COVER PAGE FOR STUDY PROTOCOL

Official Study Title: A Phase III Double-blind, Randomised, Parallel-Group Comparison of the Efficacy and Safety of FP-1201-lyo (Recombinant Human Interferon Beta-1a) and Placebo in the Treatment of Patients with Moderate or Severe Acute Respiratory Distress Syndrome

Unique Protocol ID: FPCLI002 (INTEREST Study)

Date of the Study Protocol: 29 August 2017 (version 6.0)

NCT Number: NCT02622724

TITLE: A Phase III Double-blind, Randomised, Parallel-Group Comparison of the Efficacy and Safety of FP-1201-lyo (Recombinant Human Interferon Beta-1a) and Placebo in the Treatment of Patients with Moderate or Severe Acute Respiratory Distress Syndrome

INTEREST study

Sponsor:	Faron Pharmaceuticals Ltd Joukahaisenkatu 6 FIN-20520 Turku Finland
Global Co-ordinating Investigators	███████████ ██████████
Sponsor Protocol No.:	FPCLI002
EudraCT No.:	2014-005260-15
Study Drug Name:	FP-1201-lyo
Development Phase:	III
Date of Protocol:	29 Aug 2017, Final Version 6.0 Includes: Amendment 1 (19 October 2015) Amendment 2 (22 October 2015) Non-Substantial Amendment 3 (30 June 2016) Non-Substantial Amendment 4 (2 May 2017) Amendment 5 (29 Aug 2017)
Date of Previous Protocol:	2 May 2017, Final Version 5.0

The study will be conducted according to the protocol and in compliance with Good Clinical Practice, with the Declaration of Helsinki and with other applicable regulatory requirements.
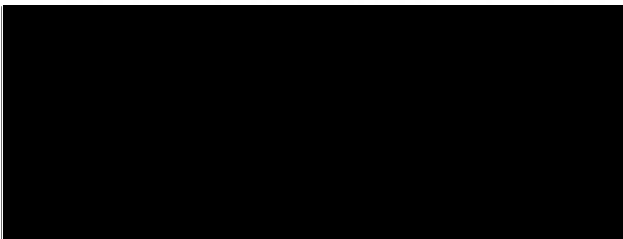
SIGNATURE PAGE

Declaration of Sponsor or Responsible Medical Officer

Title: A Phase III Double-blind, Randomised, Parallel-Group Comparison of the Efficacy and Safety of FP-1201-lyo (Recombinant Human Interferon Beta-1a) and Placebo in the Treatment of Patients with Moderate or Severe Acute Respiratory Distress Syndrome

This study protocol including Amendments 1, 2, 3, 4 and 5 was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, as amended in 2013, and the ICH guidelines on Good Clinical Practice.
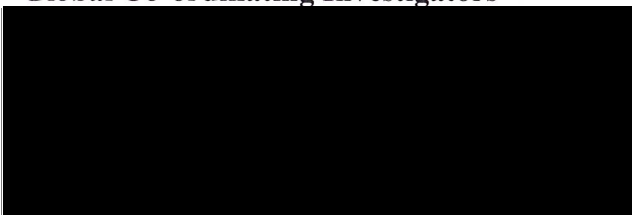
11th Sep 2017
_____
Date

Faron Pharmaceuticals Ltd

Declaration of the Global Co-ordinating Investigators

Title: A Phase III Double-blind, Randomised, Parallel-Group Comparison of the Efficacy and Safety of FP-1201-lyo (Recombinant Human Interferon Beta-1a) and Placebo in the Treatment of Patients with Moderate or Severe Acute Respiratory Distress Syndrome

This study protocol including Amendments 1, 2, 3, 4 and 5 was subjected to critical review and has been approved by the Sponsor. The information it contains is consistent with the current risk/benefit evaluation of the investigational product as well as with the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, as amended in 2013, and the ICH guidelines on Good Clinical Practice.

Global Co-ordinating Investigators

11ʰ Sept 2017
Date

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11ᵗʰ Sep 2017
Date

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Declaration of the National Co-ordinating Investigator

Title: A Phase III Double-blind, Randomised, Parallel-Group Comparison of the Efficacy and Safety of FP-1201-lyo (Recombinant Human Interferon Beta-1a) and Placebo in the Treatment of Patients with Moderate or Severe Acute Respiratory Distress Syndrome

This study protocol including Amendments 1, 2, 3, 4 and 5 was subjected to critical review and has been approved by the Sponsor. The information it contains is consistent with the current risk/benefit evaluation of the investigational product as well as with the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, as amended in 2013, and the ICH guidelines on Good Clinical Practice.
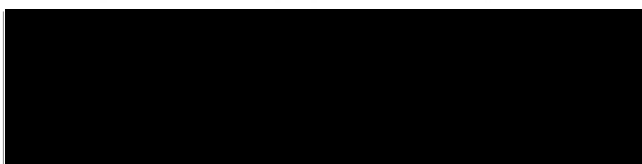
National Co-ordinating Investigator

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Signature   Date

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Title (block letters)

_____

Institution (block letters)

_____

Phone number

Declaration of the Investigator

Title: A Phase III Double-blind, Randomised, Parallel-Group Comparison of the Efficacy and Safety of FP-1201-lyo (Recombinant Human Interferon Beta-1a) and Placebo in the Treatment of Patients with Moderate or Severe Acute Respiratory Distress Syndrome

All documentation for this study that is supplied to me, and that has not been previously published will be kept in the strictest confidence. This documentation includes this study protocol, Investigator's Brochure, electronic Case Report Form, and other scientific data.

The study will not commence without the prior written approval of a properly constituted Independent Ethics Committee (IEC). No changes will be made to the study protocol without the prior written approval of the Sponsor and the IEC, except where necessary to eliminate an immediate hazard to the patients.

I have read and understood and agree to abide by all the conditions and instructions contained in this protocol including Amendments 1, 2, 3, 4 and 5.

Responsible Investigator of the local study centre

_____   _____
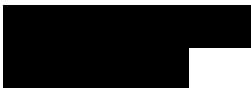Signature   Date

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Name (block letters)

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Title (block letters)

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Institution (block letters)

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Phone number

PROTOCOL SYNOPSIS

Title	A Phase III Double-blind, Randomised, Parallel-Group Comparison of the Efficacy and Safety of FP-1201-lyo (Recombinant Human Interferon Beta-1a) and Placebo in the Treatment of Patients with Moderate or Severe Acute Respiratory Distress Syndrome
Sponsor Study No.	FPCLI002
Phase	III
Sponsor	Faron Pharmaceuticals Ltd
Global Co-ordinating Investigators	██████████████
Study Centre(s)	Approximately 70-80 investigational sites located in Europe will participate in the study

Objectives

Primary Objective To demonstrate the efficacy of FP-1201-lyo in improving the clinical course and outcome based on survival and need for mechanical ventilation in patients with moderate or severe acute respiratory distress syndrome (ARDS)

Secondary Objectives Safety

- To assess the safety of FP-1201-lyo compared with placebo

Efficacy

- To evaluate 28-day all-cause mortality of FP-1201-lyo compared with placebo

- To evaluate all-cause mortality at other selected time points

- To evaluate the efficacy of FP-1201-lyo compared with placebo by assessing:
    - days free of organ failure
    - days free of renal support
    - days free of vasoactive support
    - days free of mechanical ventilation
    - number of intensive care unit (ICU)-free days
    - number of days in hospital

- To evaluate the immunogenicity of FP-1201-lyo by monitoring neutralising antibodies to interferon (IFN) beta-1a

- To evaluate the pharmacodynamics (PD) of FP-1201-lyo with myxovirus resistance protein A (MxA)

- To evaluate patient outcomes for respiratory (forced expiratory volume in 1 second [FEV_1]) and neurological functioning (6-minute walk test [6MWT]) and quality of life (QoL) (EuroQol 5-Dimensions 3-Levels questionnaire [EQ-5D-3L]) measured at selected time points

Pharmacoeconomics

- To evaluate selected pharmacoeconomic parameters

Exploratory Objectives

Exploratory

- To evaluate gas exchange (partial pressure of oxygen/fraction of inspired oxygen [PaO_2/FiO_2] ratio) during mechanical ventilation as an indicator of improving lung function on treatment
- To evaluate the PD of FP-1201-lyo using the cluster of differentiation (CD)73 biomarker
- To evaluate selected potential inflammatory markers (PIMs)
- To obtain a blood sample for future pharmacogenetic analysis
- To evaluate all-cause mortality, QoL, and respiratory and neurological functioning at extended follow-up (Day [D]360)

Design   Double-blind, randomised, parallel-group evaluation of FP-1201-lyo compared with placebo

Patients in the ICU will undergo screening during which informed consent will be obtained and eligibility assessed. No more than 48 hours may elapse between confirmation of moderate or severe ARDS and administration of the first dose of study drug

Following randomisation, stratified by country and ARDS severity, patients will be treated daily with FP-1201-lyo 10 μg or placebo for 6 days and will undergo daily assessments while in the ICU for a maximum of 28 days. Long-term follow-up will occur at D90 (visit or telephone contact), D180, and an extended follow-up visit at D360, which is the final visit. The main analysis and reporting will use D28 and long-term follow up D90 data. The results of the long-term follow up visit (D180) and the extended follow-up visit (D360) will each be reported separately as addendums to the Clinical Study Report

An Independent Data Monitoring Committee will be established to monitor safety

Treatment	FP-1201-lyo 10 µg (recombinant human IFN beta-1a) or placebo will be administered once daily as an intravenous bolus injection for 6 days
	FP-1201-lyo is a lyophilised powder reconstituted in water for injection
Number of Patients	300 patients will be randomised with the aim of having 272 evaluable patients at 70–80 investigational sites
Population	Adult patients diagnosed with moderate or severe ARDS.
	The inclusion and exclusion criteria apply during screening and prior to administration of the first dose of study drug on D1
Inclusion Criteria	All patients must be intubated and mechanically ventilated to diagnose ARDS and be eligible for the study

1. Patient has a diagnosis of moderate or severe ARDS according to the Berlin definition of ARDS:

1.1 Acute onset of respiratory failure within 1 week of a known clinical insult or new or worsening respiratory symptoms

1.2 Respiratory failure associated with known ARDS risk factors and not fully explained by either cardiac failure or fluid overload (an objective assessment of cardiac failure or fluid overload is needed if no risk factors for ARDS [moderate or severe ARDS] are present)

1.3 Radiological abnormalities on chest X-ray or on computerised tomography scan, i.e., bilateral opacities that are not fully explained by effusions, nodules, masses or lobar/lung collapse

1.4 Hypoxaemia:

- Moderate ARDS: PaO_2/FiO_2 >100 mmHg (>13.3 kPa) to ≤200 mmHg (≤26.6 kPa) with positive end expiratory pressure (PEEP) ≥5 cmH_2O

- Severe ARDS: PaO_2/FiO_2 ≤100 mmHg (≤13.3 kPa) with PEEP ≥5 cmH_2O

2. The radiological and hypoxaemia criteria (1.3 and 1.4) must be met within the same 24-hour period. The time of onset of ARDS is when the last of the two specified ARDS criteria is met

3. Administration of the first dose of study drug must be planned to take place within 48 hours of moderate or severe ARDS diagnosis.

4. Patient is intubated and mechanically ventilated

5. A signed informed consent form from the patient or the patient's personal legal representative or a professional legal representative must be available

6. Patient is aged ≥18 years

Exclusion Criteria

1. Woman known to be pregnant, lactating or with a positive (urine or serum test) or indeterminate (serum test) pregnancy test
2. Patient is simultaneously taking part in another pharmacotherapy protocol
3. Patient is not expected to survive for 24 hours
4. Patient has an underlying clinical condition where, in the opinion of the Investigator, it would be extremely unlikely that the patient would come off ventilation, e.g., motor neurone disease, Duchenne muscular dystrophy or rapidly progressive interstitial pulmonary fibrosis
5. Patient has severe chronic obstructive pulmonary disease requiring long-term home oxygen therapy or mechanical ventilation (non-invasive ventilation or via tracheotomy) except for continuous positive airway pressure (CPAP) or bi-level positive airway pressure used solely for sleep-disordered breathing
6. Patient has congestive heart failure, defined as New York Heart Association class IV
7. Patient has acute left ventricular failure
8. Patient has liver failure (Child–Pugh grade C)
9. Patient has received any prior interferon
10. Patient has known hypersensitivity to natural or recombinant IFN beta or to any of the excipients
11. Patient is receiving renal dialysis therapy for chronic renal failure
12. Patient is receiving extra-corporeal membrane oxygenation, high-frequency oscillatory ventilation or any form of extra-corporeal lung support
13. Patient has had any form of mechanical ventilation (invasive or non-invasive, excluding CPAP alone) for longer than 48 hours prior to the diagnosis of ARDS
    Non-invasive ventilation has to be continuously applied for at least 12 hours per day in these 48 hours
14. Patient has burns to ≥15% of their total body surface area

Criteria for Evaluation of Efficacy

Primary Efficacy Endpoint:

- Composite endpoint including any cause of death at D28 and days free of mechanical ventilation (VFDsurv) within 28 days among survivors

Secondary Efficacy Endpoints:

- Secondary endpoints relating to the efficacy of FP-1201-lyo treatment:
  - All-cause mortality at D28, D90 and D180
  - Mortality in ICU up to D28
  - Mortality in hospital up to D28

