'aide d'une pompe à infusion en infusion continue i.v. dans une veine centrale (haut débit). Le flux de la pompe pour maintenir le dosage (infusion 24-48 heures) devra être ajusté en fonction du poids du patient comme indiqué dans l'Appendice 3 du Protocole d'Essai.*

CAUTION: New Drug-Limited by Federal law to investigational use. Investigational drug to be used by a qualified investigator only.
ATTENTION: *Nouvelle substance - Limitée à la recherche par la loi Fédérale. Produit de recherche, ne doit être utilisé que par un investigateur qualifié.*

Specimen label for each ampoule - repertaxin or placebo

STUDY REP0104 Dompé s.p.a., Italy

PATIENT No. XXYY

10 mL of repertaxin (33 mg/mL) or placebo

DO NOT STORE AT >30°C (86°F)
Blinded batch No.
Expiry date mm/yyyy

New Drug-Limited by Federal law to investigational use. Investigational drug to be used by a qualified investigator only.

14.3. APPENDIX 3 - STUDY DRUG INFUSION RATE BY BODY WEIGHT

Loading dose = 4.488 mg/kg /hour Dosing solution (repertaxin) = 12.65 mg/mL
Maintenance dose = 2.772 mg/kg /hour

BODY WEIGHT(kg) (each figure is from 0.00 to 0.99)	LOADING DOSE (mL/hour x 30 min)	MAINTENANCE DOSE (mL/hour x 47.5 hours)
30	10,6	6,6
31	11,0	6,8
32	11,4	7,0
33	11,7	7,2
34	12,1	7,5
35	12,4	7,7
36	12,8	7,9
37	13,1	8,1
38	13,5	8,3
39	13,8	8,5
40	14,2	8,8
41	14,5	9,0
42	14,9	9,2
43	15,3	9,4
44	15,6	9,6
45	16,0	9,9
46	16,3	10,1
47	16,7	10,3
48	17,0	10,5
49	17,4	10,7
50	17,7	11,0
51	18,1	11,2
52	18,4	11,4
53	18,8	11,6
54	19,2	11,8
55	19,5	12,1
56	19,9	12,3
57	20,2	12,5
58	20,6	12,7
59	20,9	12,9

BODY WEIGHT(kg) (each figure is from 0.00 to 0.99)	LOADING DOSE (mL/hour x 30 min)	MAINTENANCE DOSE (mL/hour x 47.5 hours)
60	21,3	13,1
61	21,6	13,4
62	22,0	13,6
63	22,4	13,8
64	22,7	14,0
65	23,1	14,2
66	23,4	14,5
67	23,8	14,7
68	24,1	14,9
69	24,5	15,1
70	24,8	15,3
71	25,2	15,6
72	25,5	15,8
73	25,9	16,0
74	26,3	16,2
75	26,6	16,4
76	27,0	16,7
77	27,3	16,9
78	27,7	17,1
79	28,0	17,3
80	28,4	17,5
81	28,7	17,7
82	29,1	18,0
83	29,4	18,2
84	29,8	18,4
85	30,2	18,6
86	30,5	18,8
87	30,9	19,1
88	31,2	19,3
89	31,6	19,5
90	31,9	19,7
91	32.3	19.9
92	32.6	20.2

BODY WEIGHT(kg) (each figure is from 0.00 to 0.99)	LOADING DOSE (mL/hour x 30 min)	MAINTENANCE DOSE (mL/hour x 47.5 hours)
93	33.0	20.4
94	33.3	20.6
95	33.7	20.8

14.4. APPENDIX 4 - STUDY FLOW CHART

Test/Examination	Screening	Tx	ICU admission	Time after ICU admission							ICU discharge	Hospital discharge	1 month	6 months	12 months
				12 h	24 h	36 h	48 h	54 h	60 h	72 h					
Informed consent	X														
Inclusion/exclusion criteria[1]	X														
Laboratory tests	X											X			
Renal/hepatic function[2]	X			X^2	X^2	X^2									
Study drug administration[3]		X----------X^2													
Pharmacokinetic sampling[2]					X^2		X^2	X^2							
PaO_2/FiO_2 ratio			X		X		X			X					
Chest X-ray			X		X		X			X					
Vital signs (BP & HR)[4]	X		X	X	X	X	X		X	X	X				
Cardiopulmonary measurements[4]			X	X	X	X	X		X	X	X				
BAL[5]					X^5										
Vital status											X	X	X	X	X
FEV_1 & FVC													X	X	X
BOS score														X	X
Cumulative acute rejection														X	X
Adverse Events[6]		X----------X^6											X----------X^6		
Prior and concomitant medications[7]	X----------X^7												X----------X^7		