- Other secondary efficacy endpoints at D28 :
  - Days free of organ failure (Sequential Organ Failure Assessment methodology) (D28 or last day in ICU if patient has left the ICU earlier than D28, or at withdrawal)
  - Days free of renal support
  - Days free of vasoactive support
  - Days free of mechanical ventilation
  - Number of ICU-free days
  - Number of days in hospital
- Presence of neutralising antibodies to IFN beta-1a at baseline and D28 (or last day in ICU if patient has left the ICU earlier than D28, or at withdrawal)
- Evaluation of PD using MxA biomarker from baseline to D14
- Long-term secondary endpoints, relating to QoL, respiratory and neurological functioning at D180:
  - EQ-5D-3L
  - 6MWT
  - FEV_1

Criteria for Evaluation of Safety	- Adverse events up to D28, AEs occurring after D28 if the investigator considers there is a causal relationship with the study drug and all deaths up to D360 - Physical examination, vital signs and laboratory results (biochemistry, haematology and urinalysis) up to D28 (or last day in ICU if patient has left the ICU earlier than D28, or at withdrawal)
Criteria for Evaluation of Pharmacoeconomics	- Endpoints for the evaluation of pharmacoeconomics at D28:

	– Days free of organ failure (D28 or last day in ICU if patient has left the ICU earlier than D28, or at withdrawal) – Days free of renal support – Days free of vasoactive support – Days free of mechanical ventilation – Number of ICU-free days – Number of days in hospital

Criteria for Evaluation of Exploratory Variables	• Exploratory endpoints relating to the efficacy of FP-1201-lyo treatment:
	  – Composite endpoint including mortality and days free of mechanical ventilation (VFDsurv) within 90 days among survivors
	  – Ordered categorical endpoint defined as improvement (severe to moderate/mild; from moderate to mild ARDS), no change or worsening (from moderate to severe/death; from severe to death) in terms of gas exchange (PaO_2/FiO_2 ratio) from baseline to D28 (or last day in ICU if patient has left the ICU earlier than D28, or at withdrawal)
	• Change in the treatment-specific exploratory biomarker CD73 concentration from baseline to D14
	• Changes in levels of PIMs, including but not limited to, interleukin-6 and -8 from baseline to D14
	• A blood sample will be taken for pharmacogenetic analysis and correlation with other markers of the activity of FP-1201-lyo
	• Extended long-term follow-up: 12-month mortality rate, EQ-5D-3L, 6MWT and FEV_1

Statistical Methods	For 90% power and a two-sided Mann–Whitney U-test at the significance level of 0.05, a total of 272 patients are required based on the following assumptions: • mortality rate of 30% in the control group and 15% in the FP-1201-lyo group at D28 • 20% of patients survive but with zero ventilator-free days (VFDs) in the control group • A mean difference (FP-1201-lyo minus control) of 3.0 days in mean ventilator-free days where patients who die are assigned a score of 0 However, assuming 5% of patients (16) drop-out and a further 4% (12) of the remaining patients will not be evaluable for the efficacy analysis, the study will

randomise 300 patients to build in some flexibility around the assumptions listed above.

Analysis Sets:

The Full Analysis Set (FAS) will consist of all randomised and treated patients

The Per-Protocol Set (PPS) will consist of those patients in the FAS excluding patients with major protocol violations. A list of major protocol violations relevant for excluding data from the PPS will be detailed in the Statistical Analysis Plan. The precise definition of the PPS at the patient level will be identified at the blinded data review meeting

Statistical analyses for the primary and secondary endpoints will be performed on both the FAS and PPS

The safety set will consist of all patients who receive at least one dose of study drug. The safety and tolerability analyses will be based on this analysis set. A patient who receives the wrong treatment according to the randomisation will be analysed for safety and tolerability in the treatment group corresponding to the treatment received

Primary Endpoint Analysis

The primary composite endpoint includes death and days free of mechanical ventilation within 28 days among survivors. VFDs to D28 is defined as the number of calendar days during which the patient is ventilator-free including two unassisted breathing (UAB) days to D28, assuming that a patient survives for at least two consecutive calendar days after initiating UAB. The non-parametric analysis of the primary composite endpoint VFDsurv is based on a scoring scheme with patients who do better getting a higher score. All patients who die before 28 days will be assigned a VFDsurv score of -1. For those patients who survive to D28 the VFDsurv score will be equal to the number of VFDs calculated according to the above definition. The statistical method for group comparison of this endpoint will be based on the van Elteren test adjusting for the country, ARDS severity and key baseline characteristics. The statistical methodology for the scoring scheme is as set down in Finkelstein and Schoenfeld.

LIST OF STUDY PERSONNEL

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	Medical Monitor local rate phone numbers:
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	Czech Republic: █████
	France: █████
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Responsible Statistician	▮▮▮
	Data Magik Ltd., UK
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| **Interactive Web-Response System (IWRS)** | For contact details please refer to the IWRS User Manual in the Investigator Package |

TABLE OF CONTENTS

Tables

Figures

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

A-aDO$_2$	Alveolar-arterial oxygen difference
AE	Adverse Event
ALI	Acute Lung Injury
APACHE II	Acute Physiology and Chronic Health Evaluation II
ARDS	Acute Respiratory Distress Syndrome
BIPAP	Bilevel Positive Airway Pressure
BP	Blood Pressure
CAPD	Continuous Ambulatory Peritoneal Dialysis
CD73	Cluster of differentiation 73
CDMS	Clinical Data Management System
CI	Confidence Interval
CPAP	Continuous Positive Airway Pressure
CRA	Clinical Research Associate (Monitor)
CT	Computerised Tomography
D	Day (as in treatment day)
ECG	Electrocardiogram
e-CRF	Electronic Case Report Form
EQ-5D-3L	EuroQol 5-Dimensions 3-Levels questionnaire
FAS	Full Analysis Set
FEV$_1$	Forced Expiratory Volume in 1 second
FiO$_2$	Fraction of inspired oxygen
FU	Follow-up
GCS	Glasgow Coma Scale
HD	Haemodialysis
HFOV	High-Frequency Oscillatory Ventilation
HR	Heart Rate
ICD	Informed Consent Document
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IFN	Interferon
IVRS	Interactive Voice-Response System
IWRS	Interactive Web-Response System

MM	Medical Monitor
MIU	Million International Units
MxA	Myxovirus resistance protein A
NOAEL	No Observed Adverse-Effect Level
OTD	Optimum Tolerated Dose
PaO$_2$	Partial pressure of oxygen
PBW	Predicted Body Weight
PEEP	Positive End Expiratory Pressure
PerLR	Personal Legal Representative
PD	Pharmacodynamics
PHT	Portal hypertension
PIM	Potential Inflammatory Marker
PPS	Per-Protocol Set
PrfLR	Professional Legal Representative
QoL	Quality of Life
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOFA	Sequential Organ Failure Assessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-Emergent Adverse Event
UAB	Unassisted breathing
VFD	Ventilator-Free Day
VFDsurv	Composite endpoint including any cause death at D28 and days free of mechanical ventilation within 28 days among survivors
WFI	Water For Injection
6MWT	6-Minute Walk Test

1   INTRODUCTION

This is a Phase III clinical study to investigate the efficacy and safety of FP-1201-lyo (recombinant human interferon [IFN] beta-1a) in patients diagnosed with moderate or severe acute respiratory distress syndrome (ARDS). FP-1201-lyo is a lyophilised powder form of recombinant human IFN beta-1a reconstituted in water for injection and is administered intravenously. Recombinant human IFN beta-1a is an approved treatment for patients with relapsing-remitting multiple sclerosis and its safety profile in such patients is well characterised.

1.1   Background

1.1.1   Acute Respiratory Distress Syndrome

ARDS is a type of acute diffuse lung injury associated with recognised risk factors that is characterised by inflammation leading to increased pulmonary vascular permeability and loss of aerated lung tissue. ARDS is a serious clinical disorder, which follows a variety of severe lung insults. Such insults include, among others, pneumonia, aspiration of gastric contents, non-pulmonary sepsis and major trauma.

ARDS is characterised by injury to the endothelial barriers and alveolar epithelium of the lung, acute lung inflammation and protein-rich pulmonary oedema leading to acute respiratory failure. In the Berlin definition of ARDS (see Table 1 [Ref: ARDS Definition Task Force 2012]) severity can range from mild, through moderate, to severe ARDS.

Table 1   The Berlin ARDS Definition

Characteristic	Mild ARDS	Moderate ARDS	Severe ARDS
Timing	Acute onset within 1 week of a known clinical insult or new or worsening respiratory symptoms		
Hypoxaemia	PaO_2/FiO_2 >200–≤300 mmHg with PEEP or CPAP ≥5 cmH$_2$O	PaO_2/FiO_2 >100– ≤200 mmHg with PEEP ≥5 cmH$_2$O	PaO_2/FiO_2 ≤100 mmHg with PEEP ≥5 cmH$_2$O
Origin of oedema	Respiratory failure associated with known ARDS risk factors and not fully explained by cardiac failure or fluid overload. An objective assessment of cardiac failure or fluid overload is needed if no ARDS risk factors are present		
Radiological abnormalities (chest X-ray or CT scan)	Bilateral opacities not fully explained by effusions, nodules, masses or lobar/lung collapse		

Abbreviations: ARDS=acute respiratory distress syndrome; CPAP=continuous positive airway pressure; CT=computerised tomography; PaO2/FiO2=partial pressure of oxygen/fraction of inspired oxygen; PEEP=positive end expiratory pressure.

The mortality rates in patients with ARDS range from approximately 20% to 40% across all severities, and can be even higher when associated with dysfunction in other organs. Although mortality from ARDS has decreased in the last decade due to improvements in supportive care and in the treatment of underlying conditions, it still remains at a high level.

ARDS is also costly in health economics terms. Patients with ARDS consume significantly more resources than matched patients without moderate or severe ARDS

because they require longer intensive care unit (ICU) and hospital stays. The quality of life (QoL) of these patients may also be significantly impacted, with 35% of patients with moderate or severe ARDS unable to return to work 24 months after hospital discharge.

There are currently no approved pharmacological therapies for ARDS. ARDS has no primary treatments proven to improve outcomes other than supportive care.

1.1.2 FP-1201-lyo in Moderate or Severe Acute Respiratory Distress Syndrome

In serious life-threatening situations, such as infection leading to sepsis or trauma causing massive tissue injuries, an escalation of the systemic inflammatory response leads to multiple organ failure including ARDS. In the case of ARDS, a key pathophysiological result is increased vascular leakage, which has been suggested to be related to a lack of adenosine, which acts to enhance endothelial barrier function. Therefore any biological substances that act to increase adenosine levels should reduce vascular leakage and be of benefit in ARDS. Such a substance is cluster of differentiation 73 (CD73) – a cell surface enzyme. Interferons, such as IFN beta-1a, have been shown to up-regulate CD73 and therefore could be a potential treatment for moderate or severe ARDS. Preclinical studies have shown that CD73 expression on endothelial cells is up-regulated by IFN beta-1a treatment in a time- and dose-dependent fashion. [Ref: Kiss et al 2007] Furthermore, in a mouse multi-organ failure model, IFN beta-1a was shown to be of benefit in protecting the alveolar structure from damage compared with controls. In addition, IFN beta-1a treatment has been shown to prevent leakage in animal models of acute lung injury (ALI). Enhanced adenosine production also controls leucocyte infiltration, thus reducing the escalation of inflammation in lungs.

The studies conducted by Faron Pharmaceuticals Ltd to date have been performed with the lyophilised product (FP-1201-lyo) intended to be used in clinical studies and subsequent commercial use. A 28-day safety study was conducted in cynomolgus monkeys at three dose levels. This study showed that treatment with intravenous FP-1201-lyo at dose levels of 0.25, 1.0 and 3.0 million international units (MIU)/kg/d was well tolerated. FP-1201-lyo treatment was associated with minor changes in haematological and clinical chemistry variables, including an expected increase in concentrations of myxovirus resistance protein A (MxA) in the blood and increased neutralising antibody activity on completion of treatment, particularly at the highest dose.

The no observed adverse-effect level (NOAEL) for IFN beta-1a in cynomolgus monkeys was considered 3.0 MIU/kg/d for 28 days. In a 70 kg human this would translate to a dose of 210 MIU/d or a total dose of 1260 MIU over 6 days. The proposed daily dose for this clinical study is 2.7 MIU/d (10 µg/d), i.e., a total of 16.2 MIU (60 µg) over 6 days. The NOAEL is therefore 77.7 times the proposed 6-day exposure to IFN beta-1a.

Recombinant human IFN beta-1a (FP-1201) was assessed for the treatment of ALI and ARDS (1994 American-European Consensus Conference definition of ALI/ARDS [Ref: Bernard et al 1994]) in a Phase I/II study (FPCLI001) [Ref: Bellingan et al 2014]. This open-label study included 37 patients with ALI/ARDS and the optimum tolerated dose (OTD) was shown to be FP-1201 10 µg daily for 6 days. The primary efficacy endpoint was the Day 28 mortality rate, which was 8.1% in the safety population – well below that normally seen in ICUs in the UK for patients with ALI/ARDS. From the literature, Day 28 mortality rates for ALI/ARDS vary from

20% (ALI) to over 60% (ARDS), with a generally accepted figure of approximately 40%. [Ref: Phua et al 2009, Doyle et al 1995, Zilberberg et al 1998, Sloane et al 1992] The long-term efficacy endpoint of 6-month mortality also demonstrated a mortality rate well below that expected for this population of patients. Four of the 37 patients died before the 6-month time point.

Pyrexia was the most common drug-related treatment-emergent adverse event (TEAE) in the study. All pyrexia events resolved rapidly without sequelae. Clinically significant low haemoglobin values were recorded in all cohorts at different time points during the study (including both at screening and at Day 28). No other trends in laboratory values or other safety variables were observed from baseline to Day 28. Data from the measurements of vital signs, electrocardiograms (ECGs), other laboratory data (biochemistry and haematology) and physical examinations showed no clear trends. At Day 28, chest X-rays for 19 patients showed improvements since the previous assessment.

Since the completion of the Phase I/II study the terminology relating to ALI and ARDS has been reviewed and revised. The term ALI has been dropped and ARDS is now defined as mild, moderate or severe, with clearly defined criteria applied for: timing of symptom onset; severity of hypoxaemia; origin of lung oedema; and level of radiological abnormality as assessed by chest X-ray. The revision of the American-European Consensus Conference definition of ARDS was presented at the meeting of the European Society of Intensive Care Medicine held in Berlin, Germany, on 5 October 2011. The Berlin definition [Ref: ARDS Definition Task Force 2012] (see Table 1) will be used in this Phase III study.

Further details can be found in the Investigator's Brochure, which contains comprehensive information on the investigational product. Also see Section 3.1 for details concerning the design of the current study and Section 3.3 for justification of the design of this study.

1.2   Rationale

The results of the Phase I/II study (FPCLI001) provided evidence for the beneficial effect of FP-1201 –intravenously administered IFN beta – in patients with ARDS and fully supported the conduct of a pivotal and well-controlled Phase III study.

The dose selected for this study (10 μg) is based on information from the previous study, where the maximum tolerated dose was found to be 22 μg. A dose of 10 μg was shown to be the OTD based on information from dose-limiting toxicity and proven markers of IFN beta-1a biological activity. An expansion cohort of 22 patients treated with the OTD (10 μg) demonstrated clear preliminary evidence for the efficacy and biological activity of IFN beta-1a using proven surrogate markers without major safety concerns.

Early diagnosis and treatment is essential for effectively treating ARDS before it becomes so severe that organ damage or death is almost inevitable. The rapid systemic exposure of medication provided by administering the drug intravenously makes this the appropriate route of administration in this patient population. In severely ill sedated patients, poor peripheral circulation may result in drugs given subcutaneously not being adequately distributed.

In relation to the treatment duration of only 6 days, the pathogenesis of ARDS is divided into three distinct phases – acute (days 1–6), sub-acute (days 7–14) and chronic (day 15+) – with fibrosis of the lungs beginning within the first week after

initial insult. Almost all patients who fail to improve or deteriorate after 1 week of ventilation have evidence of lung fibrosis. Administering treatment beyond 6 days would add little value to patients included in this study. This 6-day dosing regimen has therefore been selected as the optimal treatment regimen.

1.3   Risk/Benefit Assessment

Non-clinical data reveal no special hazard for humans treated with FP-1201 in conventional studies of safety, pharmacology, repeated-dose toxicity and genotoxicity.

ARDS is a severe condition with a high mortality rate, despite progress in ICU medicine. Currently there is no approved pharmacological therapy available for ARDS. Patients with ARDS are treated with intensive support, which includes various strategies for assisted ventilation. The results of the Phase I/II study (FPCLI001) provided evidence for the beneficial effect of FP-1201 in patients with ARDS and fully supported the conduct of a pivotal and well-controlled Phase III study.

IFNs were the first cytokines to be administered to humans; they are endogenous pyrogens and fever has been recognised as a frequent event. The febrile response varies with the dose, type and route of administration. Fever is characterised by preceding mild malaise and shaking chills that lead to elevation of body temperature. IFNs only occasionally cause rigors, in contrast to tumour necrosis factors. There is a well-described thermal ceiling, which means that fever does not exceed certain levels, regardless of the dose of IFN used. In addition, pyrogenic tolerance often develops with daily administration. This gradual diminution in pyrogenicity is one of the hallmarks of IFN therapy, a characteristic not shared by therapy with other pyrogenic cytokines.

On the first day of IFN therapy, the patient's temperature characteristically reaches 38–39°C (orally) 5–6 hours after IFN administration, and this elevation persists for around 2 hours. The temperature elevations experienced by patients vary substantially between individuals. The pyrogenic effect (high fever, rigors and chills) of IFN beta-1a was observed in the Phase I/II study at the highest dose of FP-1201 22 µg; this was shown to be the maximum tolerated dose.

IFN-induced fever responds to antipyretic analgesic drugs and they are recommended to decrease or prevent the flu-like symptoms associated with FP-1201-lyo administration.

The objectives of the Phase I/II clinical study were to assess the safety, tolerability and preliminary efficacy of the optimal tolerated dose, which was shown to be 10 µg daily for 6 days. The scientific rationale is that a biological substance, which acts to increase adenosine levels, should reduce vascular leakage and be of benefit in ARDS. The benefit of interferon beta-1a in the Phase I/II was shown by the fall in the mortality rate and the number of ICU days. The pivotal clinical study, FPCLI002, is being conducted to confirm the safety and efficacy of interferon beta-1a (FP-1201-lyo) in patients with moderate to severe ARDS.

The available information suggests that the present study has a favourable risk/benefit ratio.

2   STUDY OBJECTIVES

2.1   Primary Objective

The primary objective of this study is to demonstrate the efficacy of FP-1201-lyo in improving the clinical course and outcomes based on survival and need for mechanical ventilation in patients with moderate or severe ARDS.

2.2   Secondary Objectives

2.2.1   Safety Objectives

- To assess the safety of FP-1201-lyo compared with placebo

2.2.2   Efficacy Objectives

- To evaluate 28-day all-cause mortality of FP-1201-lyo compared with placebo
- To evaluate all-cause mortality at other selected time points
- To evaluate the efficacy of FP-1201-lyo compared with placebo by assessing:
    - Days free of organ failure
    - Days free of renal support
    - Days free of vasoactive support
    - Days free of mechanical ventilation
    - Number of ICU-free days
    - Number of days in hospital
- To evaluate the immunogenicity of FP-1201-lyo by monitoring neutralising antibodies to IFN beta-1a
- To evaluate the pharmacodynamics (PD) of FP-1201-lyo with MxA
- To evaluate patient outcomes for respiratory (forced expiratory volume in 1 second [FEV_1]) and neurological functioning (6-minute walk test [6MWT] [Ref: Holland et al 2014]) and QoL (EuroQol 5-Dimensions 3-Levels questionnaire [EQ-5D-3L]) measured at selected time points

2.2.3   Pharmacoeconomic Objectives

- To evaluate selected pharmacoeconomic parameters

2.2.4   Exploratory Objectives

- To evaluate gas exchange (partial pressure of oxygen/fraction of inspired oxygen [PaO_2/FiO_2] ratio) during mechanical ventilation as an indicator of improving lung function on treatment
- To evaluate the PD of FP-1201-lyo using the biomarker CD73
- To evaluate selected potential inflammatory markers (PIMs)
- To obtain a blood sample for future pharmacogenetic analysis
- To evaluate all-cause mortality, QoL, and respiratory and neurological functioning at extended follow-up (Day [D]360)

3   OVERALL DESIGN AND PLAN OF THE STUDY

3.1   Overview

This is a multicentre, Phase III, double-blind, randomised, parallel-group comparison study of the efficacy and safety of FP-1201-lyo compared with placebo in adult patients diagnosed with moderate or severe ARDS. A composite endpoint of death and days free of mechanical ventilation within 28 days among survivors is the primary endpoint; all-cause mortality at D28 is one of the secondary endpoints. Both treatment groups will receive supportive care (see Appendix 1, Section 11.1).

A total of 300 patients will be randomised into the study to achieve 272 patients are evaluable for efficacy.

Patients in the ICU will undergo screening during which written informed consent will be obtained and eligibility assessed. Not more than 48 hours may elapse between confirmation of moderate or severe ARDS and administration of the first dose of study drug. An interactive web-response system (IWRS) will be used to randomise the patients using country and ARDS severity as stratification parameters.

Following randomisation, patients will be treated daily with FP-1201-lyo 10 μg or placebo intravenously as a bolus for 6 days and will undergo daily assessments while in the ICU for a maximum of 28 days. The main analysis and reporting will use D28 and long-term follow-up D90 data. Long-term follow-up will occur at D180 and an extended follow-up at D360, the results of which will each be reported separately as addendums to the Clinical Study Report. The overall duration of the study for a patient is therefore 12 months, including the 6-month extended follow-up phase. During the long-term and extended follow-up periods, and after the end of the extended follow-up visit (D360), patient care will follow normal hospital procedures, as appropriate. The study will be completed when the final patient completes their D360 study assessment. No interim analyses are planned. The study design is summarised in Figure 1, and a more detailed flow chart showing the screening and randomisation procedures is shown in Figure 2.

Discontinuation criteria for individual patients and the entire study are described in Section 4.4.1.

The study design incorporates an Independent Data Monitoring Committee (IDMC) that will review ongoing safety (i.e., adverse events [AEs] and serious AEs [SAEs]), and will also make recommendations to the Sponsor as described in the IDMC charter. Details of the IDMC are given in Section 9.8.

A schedule for the tests and assessments to be conducted during this study is given in Section 7.1 (Table 5).

Figure 1: Study Design

Day Visit	-48 h Screening	Randomisation	pre-dose on D1 --- D1–D6 Double-blind treatment period	D7–D28 Short-term FU	D90[c] Long-term FU	D180	D360 Extended FU
		Baseline includes pre-dose on D1[a]	Daily assessments up to D28 while in ICU[b]				End of Study
					CSR	*Addendum to CSR*	*Addendum to CSR*
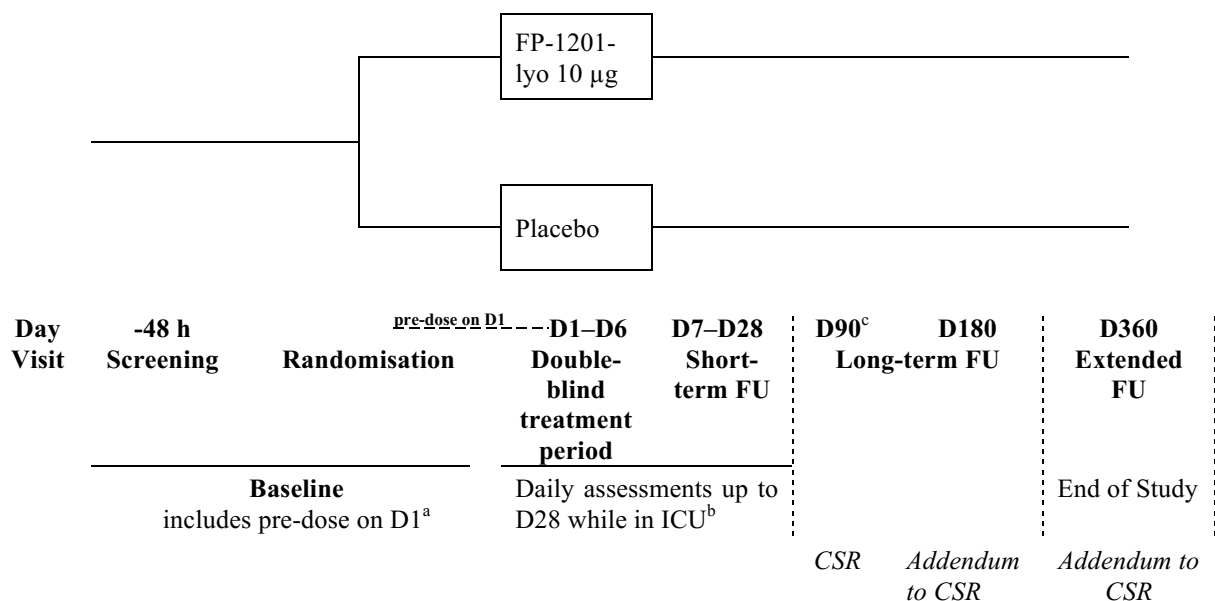
[a] *Not more than 48 hours may elapse between confirmation of moderate or severe ARDS during screening and administration of the first dose of study drug on D1. Once eligibility has been met, randomisation can occur during screening or pre-dose on D1.*

[b] *Assessments are described in the Schedule of Procedures in Table 5 for the patient's last day in the ICU. These will be done on D28 or earlier, according to the clinical progress of the patient. If a patient leaves the ICU before D28, their survival status and other endpoints must be assessed on D28 (see schedule in Table 5).*

[c] *Can be visit or telephone contact.*

Abbreviations: CSR=Clinical Study Report; D=day; FU=follow-up; ICU=intensive care unit.

Figure 2: Flow Chart of Screening and Randomisation Procedures

Maximum 48 hours continuous NIV (>12 hours/day) or mechanical ventilation to diagnosis of ARDS

Max 48 hours from ARDS diagnosis to first DOSE

PATIENT IS INTUBATED AND MECHANICALLY VENTILATED (exclude elective post-operative patients)
Does the patient have a PaO₂/FiO₂ ratio consistent with moderate or severe ARDS?
Has mechanical ventilation been for less than 48 hours?

YES

Patient assessed by ICU clinician for diagnosis of moderate or severe ARDS.
Radiological and hypoxaemic criteria must occur within the same 24-hour period.
Record the times of the P/F ratio AND chest X-ray/CT scan.
The later time is the time of first ARDS diagnosis.

Moderate or severe ARDS diagnosed? — NO

Has the patient been assessed against the other inclusion and exclusion criteria? — NO

Excluded:
- not meeting inclusion criteria
- meeting exclusion criteria
- declined consent
- no pre-dose confirmation
- chest X-ray not consistent

YES

Trial is discussed with patient or legal representative and trial information provided

Written informed consent obtained? — NO

YES

Chest X-ray not consistent?
Patient is definitely excluded

Eligibility confirmation process (section 4.3):
Fill out **all** orange boxes of Scr-Elig 1 page.
More than 36 hours from first diagnosis?
Night time (22:00-08:00)?