1 = Confirmatory screening to take place upon admission to the center prior to randomization
2 = (shadowed boxes) Time is from the start of study drug infusion. The '24 hours' sample is taken @ 24±6 hours; the '54 hours' sample is taken in the interval 2-6 hours after the infusion end
3 = Study drug infusion will start approximately 2 hours before the anticipated time of reperfusion
4 = Vital signs and cardiopulmonary measurements (when a PA catheter is present) will be measured up to ICU discharge or up to 5 days after ICU admission, whichever occurs earlier
5 = BAL will be performed during mechanical ventilation, in the interval between 12 and 24 hours after reperfusion
6 = All AEs will be reported up to 10 days or hospital discharge. Thereafter, only untoward events that the Investigator assesses as at least possibly related to the study drug will be recorded
7 = After 10 days or hospital discharge, only drugs used for immunosuppression, infection prophylaxis, and AEs possibly related to the study drug (see 6 above) will be recorded

14.5. APPENDIX 5 - WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983,
41st WMA General Assembly, Hong Kong, September 1989,
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000
Note of clarification on Paragraph 29 added by WMA General Assembly, Washington 2002

A. INTRODUCTION

The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognised. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

The subjects must be volunteers and informed participants in the research project.

The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimise the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorised representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorised representative.

Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorised surrogate.

Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Note of clarification on Paragraph 29 of the WMA Declaration of Helsinki

The WMA hereby reaffirms its position that extreme care must be taken in making use of placebo-controlled trials and that in general this methodology should only be used in the absence of existing proven therapy. However. a placebo-controlled trial may be ethically acceptable, even if proven therapy is available, under the following circumstances:

- Where for compelling and scientifically sound methodological reasons its use is necessary to determine the efficacy or safety of a prophylactic, diagnostic or therapeutic method

or

- Where a prophylactic, diagnostic or therapeutic method is being investigated for a minor condition and the patients who receive placebo will not be subject to any additional risk or serious irreversible harm.

All other provisions of he Declaration of Helsinki must be adhered to, especially the need for appropriate ethical and scientific review. (6.10.02)

14.6. APPENDIX 6 - ACCEPTABLE TIME WINDOWS FOR ASSESSMENTS/ PROCEDURES

Assessment/Procedure	Window
Infusion Bag 1 begins approximately 2 hrs prior to reperfusion	> 1 hour prior to reperfusion
Change loading dose to maintenance dose @ 30 mins	30-35 minutes after start of infusion
Change Infusion Bag 1 to Infusion Bag 2 @ 24 hrs after start of infusion	± 1 hour
End of Infusion @ 48 hrs	± 1 hour
PaO2/FiO2 ratio measurements	
@ ICU Admission	± 2 hour
@ 24 hrs	± 2 hour
@ 48 hrs	± 4 hours
@ 72 hrs	± 4 hours
X-ray to calculate PGD Score	
@ ICU Admission	± 4 hour
@ 24 hrs	± 4 hours
@ 48 hrs	± 8 hours
@ 72 hrs	± 8 hours
Vitals & Cardiopulmonary measurements @ ICU admission and every 12 hours after ICU admission for 5 days or ICU discharge (whichever occurs earlier)	± 4 hours
Renal & Hepatic Function after infusion start	
@ 12 hrs	± 4 hours
@ 24 hrs	± 4 hours
@ 36 hrs	± 4 hours
PK samples	
After infusion start	24 ± 6 hours
Just prior to end of infusion	Up to 1 hour prior to infusion end
Post infusion end	2-6 hours
Mechanical Ventilation/Discharge	
@ 8 am every morning	± 4 hours
Hospital discharge blood sample	Up to 24 hours prior to discharge
Month 1 visit post transplant	30-35 days
Month 6 & Month 12 visits	± 2 weeks