Chest X-ray equivocal?
Patient is reassessed (new CXR/CT scan)

YES   NO

Direct call to MM for fast procedure → Wait for MM confirmation
Call MM if no response in 60 min

ELIGIBILITY CONFIRMED? — NO → Telephone or email discussion with MM

YES

PRE-DOSE CHECK OF ELIGIBILITY: Get a new P/F ratio

P/F ≤100?   P/F >100 and ≤200   P/F >200

RANDOMIZATION Severe group   RANDOMIZATION Moderate group   ITT starts from here

Patient allocated randomization number; IMP number

First DOSE

3.2   Criteria for Evaluation of the Study

3.2.1   Criteria for Evaluation of Efficacy

Primary Efficacy Endpoint:

- Composite endpoint including any-cause death at D28 and days free of mechanical ventilation (VFDsurv) within 28 days among survivors

Secondary Endpoints:

- Secondary endpoints relating to the efficacy of FP-1201-lyo treatment:
    - All-cause mortality at D28, D90 and D180
    - Mortality in ICU up to D28
    - Mortality in hospital up to D28

- Other efficacy endpoints at D28:
    - Days free of organ failure (Sequential Organ Failure Assessment [SOFA] methodology) (D28 or last day in ICU if patient has left the ICU earlier than D28, or at withdrawal)
    - Days free of renal support
    - Days free of vasoactive support
    - Days free of mechanical ventilation
    - Number of ICU-free days
    - Number of days in hospital

- Presence of neutralising antibodies to IFN beta-1a at baseline and D28 (or last day in ICU if patient has left the ICU earlier than D28, or at withdrawal)

- Evaluation of PD using MxA biomarker from baseline to D14

- Long-term secondary endpoints, relating to QoL, respiratory and neurological functioning at D180:
    - EQ-5D-3L
    - 6MWT
    - FEV_1

3.2.2   Criteria for Evaluation of Safety

- AEs up to D28, AEs occurring after D28 if the investigator considers there is a causal relationship with the study drug and all deaths up to D360

- Physical examination, vital signs and laboratory results (biochemistry, haematology and urinalysis) up to D28

3.2.3   Criteria for Evaluation of Pharmacoeconomics

- Days free of organ failure up to D28 (or last day in ICU if patient has left the ICU earlier than D28, or at withdrawal)
- Days free of renal support up to D28
- Days free of vasoactive support up to D28
- Days free of mechanical ventilation up to D28
- Number of ICU-free days up to D28

- Number of days in hospital up to D28

3.2.4 Criteria for Evaluation of Exploratory Endpoints

- Exploratory endpoints relating to the efficacy of FP-1201-lyo treatment:
    - Composite endpoint including mortality and days free of mechanical ventilation (VFDsurv) within 90 days among survivors
    - Ordered categorical endpoint defined as improvement (from severe to moderate/mild; from moderate to mild ARDS), no change or worsening (from moderate to severe/death; from severe to death) in terms of gas exchange (PaO_2/FiO_2 ratio) from baseline to D28
- Change in the treatment-specific exploratory biomarker CD73 concentration from baseline to D14
- Change in PIMs, including but not limited to, interleukin-6 and -8 from baseline to D14
- A blood sample will be taken for pharmacogenetic analysis and correlation with other markers of the activity of FP-1201-lyo
- Extended long-term follow-up: 12-month mortality rate, EQ-5D-3L, 6MWT and FEV_1

3.3 Justification of the Study Design

This is a Phase III study to evaluate the efficacy and safety of FP-1201-lyo in the treatment of patients with moderate or severe ARDS. The study is designed as a double-blind, randomised, parallel-group, placebo-controlled, multicentre clinical study.

At present there is no approved pharmacological treatment that could be considered as an active comparator for studies of ARDS. The only currently available treatment for ARDS patients is intensified supportive care. Therefore, the current approach to ARDS study design should be to show superiority of the investigated study drug to the best method of care. The study drug will be used as additive treatment to supportive care and therefore it is most appropriate to use placebo as a comparator.

A different primary endpoint is used in this study to that described in the European Medicines Agency guideline on clinical investigation of medicinal products in the treatment of patients with ARDS. The guideline recommends using all-cause mortality at D28. [Ref: Committee for Medicinal Products for Human Use 2006] The Sponsor has discussed the primary endpoint with the European Medicines Agency and it was agreed that the composite endpoint would be more sensitive in picking up effect signals. In addition, this study has a double-blind design, so the decision to wean the patient from mechanical ventilation should not be influenced by the treatment group.

Owing to the randomisation procedure and the double-blind nature of this study the potential bias of the study results is minimised.

4 STUDY POPULATION

The study population will consist of patients with moderate or severe ARDS. Patients must meet all the inclusion criteria and none of the exclusion criteria to be enrolled in the study. The inclusion and exclusion criteria apply during screening and prior to administration of the first dose of study drug on D1.

4.1 Inclusion Criteria

All patients must be intubated and mechanically ventilated to diagnose ARDS and be eligible for the study.

1. Patient has a diagnosis of moderate or severe ARDS according to the Berlin definition of ARDS [Ref: ARDS Definition Task Force 2012]

    1.1 Acute onset of respiratory failure within 1 week of a known clinical insult or new or worsening respiratory symptoms

    1.2 Respiratory failure associated with known ARDS risk factors and not fully explained by either cardiac failure or fluid overload (an objective assessment of cardiac failure or fluid overload is needed if no risk factors for ARDS [moderate or severe ARDS] are present)

    1.3 Radiological abnormalities on chest X-ray or on computerised tomography (CT) scan, i.e., bilateral opacities that are not fully explained by effusions, nodules, masses or lobar/lung collapse

    1.4 Hypoxaemia:

    - Moderate ARDS: PaO_2/FiO_2 >100 mmHg (>13.3 kPa) to ≤200 mmHg (≤26.6 kPa) with positive end expiratory pressure (PEEP) ≥5 cmH$_2$O
    - Severe ARDS: PaO_2/FiO_2 ≤100 mmHg (≤13.3 kPa) with PEEP ≥5 cmH$_2$O

2. The radiological and hypoxaemia criteria (1.3 and 1.4) must occur within the same 24-hour period. The time of onset of ARDS is when the last of the two specified ARDS criteria is met

3. Administration of the first dose of study drug must be planned to take place within 48 hours of moderate or severe ARDS diagnosis.

4. Patient is intubated and mechanically ventilated

5. A signed written informed consent form from the patient or the patient's personal legal representative (PerLR) or a professional legal representative (PrfLR) must be available

6. Patient is aged ≥18 years

4.2 Exclusion Criteria

1. Woman known to be pregnant, lactating or with a positive (urine or serum test) or indeterminate (serum test) pregnancy test

2. Patient is simultaneously taking part in another pharmacotherapy protocol

3. The patient is not expected to survive for 24 hours

4. Patient has an underlying clinical condition where, in the opinion of the Investigator, it would be extremely unlikely that the patient would come off ventilation, e.g., motor neurone disease, Duchenne muscular dystrophy, or rapidly progressive interstitial pulmonary fibrosis

5. Patient has severe chronic obstructive pulmonary disease requiring long-term home oxygen therapy or mechanical ventilation (non-invasive ventilation or via tracheotomy) except for continuous positive airway pressure (CPAP) or bi-level positive airway pressure (BIPAP) used solely for sleep-disordered breathing

6. Patient has congestive heart failure, defined as New York Heart Association class IV (Ref: Committee for Medicinal Products for Human Use 1994)

7. Patient has acute left ventricular failure

8. Patient has liver failure (Child–Pugh grade C)

9. Patient has received any prior IFN

10. Patient has known hypersensitivity to natural or recombinant IFN beta or to any of the excipients

11. Patient is receiving renal dialysis therapy for chronic renal failure

12. Patient is receiving extra-corporeal membrane oxygenation, high-frequency oscillatory ventilation (HFOV) or any form of extracorporeal lung support

13. Patient has had any form of mechanical ventilation (invasive or non-invasive, excluding CPAP alone) for longer than 48 hours prior to the diagnosis of ARDS

    Non-invasive ventilation has to be continuously applied for at least 12 hours per day in these 48 hours

14. Patient has burns to ≥15% of their total body surface area

4.3 Confirmation of Patient Eligibility

To ensure appropriate enrolment into the study there will be a formal confirmation of eligibility process utilising a two-step procedure:

In Step 1, there will be an electronic Case Report Form (e-CRF) with mandatory fields for completion and a checklist of inclusion and exclusion criteria; all items will need to be completed. The Clinical Data Management System (CDMS) will not allow a patient to progress to randomisation if these have been left unpopulated.

In Step 2, for situations where a chest CT scan is not available for confirming the radiological aspect of the diagnosis and chest X-ray is used for diagnosis, the enrolling clinician will match their patient's chest X-ray against a panel of 12 chest X-rays from the Berlin ARDS Definition [ARDS Definition Task Force 2012] and tick to show which lung fields are the closest match. After including their name, direct e-mail and telephone contact details, and on requesting 'Eligibility Confirmation', the CDMS will automatically inform the Medical Monitor (MM) who will then review the e-CRF:

> 1. If the tick corresponds to a positive Moderate/Severe ARDS image, the MM will make the patient available to be randomised and the CDMS will send an automatic email to the site that the patient has

passed screening.

2. If the tick is against an equivocal Moderate/Severe ARDS image, the MM uses the contact details for the site and contacts the sender to continue standard therapy and possibly undertake further thoracic imaging after 8–12 hours. This may be repeated twice as long as the imaging is clinically indicated and the time to diagnosis does not exceed 48 hours from the start of mechanical ventilation.

3. If the tick is against a negative Moderate/Severe ARDS image, the MM will contact the sender to inform them that the patient cannot be randomised and discuss further options.

With this method, only those patients whose data are recorded as meeting eligibility criteria can be randomised.

4.4   Patient Withdrawal and Replacement

4.4.1   Discontinuation Criteria

Patients may withdraw from the entire study, including follow-up, at any time without penalty and for any reason without prejudice to their future medical care; they are not obliged to state their reasons for withdrawing. The decision to withdraw can be made by the patient or their PerLR or PrfLR.

Patients may be **withdrawn from the study** under the following circumstances:

- Protocol violations including non-compliance with study procedures or patient lost to follow-up
- AEs
- Administrative reasons
- Patient, PerLR or PrfLR request
- Sponsor request
- Investigator request

The Investigator must ensure that the status page in the e-CRF for the **end of the study** is completed.

Patients may be required to **withdraw from study drug** after discussion with the Sponsor and/or Investigator for the following reasons:

- AEs
- At the discretion of the Investigator or if it is considered to be in the patient's best interest

Patients who discontinue study drug may continue in the study. The Investigator must ensure that the status page in the e-CRF for the **end of study** is completed.

In all cases, the reason(s) for withdrawal, including the primary reason, must be recorded in the e-CRF. If a patient is prematurely withdrawn from the study drug for any reason, the Investigator must make every effort to perform the evaluations described for the follow-up visits. Any on-going AEs should be followed up to resolution or D28 whichever is the sooner. See also Sections 9.6.5 and 9.6.6.

If a patient (or their representative) withdraws consent and still agrees to undergo a final examination, this will be documented in the e-CRF and the Investigator's copy of the Informed Consent Document (ICD), which will be countersigned and dated by the patient (or PerLR or PrfLR).

Patients who withdraw consent for their data to be analysed will be identified in the CDMS. Any data collected will not be deleted, but will not be used in any subsequent outputs.

Withdrawn patients will not be replaced.

The study will be terminated if, in the opinion of the Sponsor, significant safety concerns arise during the conduct of the study.

4.4.1.1   Non-attendance of Follow-up Assessments

Attempts should be made to contact patients discharged from hospital who do not attend their study follow-up assessments to ensure their well-being. In such cases, the patient will be contacted at least twice by telephone and once by letter to request that they attend the scheduled follow-up assessment. If patients do not respond they will be considered as withdrawn at that time point (lost to follow-up). If a patient is lost to follow-up, wherever possible mortality data will be sought at all remaining mortality time points from alternative sources such as the patient's local physician.

4.4.2   Replacement Policy

A screen failure patient is defined as any patient who did not comply with the inclusion and exclusion criteria during screening **and** prior to receiving the first dose of study drug. Patients who become ineligible after consenting, and before treatment, will be deemed to be screen failures and will be replaced.

It is possible that a patient may be randomised to a study group following successful screening but may become ineligible prior to administration of the first dose of study drug. For example, prior to the first dose of study drug a patient may be extubated and so is no longer being mechanically ventilated or a patient may have an improved oxygenation status, which no longer meets the moderate or severe ARDS criteria. Such patients will be classed as screen failures and will be replaced.

Patient numbers (and randomisation numbers if applicable) of screen failures will not be reallocated. A new patient will be enrolled and assigned the next available number.

Patients who complete screening and are randomised and receive the first dose of study drug will not be replaced even if later withdrawn or lost to follow-up.

4.5   Planned Sample Size and Number of Study Centres

It is planned to randomise 300 patients at approximately 70 - 80 centres in 9 countries for this study. See Section 8.10 for a discussion of sample size.

4.6   Patient Identification and Randomisation

4.6.1   Patient Identification

Patients will be allocated a unique 5-digit patient number (patient identification number), which will include the country number (1 digit), the site number (2 digits), as well as a consecutive individual number (2 digits). The patient number will be assigned at the study centre on enrolment (i.e., provision of written informed consent)

in chronological order of screening and will be used throughout the study. If a patient is not subsequently randomised, their screening number will not be reallocated. Each screened patient will therefore have a unique identifier.

4.6.2   Randomisation Scheme

Patients will be randomised on a 1:1 basis to FP-1201-lyo or placebo. IWRS will be used to obtain randomisation details.

To ensure that conclusions are not dominated by data from a small number of centres, and also to obtain a broad spread of patients and centres within the constraints of the inclusion/exclusion criteria, each centre will be allowed to include up to, but no more than, 30 patients.

Randomisation will be stratified by country and ARDS severity (moderate or severe), and a sequence of randomisation numbers will be assigned to each study centre.

Refer to Section 5.4 for details of blinding and breaking the blind.

4.6.3   Randomisation of Patients to Treatment

Randomisation of patients to treatment will occur during screening or on D1 after all screening procedures have been performed and eligibility for inclusion in the study has been confirmed. Each randomised patient will receive a unique randomisation number. Randomised patients who terminate their study participation for any reason, regardless of whether study drug was taken or not, will retain their randomisation number.

The Investigator will use IWRS for randomisation of patients. Details can be found in the study file.

5 STUDY DRUG

5.1 Identity

Rentschler Biotechnologie GmbH, Laupheim, Germany manufacture lyophilised FP-1201-lyo (recombinant human IFN beta-1a) and the matched placebo. Both FP-1201-lyo and placebo powders are free of animal serum and human serum albumin. Vetter Pharma International Services, Ravensburg, Germany manufacture pre-filled water for injection (WFI) diluent syringes.

A MixJect® transfer device will be used in this study. This MixJect® transfer device is a single unit for reconstituting a powdered drug with a diluent pre-filled syringe. Upon reconstitution, the drug is available for immediate injection. The MixJect® transfer device enables the safe, rapid and easy preparation of lyophilised drugs. The MixJect® transfer device is manufactured by West Pharmaceutical Services GmbH, Germany/Medimop Medical Projects, Ra'anana, Israel. The device carries the CE mark and has 510(k) approval by the United States Food and Drug Administration.

Fisher Clinical Services Ltd, Horsham, UK, is responsible for the packaging of FP-1201-lyo and matched placebo in a carton box. The pre-filled WFI diluent syringe, the MixJect® transfer device and the reconstitution instructions are packaged in the accessorial carton kit. Fisher Clinical Services Ltd is responsible for distributing the investigational medicinal product to the study sites.

5.2 Administration

FP-1201-lyo 10 μg (or placebo) will be diluted in WFI near the patient/in the ICU. Once prepared, the dose must be administered to the patient immediately. The diluted FP-1201-lyo or placebo will be administered as an intravenous bolus injection via a central or peripheral line. The injection will be followed with a 5 mL flush of sterile saline (not provided).

FP-1201-lyo and placebo injections will be given once daily for 6 days. The injection should be given at the same time each day ±1 hour providing the patient's condition allows this. If for any reason this is not possible, the treatment window may be extended by up to 4 hours. The reason for the delay must be entered in the e-CRF. Subsequent doses should not be delayed and should revert to the original time schedule (e.g., if the D1 dose was at 13:00, the D2 dose was delayed and given at 17:00, the D3 dose should be given at 13:00 ±1 hour).

No dose modifications or temporary cessations of study drug administration are allowed. If a delay beyond the 4-hour window described above is required, the patient must be withdrawn from study drug but all data must continue to be collected per protocol. Administration of a second course of study drug is not permitted.

5.3 Packaging, Labelling and Storage

FP-1201-lyo and matched placebo are packaged in a carton. The pre-filled WFI diluent syringe and the MixJect® transfer device are packaged in an accessorial carton kit.

Six investigational medicinal products and six accessorial carton kits are reserved for each patient to cover the 6-day treatment period.

Labelling will be prepared by Fisher Clinical Services Ltd to meet the local regulatory requirements.

All study drug supplies must be stored in accordance with the manufacturer's instructions, i.e., FP-1201-lyo and placebo are to be stored at 2–8°C and the accessorial carton kits are to be stored at room temperature. Until dispensed to the patients, the study drug will be stored in a securely locked area, accessible to authorised personnel only.

The investigational medicinal product and the accessorial carton kits are dispensed only by the Investigator or by a member of staff specifically authorised by the Investigator, or by a pharmacist, as appropriate.

5.4   Blinding and Breaking the Blind

The study will be performed in a double-blind manner. All study drugs will be supplied in identical vials and will be similar in colour and appearance, thereby enabling double-blind conditions.

The treating physician (investigator) is responsible for the medical care of the trial patient and the study set up allows the investigator to rapidly break the treatment code in an emergency situation.

The study blind should only be broken in a medical emergency (where knowledge of the study drug received would affect the treatment of the emergency) or as a regulatory requirement (e.g., for SAEs or death). Note that there is no specific antidote or method of removing the study drug from the body (such as dialysis) and the best available care for the patient should be continued.

If the blind is broken, the date, time and reason must be recorded in the patient's e-CRF and any associated AE report. It is the responsibility of the investigator to promptly document and explain any unblinding to the sponsor/CRO.

Detailed instructions for the use of the IWRS in order to break the study blind for a patient are provided in a separate document that will be filed in the Site File and Trial Master File. As well as the IWRS, a backup system enabling unblinding of treatment is provided to the sites.

After a patient has been unblinded data collection should continue as per protocol.

Suspected unexpected serious adverse reactions (SUSARs), which are subject to expedited reporting, should be unblinded before submission to the regulatory authorities and Independent Ethics Committees (IECs).

The overall randomisation code will be broken only for reporting purposes. This will occur once all D90 clinical data have been entered into the database and all data queries related to D90 have been resolved, and the assignment of patients to the analysis sets has been completed.

5.5   Drug Accountability

The Investigator is responsible for maintaining accurate study drug accountability records throughout the study.

Each dispensing of study drug will be documented in the e-CRF.

The Investigator is responsible for ensuring all unused medication is destroyed at the investigational site following the appropriate drug accountability procedures.

5.6   Compliance

The study drug is administered intravenously at the study site, so it is not necessary to monitor patient compliance with the study drug regimen.

5.7   Previous and Concomitant Medications

Any medication the patient takes other than the study drug between screening and D28, including herbal and other non-traditional remedies, is considered to be a concomitant medication. All concomitant medications must be recorded in the e-CRF except for nutritional and volume therapy, electrolyte support, vitamins and supportive therapies such as artificial tears, ointments, etc. The following information must be recorded in the e-CRF for each concomitant medication: generic name, route of administration, start date, stop date, dosage and indication. Any changes in the dosage or regimen of a concomitant medication must be recorded in the e-CRF. If a patient is discharged from the hospital prior to D28 then the patient must be instructed that the Investigator should be informed about additional medication up to D28.

Concomitant medications or therapies that are considered necessary for the patient's welfare and that will not interfere with the study drug may be given at the discretion of the Investigator.

All patients in this study will be managed with supportive care measures according to the best practice established locally in each ICU. Due consideration must be given to the study guidance on supportive measures given in Appendix 1, Section 11.1.

At screening, patients (or their relatives) will be asked what medications they have taken during the last month. In connection with the 6MWT at D180 and D360, patients (or their carers or relatives) will be asked what medications the patients have taken during the last 24 hours.

There are no prohibited co-medications in this study.

6   VARIABLES AND METHODS OF ASSESSMENT

The timing of the assessments of the variables is shown in the Schedule of Procedures (Table 5 in Section 7.1).

6.1   Efficacy Variables

The following efficacy variables will be assessed:

- Mortality
- Days on mechanical ventilation
- SOFA
- Days in ICU
- Days in hospital
- Days on vasoactive support
- Days on renal support
- Neutralising antibodies
- Biomarker MxA
- QoL
- Respiratory functioning (FEV_1)
- Neurological functioning (6MWT)

6.1.1   Primary Efficacy Variables

All-cause mortality will be assessed at D28 (primary) and also at D90 and D180, and at the extended long-term follow-up at D360. All deaths shall be recorded as SAEs up to D360. The patient's location at the time of death (ICU or hospital) will also be recorded.

For the composite primary endpoint, ventilator-free days (VFDs) will be calculated. A patient will be reported as ventilator free after **two consecutive calendar days** of unassisted breathing (UAB). UAB is defined as:

- Spontaneously breathing with face mask, nasal prong oxygen or room air
- T-piece breathing
- Tracheostomy mask breathing
- CPAP ≤5 cmH_2O without pressure support or intermittent mandatory ventilation assistance
- Use of CPAP or BIPAP solely for sleep apnoea management

Patients still on positive pressure ventilation/receiving assisted breathing who are transferred to another hospital or healthcare facility prior to D28 will be followed up to assess the VFD outcome at D28.

These variables will also be assessed at D90 in an exploratory analysis.

6.1.2   Secondary Efficacy Variables

6.1.2.1   Sequential Organ Failure Assessment

Organ failure status will be assessed using the SOFA score, which assesses six organ systems: respiration, coagulation, liver, cardiovascular, central nervous system and

renal (Table 2). [Ref: Grissom et al 2010] A patient will be defined as being free of organ failure when the SOFA score is zero. The score will be assessed pre-dose on D1 and daily up to D14, at D21 and at D28 while the patient is in the ICU, based on worst daily values.

The SOFA score variables to be assessed daily from baseline to D28/leaving the ICU are:

- PaO_2/FiO_2 (mmHg)
- Platelets $\times 10^3/mm^3$
- Bilirubin (µmol/L or mg/dL)
- Hypotension/use of vasopressors
- Glasgow Coma Scale (GCS)
- Creatinine (µmol/L or mg/dL)

Table 2   SOFA Scoring

SOFA score	0	1	2	3	4
Respiration					
PaO_2/FiO_2, mmHg	>400	>300–≤400	>200–≤300	>100–≤200	≤100
				---with respiratory support---	
Coagulation					
Platelets $\times 10^3/µL$	>150	>100–≤150	>50–≤100	>20–≤50	≤20
Liver					
Bilirubin, mg/dL	<1.2	≥1.2–<2.0	≥2.0–<6.0	≥6.0–<12.0	≥12.0
(µmol/L)	(<20)	(≥20–<33)	(≥33–<102)	(≥102–<204)	(≥204)
Cardiovascular					
Hypotension[a]	No hypo-tension	MAP <70 mmHg	Dopamine ≤5 or dobutamine (any dose)	Dopamine >5 or epinephrine ≤0.1 or norepinephrine ≤0.1	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
Central nervous system					
Glasgow Coma Scale[b, c]	15	13–14	10–12	6–9	<6
Renal					
Creatinine, mg/dl	<1.2	≥1.2–<2.0	≥2.0–<3.5	≥3.5–<5.0	≥5.0
or µmol/L or urine output	<110	≥110–<171	≥171–<300	≥300–<440 or <500 mL/d	≥440 or <200 mL/d

[a] *Adrenergic agents administered for at least 1 hour (doses are given in µg/kg/min).*

[b] *For patients who are intubated, the verbal response is scored as:*

    5 – Seems able to talk

    3 – Questionable ability to talk

    1 – Generally unresponsive.

[c] *For patients who are sedated use an estimated score for GCS (the assumption is 15, if no other factors than sedation affect GCS)*

Abbreviations: MAP=mean arterial pressure; PaO_2/FiO_2=partial pressure of oxygen/fraction of inspired oxygen; SOFA=Sequential Organ Failure Assessment.

6.1.2.2 Renal Support

Any renal support given to the patient will be recorded on a specific page in the e-CRF.

6.1.2.3 Vasoactive Support

Any vasoactive support given to the patient will be recorded on a specific page in the e-CRF. Vasoactive support includes: catecholamine and non-catecholamine vasopressors, inotropes and vasodilating agents.

6.1.2.4 Neutralising Antibodies to Interferon Beta-1a

Blood samples (2.5 mL) will be taken on D1 pre-dose (baseline) and D28 (or on last day in ICU or at withdrawal, if earlier) to determine the presence of neutralising antibodies to IFN beta-1a.

Neutralising antibody blood sample preparation and sample storage details are provided Laboratory Manual. It is essential that the actual time and date of collection of each antibody sample be recorded in the sample collection form provided with the laboratory kits.

Neutralising antibody samples will be analysed centrally at Wieslab AB, Malmö, Sweden.

6.1.2.5 Myxovirus Resistance Protein A

Whole blood samples (2 mL) will be taken pre-dose on D1 and daily from D2 to D14 for analysis of MxA levels. For details of sample preparation and storage refer to the Laboratory Manual. MxA samples will be analysed centrally at Wieslab AB.

6.1.2.6 Quality of Life, Respiratory Functioning and Neurological Functioning

Health-related QoL (EQ-5D-3L), respiratory functioning via FEV_1 and neurological functioning via the 6MWT, will be assessed at D180 (6 months). If the patient is unable to come to the hospital for the FEV_1 and 6MWT assessments, the QoL information should still be obtained by telephone interview. Additional data will be collected at D360 (12 months) to form part of the extended long-term follow-up.

<u>EQ-5D-3L Questionnaire</u>

EQ-5D-3L provides a simple descriptive profile and a single index value of health status that can be used in clinical research and economic evaluation of healthcare as well as in population health surveys. The questionnaire is cognitively undemanding, designed for self-completion and takes only a few minutes to complete.

At the time when the patients are screened/enrolled in the study it may not be possible to directly obtain baseline information on their pre-admission QoL. In these cases, a description of the patient's pre-study functioning at home should be obtained from their relatives, recorded in the e-CRF and used as the reference value. This information will be checked by the patient at a later stage and correspondingly updated in the e-CRF.

Forced Expiratory Volume in 1 second

Measuring FEV_1 assesses pulmonary function (airway obstruction, bronchoconstriction or bronchodilation). FEV_1 is the volume exhaled during the first second of a forced expiratory manoeuvre, starting from the level of total lung capacity.

Six-minute Walk Test

The 6MWT measures [Ref: Holland et al 2014] the distance that a patient can quickly walk on a flat, hard surface in a period of 6 minutes. According to the current standard the test should be done twice and the best result is used. It evaluates the global and integrated responses of all systems involved during exercise, including the pulmonary and cardiovascular systems, systemic circulation, peripheral circulation, blood and neuromuscular units and muscle metabolism. The self-paced 6MWT assesses the sub-maximal level of functional capacity and may better reflect the functional exercise level for daily activities.

6.2   Safety Variables

6.2.1   Adverse Events

6.2.1.1   Collection of Adverse Events

It is the responsibility of the Investigator to collect all AEs (both serious and non-serious) derived by observation, by spontaneous unsolicited reports of patients, and, where appropriate, by routine open questioning, e.g., "How have you felt since I last saw you?"

6.2.1.2   Definitions

Definitions of AEs and SAEs and their documentation and reporting within this study follow International Conference on Harmonisation (ICH) Good Clinical Practice, European Union, and national regulations and requirements.

An AE is defined as any untoward medical occurrence that occurs in a patient or clinical investigation subject administered a pharmaceutical product, and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product.

All AEs up to D28 are collected. AEs occurring after D28 should be reported to the Sponsor if the Investigator considers there is a causal relationship with the study drug. All AEs up to D360, which lead to death, are reported as SAEs. Concomitant illnesses, which existed before entry into the study, will not be considered AEs unless there is any deterioration from baseline that is considered clinically relevant or significant during treatment or follow-up period until D28 or discharge from ICU. All AEs must be documented, regardless of the source of identification (e.g., physical examination, laboratory assessment, ECG, reported by patient).

Pre-existing conditions will be recorded in the e-CRF on the Medical History or appropriate page.

Development of barotrauma shall be recorded as an AE.

A TEAE is defined as an AE that begins or that worsens in severity after at least one dose of study drug has been administered up to D28.

See 6.2.1.4 for the schedule of collection of adverse events

6.2.1.3 Assessment of Adverse Events

It is recognised that the patient population in the ICU will experience a number of common aberrations in laboratory values, signs and symptoms due to the severity of their underlying disease and the impact of standard therapies. These will not necessarily constitute an AE unless they require significant intervention, lead to discontinuation of blinded study drug or are considered to be of concern in the Investigator's clinical judgement.

Each AE will be assessed by the Investigator with regard to the following categories:

6.2.1.3.1 Seriousness

An SAE is defined as any <u>untoward</u> medical occurrence that at any dose:

- Results in death
- Is life-threatening. This means that the patient is at risk of death at the time of the event. It does not mean that the event hypothetically might have caused death if it were more severe
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalisation but that may jeopardise the patient or require intervention to prevent one of the above outcomes. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; or development of drug dependency or drug abuse

Medical and scientific judgment should be exercised in deciding whether a case is serious and whether expedited reporting is appropriate. However, all deaths up to D360 will be reported as a SAE.

"At any dose" does not imply that the patient is receiving study treatment at the time of the event. Study drug doses may have been given during treatment cycles or interrupted temporarily prior to the onset of the SAE, but may have contributed to the event.

6.2.1.3.2 Intensity

Classical reporting of mild, moderate and severe AEs making reference to the patient's functional status is difficult for a randomised study in critically ill patients. Patients enrolled in this study will primarily be mechanically ventilated and comatose due to their underlying condition and/or the drugs they are prescribed for sedation and analgesia in the ICU. Therefore, the classical approach to AE reporting, which requires patient communication and evaluation of the impact on functioning will be adapted to the ICU environment. The Investigator will be responsible for the assessment of severity, using the categories of mild, moderate or severe to describe each AE as:

- **Mild:** Does not interfere with patient's usual function

- **Moderate:** Interferes to some extent with patient's usual function
- **Severe:** Interferes significantly with patient's usual function

Note the distinction between serious and severe AEs. **Severe** is a measure of intensity whereas an event must meet one of the criteria for serious events listed in Section 6.2.1.3.1 to be considered **serious**; thus, a **severe** reaction is not necessarily a **serious** reaction. For example, a headache may be severe in intensity, but would not be classified as serious unless it met one of the criteria for serious events listed in Section 6.2.1.3.1.

6.2.1.3.3 Causality

The Investigator will assess the causality/relationship between the study drug and the AE and record that assessment in the e-CRF. Causality will be assessed as:

- **Not related:** AE is obviously explained by another cause; OR the time of occurrence of AE is not reasonably related to administration of the study drug
- **Possibly related:** Study drug administration and AE occurrence are reasonably related in time; AND AE is explained equally well by causes other than study drug
- **Probably related:** Study drug administration and the occurrence of the AE are reasonably related in time; AND the AE is more likely explained by exposure to study drug than by other mechanisms

The most likely cause of an AE (e.g., disease under treatment, concomitant disease, concomitant medication, other) will be indicated in the e-CRF with details of the concomitant disease or medication or other cause.

6.2.1.3.4 Clinical Laboratory Adverse Event

Abnormal laboratory findings (e.g., biochemistry, haematology, urinalysis) or other abnormal assessments (e.g., vital signs) that are judged by the Investigator as clinically significant will, if certain requirements are met, be recorded as AEs or SAEs. Clinically significant abnormal laboratory findings or other abnormal assessments that meet the definition of an AE or SAE and are detected during the study, or are present at baseline and significantly worsen following the start of the study, will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied (unless judged by the Investigator as more severe than expected for the patient's condition), or that are present or detected at the start of the study and do not worsen, will not be reported as AEs or SAEs.

The Investigator will exercise their medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

6.2.1.4 Recording Adverse Events

AE reporting will extend from signing of informed consent. AEs occurring after D28 should be reported to the Sponsor by the Investigator if the Investigator considers there is a causal relationship with the study drug. However, all deaths will be recorded and reported as SAEs throughout the study (up until D360).

All AE reports should contain a brief description of the event, date and time of onset, date and time of resolution, intensity, treatment required, relationship to study drug,

action taken with the study drug, outcome, and whether the event is classified as serious.

Recording a diagnosis (when possible) is preferred to recording a list of associated signs and symptoms. However, if a diagnosis is known but there are associated signs or symptoms not generally attributed to the diagnosis, the diagnosis and each sign or symptom must be recorded separately.

6.2.1.5 Reporting Serious Adverse Events

According to applicable European Union regulations and requirements, an SAE must be reported to the Sponsor from the trial site as soon as possible **within 24 hours** of becoming aware of the SAE. A medically qualified person at the trial site identified on the Delegation of Authority Log with this responsibility must assess the SAE. Any member of the clinical trial site staff can assist in reporting an initial SAE. The Principal Investigator or delegated sub-investigators are responsible for the SAE reporting procedures at the site during the trial, and must always sign-off on each SAE even if other site staff have reported the event on behalf of the investigators. A delegation log at each trial site will clearly show delegation of responsibilities regarding SAE reporting.

The SAE form must be completed with all the relevant information and forwarded to Crown Pharmacovigilance by:

- ███████████████████████
  █
- ████████████████████████████████████
  ██████████

If the SAE is urgently reported by telephone, a paper SAE form must always be completed and forwarded to Crown Pharmacovigilance as soon as possible.

The Investigator and the Sponsor (or Sponsor's designated agent) will review each SAE report and the seriousness and the causal relationship of the event to study treatment will be evaluated. In addition, the Sponsor (or Sponsor's designated agent) will evaluate the expectedness according to the reference document (Investigator Brochure). Based on the Investigator and Sponsor's assessment of the event, a decision will be made concerning the need for further action.

If consensus on the assessment cannot be reached between the parties (e.g., Investigator and Sponsor/Sponsor's delegate), all opinions will be provided in the Council for International Organizations of Medical Sciences Form I report and reporting to the CA and IEC should be based on the highest degree of causality provided.

Details for reporting SUSARs can be found in Section 6.2.1.7.

All SAEs will be recorded that occur between signing of informed consent and D28. Events occurring after D28 and coming to the attention of the Investigator should be reported only if they are considered in the opinion of the Investigator to be causally related to the investigational drug. However, all deaths up to D360 will be reported as SAEs.

All SAEs occurring as described above, must be reported **within 24 hours** by email or fax to Crown Pharmacovigilance.

The minimum information required for an initial report is:

- Details of person sending the report (i.e., name and address of Investigator)

- Patient identification details (screening/randomisation number, age, sex, NOT patient name)

- Protocol number

- Description of SAE

- Causality assessment

However, all points on the SAE form should be covered in the initial report and the completed SAE form itself must be emailed or faxed to Crown Pharmacovigilance.

After receipt of the initial report, Crown Pharmacovigilance will review the information and, if necessary, contact the Investigator to obtain further information for assessment of the event. Crown CRO will be responsible for all information processing and reporting according to local legal requirements.

Detailed instructions concerning SAE reporting procedures will be described in a Safety Management Plan written by Crown Pharmacovigilance. SAE Report Form and contact information for reporting SAEs will be provided to the sites.

6.2.1.6 Follow-up of Adverse Events

All AEs experienced by a patient, irrespective of the suspected causality, will be monitored until: the AE has resolved; any abnormal laboratory values have returned to baseline or stabilised at a level acceptable to the Investigator and Medical Monitor; there is a satisfactory explanation for the changes observed; the patient is lost to follow-up; or the patient has died.

6.2.1.7 Suspected Unexpected Serious Adverse Reactions

Any AE that is serious, associated with the use of the study drug, and unexpected (SUSAR) has additional reporting requirements, as described below.

- If the SUSAR is fatal or life threatening, associated with use of the study drug and unexpected, regulatory authorities and IECs must be notified **within 7 calendar days** after the Sponsor learns of the event. Additional follow-up information (cause of death, autopsy report and hospital report) should be reported **within an additional 8 days** (15 days total).

- If the SUSAR is not fatal or life threatening but is otherwise serious, associated with the use of the study drug and unexpected, regulatory authorities and IECs must be notified **within 15 calendar days** after the Sponsor learns of the event.

The Sponsor will notify the Investigators in a timely fashion of relevant information about SUSARs that could adversely affect the safety of patients. Follow-up information may be submitted if necessary.

The Sponsor will also provide annual safety updates to the regulatory authorities and IECs responsible for the study. These updates will include information on SUSARs and other relevant safety findings.

6.2.1.8 Pregnancy

Monitoring of pregnancies is not applicable to this study because pregnancy is an exclusion criterion and a pregnancy test is performed in all women of childbearing potential at screening. Owing to the nature of the study involving short (6-day) treatment with the study drug in an ICU setting, it is not possible for women to become pregnant during treatment and for there to be any foetal exposure to the study drug.

6.2.2   Laboratory Variables

The biochemistry and haematology analysis will be performed at the hospital laboratories of the individual Investigator sites. Copies of laboratory accreditation and relevant reference ranges will be provided to the Sponsor or representative prior to the analysis of the first patient sample at that site.

The laboratory variables measured in the study will be as detailed in Table 3.

Blood samples for determination of biochemistry and haematology will be taken at screening, pre-dose on D1 (baseline value) and daily up to D28 while the patient is in the ICU, as detailed in the Schedule of Procedures in Section 7.1, Table 5. The date and time of sample collection will be recorded in the e-CRF.

Dipstick urinalysis will be taken in the ICU (if urine is being produced) pre-dose on D1 (baseline value) and daily up to D28 while the patient is in the ICU, as detailed in the Schedule of Procedures in Section 7.1, Table 5. The date and time of sample collection will be recorded in the e-CRF.

Table 3   Laboratory Assessments

6.2.2.1	Haematology:	Haemoglobin
		Haematocrit
		Erythrocytes
		MCV
		Platelets
		Leucocytes
		Differential counts of:
		  Neutrophils
		  Eosinophils
		  Basophils
		  Lymphocytes
		  Monocytes
			6.2.2.2	Biochemistry:	Albumin
					Creatine kinase
					Creatinine
					Glucose
					Triglycerides
					Urea
					Bilirubin
					Total protein
					Alkaline phosphatase
					AST
					ALT
					Lactate
					Sodium (blood gas value acceptable)
					Potassium
					Bicarbonate
					Calcium (total calcium corrected for albumin level)
					Chloride

6.2.2.3	Urinalysis:	pH Protein Glucose Ketones Bilirubin Blood			
6.2.2.4	Pregnancy test:	In women with child-bearing potential only. A urine pregnancy test will be performed unless the patient is not producing urine in which case a serum pregnancy test will be performed instead			

Abbreviations: AST=aspartate transaminase; ALT=alanine transaminase; MCV=mean corpuscular volume.

A maximum of 100 mL of blood will be taken for study-specific testing during the study in addition to sampling for routine analysis of haematology and biochemistry variables.

6.2.3   Vital Signs

Vital signs will be measured supine pre-dose on D1 (baseline) and daily up to D28 while the patient is in the ICU, as detailed in the Schedule of Procedures (Section 7.1, Table 5). The date and time of collection of each parameter will be recorded in the e-CRF.

Vital sign variables are:

- Blood pressure (systolic, diastolic and mean; mmHg): to be recorded via an arterial line

- Heart rate (HR; bpm): measured as per clinical practice in each ICU

- Total respiratory rate (breaths per minute)

- Temperature (°C): core temperature to be measured according to the site's usual practices and the site of measurement should be recorded in the e-CRF

6.2.4   ECG

Refer to the Schedule of Study Procedures (Table 5) for the timings of individual measurements.

Twelve-lead ECGs will be obtained after the patient has rested in a supine position for at least 10 minutes. All 12-lead ECGs should be recorded while the patient is in the supine position. The investigator or designated physician will review the paper copies of each of the timed 12-lead ECGs on each of the study days when they are collected. ECGs will be recorded at 25 mm/s or at 50 mm/s. If anything clinically significant is observed on the ECG, the investigator will record it as part of the medical history, where the finding represents a change from baseline.

6.2.5   Physical Examinations

Physical examinations will be performed in accordance with the Schedule of Procedures (Table 5).

A physical examination covering the major body systems (general appearance, head [ear, nose and throat], cardiovascular, eyes, respiratory, abdomen, urogenital, musculoskeletal, neurological, lymph nodes and skin) will be performed at screening (baseline), last day at ICU and at D28 (or when patient withdraws from the study prior to D28).

At screening the physical examination will also include:

- Predicted body weight (PBW). Calculate the PBW (kg) using the following formulae:

  For men: 50 + 0.91(height in cm – 152.4)

  For women: 45.5 + 0.91(height in cm – 152.4)

- Actual height (in cm). The patient's height does not need to be measured immediately if recorded elsewhere in the medical records or provided 'anecdotally' by relatives (the PBW can then be calculated as described above).

6.3   Demographics and Baseline Characteristics

Demographics and baseline characteristics consist of those variables that are assessed only at screening/baseline.

6.3.1   Patient Demography

Patient demography consists of:

- Age at screening
- Race
- Sex

6.3.2   Disease History

For disease history, the following will be documented:

- Time and date of moderate or severe ARDS diagnosis
- Underlying aetiology of moderate or severe ARDS
- Chest X-ray or CT scan
- PaO_2/FiO_2 ratio element of moderate or severe ARDS diagnosis along with associated PEEP

The chest X-ray or CT scan and the confirmatory assessment of PaO_2/FiO_2 ratio along with associated PEEP must occur within the same 24-hour period.

6.3.3   Medical History

For documentation of the medical history, any relevant previous and ongoing medical conditions with a start date before the time of informed consent should be documented. Conditions that resolved long before informed consent and have no impact on the disease being studied should not be listed.

The medical history should be obtained by inspecting the patient's medical records or by interviewing the patient or their relatives.

Table 4   APACHE II score

VARIABLE	Recorded value	Points scored	POINT VALUE RANGE								
			+4	+3	+2	+1	+0	+1	+2	+3	+4
Core temperature (°C)			≥41	39.0-40.9		38.5-38.9	36.0-38.4	34.0-35.9	32.0-33.9	30.0-31.9	≤29.9
Mean arterial pressure (mmHg)			≥160	130-159	110-129		70-109		50-69		≤49
Heart rate (beats/min)			≥180	140-179	110-139		70-109		55-69	40-54	≤39
Respiratory rate (breaths/min)			≥50	35-49		25-34	12-24	10-11	6-9		≤5
If FIO$_2$ ≥ 0.5 use A-aDO$_2$ (kPa)			>67	47-66	27-46		<27				
(mmHg)			≥500	350-499	200-349		<200				
If FIO$_2$ < 0.5 use PaO$_2$ (kPa)							>9.3	8.1 – 9.2		7.3-8.0	<7.2
(mmHg)							>70	61-70		55-60	<55
Arterial pH			≥7.7	7.6-7.69		7.5-7.59	7.33 – 7.49		7.25-7.32	7.15-7.24	<7.15
If no ABG use HCO$_3$ (mmol/L)			≥52	41-51.9		32-40.9	22-31.9		18-21.9	15-17.9	<15
Sodium (mmol/L)			≥180	160-179	155-159	150-154	130-149		120-129	110-119	≤110
Potassium (mmol/L)			≥7	6-6.9		5.5-5.9	3.5-5.4	3-3.4	2.5-2.9		<2.5
Creatinine (μmol/L)*			>309	309-177	176-133		132-53		<53		
Haematocrit (%)			≥60		50-59.9	46-49.9	30-45.9		20-29.9		<20
White blood count (10^9/L)			≥40		20-39.9	15-19.9	3-14.9		1-2.9		<1
Clinical & laboratory points											

* If the patient has acute renal failure then the score for serum creatinine will be doubled

NEUROLOGICAL POINTS (15 minus the GCS)		AGE POINTS		CHRONIC HEALTH POINTS		

Eye opening	**Verbal response - not intubated**			**Does patient have any of the following?**	**Yes**	**No**
4 – spontaneously	5 – oriented and converses	≤44	0			
3 – to verbal	4 – disoriented and talks	45 – 54	2	**Liver** – Cirrhosis with PHT or encephalopathy		
2 – to painful stimuli	3 – inappropriate words	55 – 64	3	**Cardiovascular** – NYHA IV		
1 – no response	2 – incomprehensible sounds	65 – 74	5	**Respiratory** – Chronic obstructive or vascular disease resulting in restriction or chronic hypoxemia, hypercapnia or polycythaemia, severe pulmonary hypertension >400 mmHg		
Motor response	1 – no response	≥75	6	**Renal** – Chronic CAPD or HD		
6 – to verbal command	**Verbal response - tracheal tube**			**Immune** – Immunocompromised host		
5 – localizes to pain	5 – seems able to talk					
4 – withdraw to pain	3 – questionable ability to talk			If any box above is checked YES then:		
3 – flexion abnormal	1 - generally unresponsive			a. Medical or emergency postop - assign 5 points		
2 – extension abnormal	*For patients who are sedated use an estimated score for GCS (the assumption is 15, if no other factors than sedation affect GCS)*			b. Elective postop - assign 2 points		
1 – no response						

Neurological points =						
Clinical & laboratory points =						
A **Acute Physiology Points**		**B** **Age Points**		**C** **Chronic Health Points**	**Add A + B + C APACHE II score**	

6.3.4   Previous and Concomitant Medications

Previous (in the month before screening) and concomitant medications will be documented as described in Section 5.7.

6.4   Pharmacoeconomic Evaluation

Variables that will be measured as part of cost-effectiveness at D28 are:

- Days free of organ failure (SOFA methodology) (D28 or last day in ICU if patient has left the ICU earlier than D28, or at withdrawal)
- Days free of renal support
- Days free of vasoactive support
- Days free of mechanical ventilation
- Number of ICU-free days
- Number of days in hospital

6.5   Exploratory Variables

6.5.1   Improvement in Gas Exchange

The gas exchange (PaO_2/FiO_2 ratio) of patients on mechanical ventilation will be evaluated. The PaO_2/FiO_2 ratio will be used as an indicator of lung function.

6.5.2   Biomarkers: CD73 and Potential Inflammatory Markers (PIM)

Blood samples for the CD73 and PIM assessments will be collected pre-dose on D1 (baseline) and daily from D2 to D14 as detailed in the Schedule of Procedures (Section 7.1, Table 5). Fourteen samples of 2.5 mL will be collected for CD73 and PIM; from these, samples will be prepared for the CD73 and PIM testing. CD73 and PIM blood sample preparation and sample storage details are provided in the Laboratory Manual. It is essential that the actual time and date of collection of each sample is recorded in the sample collection form provided with the laboratory kits.

CD73 samples will be analysed centrally by Wieslab AB; PIM samples will be analysed at MediCity Research Laboratory, University of Turku, Turku, Finland.

6.5.3   Pharmacogenetics

The genetic sample can be taken at any time that the patient is in the ICU if consent has been obtained. The objectives of the genetic sampling are to obtain blood for extraction of DNA to identify factors that may be involved in the response or non-response of diseases to FP-1201-lyo and comparator compounds.

The blood sample will be taken for extraction of DNA. This is optional for all patients entering the study and will involve a separate patient consent procedure. Consenting to genetic sampling is not a prerequisite to participating in the main study.

A 10 mL blood sample will be collected; sample preparation and sample storage details are provided in the Laboratory Manual.

The samples and data for genetic analysis in this study will be coded. Samples will not carry any personal identifiers. DNA samples will be destroyed 15 years after

completion of this study. If the patient withdraws the consent, the sample will be destroyed without undue delay.

7   STUDY CONDUCT

For the purposes of this study, study day 1 (D1) is defined as the first calendar day (from midnight to the following midnight) of treatment with FP-1201-lyo or placebo. All days thereafter are defined as "Dn" (e.g., D2, D3, etc.) until D28. After D28 the study assessments are performed on D90 (3 months; either a clinic visit or telephone contact), D180 (6 months) and D360 (12 months).

Baseline assessments are defined as those assessments carried out in the screening period and before the first dose of study drug on D1. It is critical that the time from diagnosis of moderate or severe ARDS to administration of the first dose of FP-1201-lyo or placebo is less than 48 hours.

Unless withdrawn from study, all patients will receive study drug for 6 days.

All assessments in the period up to D28 are only to be performed when a patient is in the ICU. If an improving patient is discharged from the ICU during the short-term follow-up period then the assessments under 'last day in the ICU' will be carried out and the survival status and other endpoints must be assessed on D28.

There are three follow-up periods:

- Short term: D7 to D28
- Long term: D90 [3 months] and D180 [6 months]
- Extended: D360 [12 months] (last visit)

The end of the study will be the date of last patient/last visit at D360.

7.1 Schedule of Procedures

Table 5 Schedule of Procedures

Procedure	Screening period 48 h[a]	Baseline period D1 pre-dose	Double-blind treatment period Days 1–6 — D1	Double-blind treatment period Days 1–6 — D2–D6	Short-term follow-up until D28 or discharge from ICU (whichever is earlier) — D7–D14	D15–D27	Last day in ICU[b]	D28[c]	Long-term follow-up D90[d] +14 days	D180 +14 days	Extended follow-up D360 ±14 days
Informed consent	X										
Inclusion/exclusion criteria	X	X[e]									
Documentation of chest X-ray or CT scan	X										
Demographic details	X										
Medical history	X										
Randomisation		X[f]									
Administration of study drug			X	X							
Survival					X						X
On mechanical ventilation? (Y/N)					X						X
In ICU/in hospital? (Y/N)					X						X
SOFA		X		X	X	D21	X	X[o]			
GCS (for SOFA and APACHE II)		X		X	X	D21	X	X[o]			
APACHE II	X[g]	X									
Renal and vasoactive support? (Y/N)		X		X	X			X			

Note: In the Double-blind treatment period (Days 1–6) columns D1 and D2–D6 a spanning label "Administration of study drug" appears. Dashed continuation lines extend across the marked rows (Survival, On mechanical ventilation?, In ICU/in hospital?) from D7–D14 through to D360, and for Renal and vasoactive support? from D7–D14 through to D28.

Procedure	Screening period	Baseline period	Double-blind treatment period Days 1–6		Short-term follow-up until D28 or discharge from ICU (whichever is earlier) — D7–D28				Long-term follow-up		Extended follow-up
	48 h[a]	D1 pre-dose	D1	D2–D6	D7–D14	D15–D27	Last day in ICU[b]	D28[c]	D90[d] +14 days	D180 +14 days	D360 ±14 days
Blood sample: IFN beta-1a neutralising antibodies		X[h]					X				
Blood sample: MxA		X[h]		X	X						
EQ-5D-3L, FEV$_1$ and 6MWT		EQ-5D-3L[i]						X		X	X
Blood sample: CD73 and PIM		X[h]		X	X						
PaO$_2$/FiO$_2$	X	X		X	X	X	X				
Vital signs	X[j]	X		X	X	X	X	X[o]			
Physical examination	X	X					X	X[o]			
Pharmacoeconomics							X	X			
ECG	X[j]	X		X	D7		X	X[o]			
Haematology, chemistry, urinalysis[k]	X[j]	X		X	X	X	X	X[o]			
Pregnancy test in women of childbearing potential	X										
Previous and concomitant medications and therapies	X[l]	X	–	–	–	–	–	X			
Adverse events	X[m]	X	–	–	–	–	–	X	X[n]	X[n]	X[n]
Blood sample: genetic analysis (requires separate consent)	X	–	–	1 sample at any time while in ICU	–	–	–	X			

Administration of study drug (D1 through the double-blind treatment period Days 1–6).

[a] No more than 48 hours may elapse between confirmation of moderate or severe ARDS and administration of the first dose of study drug.

[b] These assessments will be done on the day the patient leaves the ICU, which will either be on D28 or earlier, according to the clinical progress of the patient. If the patient is still in the ICU on D28, the next visit or telephone contact will be at D90. If a patient leaves the ICU before D28, the survival status and other endpoints must be assessed on D28.

[c] D28 procedures apply for patients leaving the ICU before D28 and for patients withdrawing from the study before D28. For patients withdrawing from the study before D28 a sample should be taken for neutralising antibodies on the day they leave the ICU.

[d] D90 can either be a visit or telephone contact.

[e] Reconfirm inclusion/exclusion criteria before dosing, including that patient requires mechanical ventilation and is in the ICU (patients requiring ECMO after randomisation but before the first dose of IMP may still be included).

[f] Randomise after consent obtained and once eligibility criteria confirmed.

[g] Within 24 hours of ICU admission

[h] Within 1hour pre-dose.

[i] Baseline EQ-5D-3L to be obtained from relatives and checked later with patient.

[j] For APACHE II scoring.

[k] Samples should be taken in the morning between 04:00 and 10:00.

[l] Medicines and therapies in previous month.

[m] Adverse events will be recorded after informed consent is obtained.

[n] Deaths are reported as SAE

[o] if it is possible to be performed by the investigator

Abbreviations: APACHE II=Acute Physiology and Chronic Health Evaluation; CD=cluster of differentiation; CT=computerised tomography; D=study day; ECG=electrocardiogram; ECMO=extra-corporeal membrane oxygenation; EQ-5D-3L=EuroQol 5-Dimensions 3-Levels questionnaire; FEV_1=forced expiratory volume in 1 second; GCS=Glasgow Coma Scale; ICU=intensive care unit; IFN=interferon; MxA=myxovirus resistance protein A; PaO_2/FiO_2=partial pressure of oxygen/fraction of inspired oxygen; PIM=potential inflammatory marker; SOFA=Sequential Organ Failure Assessment; 6MWT=6-minute walk test;.

7.2   Procedures by Visit

All times should be recorded using 24-hour clock (i.e., 23:20 not 11:20 pm). Where a patient's condition precludes an assessment taking place as scheduled, the assessment should be performed within ±4 hours of the scheduled time; if this is not possible, the assessment will not be performed and the reason for non-performance should be recorded.

7.2.1   Pre-screening Evaluation and Screening Log

A complete screening and pre-first-dose evaluation will be conducted including the procedures outlined in Sections 7.2.2.1 and 7.2.2.2 below.

Patients will be considered for entry into the study when a diagnosis of moderate or severe ARDS has been made. Before being randomised, patients will be assessed to ensure that all eligibility criteria are met. Patients not meeting the eligibility criteria must not be randomised. Faron Pharmaceuticals Ltd will not permit any exceptions to the eligibility criteria and protocol waivers will not be given.

When considering patients for the study, the investigational site will review:

- The criteria for moderate or severe ARDS diagnosis to verify that all elements are present (refer to Inclusion Criterion 1 as per Section 4.1)

- The time of moderate or severe ARDS diagnosis to ensure the 48-hour study treatment window is still available (refer to Inclusion Criterion 3 as per Section 4.1)

- All eligibility criteria (refer to Sections 4.1 and 4.2)

- Provision of informed consent. Study-specific procedures must be performed <u>after</u> informed consent for the study is given. **Note:** When the ICU **normal procedures** of patients **are in line with the study procedures**, and if the timing of moderate or severe ARDS diagnosis, consent and dosing preclude repeat of procedures for the purposes of the study, then normal patient care test results may be used for screening even if **prior** to consent.

Investigational sites will review all ICU patients daily in order to identify potential patients for this study. Only patients that are intubated and mechanically ventilated are eligible for the study. Investigational sites will maintain a log for all patients that fulfil the intubation, mechanical ventilation and hypoxaemia (PEEP $\geq$ 5 cmH$_2$O and PaO$_2$/FiO$_2$ $\leq$ 200 mmHg) inclusion criteria. For patients meeting these criteria the other criteria for ARDS diagnosis according to Berlin definition [Ref: ARDS Definition Task Force 2012] as well as the other study inclusion and exclusion criteria shall be reviewed and documented on the log, whether or not the patient is entered into the study.

The log will be sent to the CRO on a weekly basis while the investigational site is open for recruitment.

7.2.2   Screening Assessments and Pre-dose Procedures

7.2.2.1   Screening Assessments to Be Performed After Moderate or Severe ARDS Diagnosis and Within 48 hours Prior to the First Dose of Study Drug

Screening assessments will be carried out after informed consent and must be completed before the first dose of study drug is administered. **The first dose of study drug must be administered within 48 hours of moderate or severe ARDS being diagnosed.**

The following parameters will be reviewed and the data will be recorded in the e-CRF once the patient has given informed consent (refer to Schedule of Procedures in Table 5):

- Date and time of informed consent

- Allocated patient screening number

- Moderate or severe ARDS disease history including:
    - Time and date of moderate or severe ARDS diagnosis
    - Underlying aetiology of moderate or severe ARDS
    - Documentation of chest X-ray or CT scan
    - PaO_2/FiO_2 ratio element of moderate or severe ARDS diagnosis along with associated PEEP

- Medical history including any concomitant diseases within the last 1 month before screening

- Date and time of entry to ICU

- Date and time of intubation and mechanical ventilation

- Review of the inclusion and exclusion criteria

- Demographic data including age at screening, race and sex

- Physical examination of major body systems including PBW (refer to Section 6.2.4 for the calculation formula) and height

- Pregnancy test: All women of childbearing potential must have a pregnancy test. The patient will be considered to lack childbearing potential if surgically sterile (e.g., hysterectomy, bilateral oophorectomy) or postmenopausal (amenorrhoea >12 months). A urine pregnancy test will be performed; if the patient is not producing urine a serum pregnancy test will be performed instead

- APACHE II score; this is based on the worst values in the first 24 hours following admission to the ICU of the following 12 variables (see Table 4) but before the first dose
    - $A\text{-}aDO_2$ or PaO_2 (depending on FiO_2)
    - Temperature (rectal or other core site)
    - Mean arterial pressure
    - Arterial pH
    - Heart rate
    - Respiratory rate
    - Sodium
    - Potassium
    - Creatinine
    - Haematocrit
    - White blood cell count
    - Glasgow Coma Scale

- Genetic sample obtained if consent has been given (refer to Section 6.5.3). The genetic sample can be taken at any time while the patient is in the ICU if consent has been obtained

- Randomisation to a study treatment group may occur when all the inclusion/exclusion criteria are met and must follow the pre-dose eligibility check as described in Figure 2. The randomisation number along with the date and time of randomisation will be recorded

- AEs

- Previous and concomitant medications and therapies

7.2.2.2 Pre-dose Procedures to Be Performed on D1 Before the First Dose of Study Drug is Administered

Note: Prior to the first administration of any study drug on D1, the patient's moderate or severe ARDS status (as per Inclusion Criterion 1 in Section 4.1) must be confirmed. That the patient continues to be on mechanical ventilation must also be confirmed. If both criteria are not confirmed, the patient should be withdrawn from the study and will be classed as a screen failure.

On D1 all pre-dose procedures and assessments are to be performed **before the first dose of study drug**.

The following procedures and assessments will be performed:

- Review of the inclusion and exclusion criteria to ensure continued eligibility, including a check that the patient is in the ICU and on ventilation

- Check PaO_2/FiO_2 ratio

- Randomisation: randomisation number and date and time of randomisation will be recorded

- SOFA scoring will be done pre-dose based on the worst value so far that day for each of the following parameters:
  - PaO_2/FiO_2 (mmHg)
  - platelets $\times 10^3/mm^3$
  - bilirubin (μmol/L or mg/dL)
  - hypotension/use of vasopressors
  - GCS
  - creatinine (µmol/L or mg/dL) or urine output

- APACHE II scoring will be done on the worst values in the 24 hours following ICU admission or up to the time of the first dose if the patient has been on the ICU less than 24 hours (see Table 4).

- The following ventilation variables should be recorded in the morning between 04:00 to 10:00 using the values closest to 10:00 (if these values are representative of the patient's condition), whenever possible:
  - Mode of ventilation (whether predominantly volume or pressure set)
  - Tidal volume (ml)
  - Respiratory rate (breaths per min) - total respiratory rate will be used if the patient is breathing spontaneously on a mechanical ventilator
  - Peak inspiratory pressure (cmH_2O)
  - Plateau pressure (cmH_2O) - if set
  - Mean airway pressure (cmH_2O)
  - PEEP

- All ventilator assessments must be taken at the same time point whenever possible and should be representative of the values over the recent past. PEEP is not available if using HFOV.

- Recording of vasoactive drugs and renal support

- Perform ECG

- Vital signs (respiratory rate, temperature, blood pressure [BP] and HR) must be measured and recorded in the morning between 04:00 and 10:00 using the values closest to 10:00 (if these values are representative of the patient's condition). All vital sign assessments must be taken at the same time point whenever possible

- Blood biochemistry: samples must be taken in the morning between 04:00 and 10:00 using the values closest to 10:00 (refer to Section 6.2.2 for specific biochemistry variables)

- Haematology: samples must be taken in the morning between 04:00 and 10:00 using the values closest to 10:00 (refer to Section 6.2.2 for specific haematology variables)

- Urinalysis (if the patient is producing urine): samples for the urinalysis dipstick must be taken in the morning between 04:00 and 10:00 using the values closest to 10:00 (refer to Section 6.2.2 for specific urinalysis variables)

- Neutralising antibody sample for central testing (refer to Section 6.1.2.4)

- MxA sample for central testing taken within 1 hour pre-dose (refer to Section 6.1.2.5)

- CD73 and PIM biomarker samples for central testing taken within 1 hour pre-dose (refer to Section 6.5.2)

- Obtain baseline EQ-5D-3L from relatives

- Recording of concomitant medications and therapies

- Recording AEs

7.2.3   Treatment Period: D1 Dosing and Post-dose

- Day 1: The first dose of study drug (FP-1201-lyo or placebo) will be administered as an intravenous bolus injection (see Section 5.2).

- Record SOFA scores. **Note:** The worst daily value of each element should be used to calculate the overall SOFA score

- Recording of concomitant medications and therapies

- Recording of AEs

7.2.4   Treatment Period: D2 to D6

Assessments from D2 to D6 are only performed when a patient is in the ICU. The following assessments are to be done daily:

- Check patient survival

- Confirm patient is in the ICU

- Check whether the patient is on ventilation (refer to Section 6.1.1 for definition of UAB)

- Administer the study drug (FP-1201-lyo or placebo) as an intravenous bolus injection **24 hours ±1 hour after the previous day's administration.** If for any

reason this is not possible, the dosing window may be extended by **up to 4 hours**. The reason for the delay must be entered in the e-CRF (see Section 5.2) Subsequent doses should not be delayed and should revert to the original time schedule.

- Record SOFA scores

- Recording of vasoactive drugs and renal support

- Vital signs (temperature, BP and HR) must be measured and recorded in the morning between 04:00 and 10:00 using the values closest to 10:00 (if these values are representative of the patient's condition). All vital sign assessments must be taken at the same time point whenever possible.

- Haematology, biochemistry and urinalysis: samples must be taken daily in the morning between 04:00 and 10:00 using the values closest to 10:00

- MxA, CD73 and PIM samples for central testing are taken within 22 hours ±2 hours after the previous day's administration of study drug, however before the next study drug dose.

- Recording of concomitant medications and therapies

- Recording of AEs

7.2.5   Short-term Follow-up Period: D7 to D14

On D8 to D14 all assessments should be performed at the same time of day as the D7 assessments were performed. The following study assessments and procedures will be performed:

- Check patient survival

- Confirm whether patient is still in hospital

- Confirm whether patient is in the ICU. Patients still requiring intensive care who are transferred to another hospital or healthcare ICU facility prior to D14 will be followed up to assess the days in ICU at D28

- Check whether the patient is on ventilation (refer to Section 6.1.1 for definition of UAB). Patients still on positive pressure ventilation who are transferred to another hospital or healthcare facility prior to D14 will be followed up to assess the VFD outcome at D28

- Record SOFA scores

- Recording of vasoactive drugs and renal support

- ECG is taken on D7

- Vital signs (temperature, BP and HR) must be measured and recorded in the morning between 04:00 and 10:00 using the values closest to 10:00 (if these values are representative of the patient's condition). All vital sign assessments must be taken at the same time point whenever possible

- Haematology, biochemistry and urinalysis: Samples must be taken daily in the morning between 04:00 and 10:00 using the values closest to 10:00

- MxA, CD73 and PIM samples for central testing taken at the same time as on D6 ±2 hours

- Recording of concomitant medications and therapies

- Recording of AEs

7.2.6   Short-term Follow-up Period: D15 to D27

- Check patient survival
- Confirm whether patient is still in hospital
- Confirm whether patient is in the ICU. Patients still requiring intensive care who are transferred to another hospital or healthcare ICU facility prior to D27 will be followed up to assess the days in ICU at D28
- Check whether the patient is on ventilation (refer to Section 6.1.1 for definition of UAB). Patients still on positive pressure ventilation who are transferred to another hospital or healthcare facility prior to D27 will be followed up to assess the VFD outcome at D28
- Record SOFA score only on D21
- Recording of vasoactive drugs and renal support
- Vital signs (temperature, BP and HR) must be measured and recorded in the morning between 04:00 and 10:00 using the values closest to 10:00 (if these values are representative of the patient's condition). All vital sign assessments must be taken at the same time point whenever possible
- Haematology, biochemistry and urinalysis: Samples must be taken daily in the morning between 04:00 and 10:00 using the values closest to 10:00
- Recording of concomitant medications and therapies
- Recording of AEs

7.2.7   Short-term Follow-up Period: Last Day in ICU

These assessments will be done on the day the patient leaves the ICU, either on D28 or earlier, according to the clinical progress of the patient. If the patient is still in the ICU on D28, the next visit or telephone contact will be at D90. If a patient leaves the ICU before D28, the survival status and other endpoints must be assessed on D28.

The following should be done:

- Check patient survival
- Confirm whether patient is in the ICU/in hospital
- Check whether the patient is on ventilation
- Record SOFA scores
- Recording of vasoactive drugs and renal support
- Neutralising antibody sample taken for central testing
- Record pharmacoeconomic assessments (see Section 6.4)
- Physical examination of major body systems
- Vital signs (temperature, BP and HR) must be measured and recorded in the morning between 04:00 and 10:00 using the values closest to 10:00 (if these values are representative of the patient's condition). All vital sign assessments must be taken at the same time point whenever possible
- Haematology, biochemistry and urinalysis: samples must be taken in the morning between 04:00 and 10:00 using the values closest to 10:00
- Recording of concomitant medications and therapies
- Recording of AEs

7.2.8 Short-term Follow-up Period: D28

These procedures apply to patients having left the ICU before D28 and for patients withdrawing from the study before D28.

- Check patient survival
- Confirm whether patient is in the ICU/in hospital
- Check whether the patient is on ventilation
- Record SOFA score, to the extent possible at the investigational site
- Recording of vasoactive drugs and renal support
- Physical examination, including vital signs if possible to be completed by the investigator
- Haematology, chemistry and urinalysis, if possible to perform at the investigational site
- Update pharmacoeconomic assessments (see Section 6.4)
- Recording of concomitant medications and therapies
- Recording of AEs and follow-up on previously reported AEs.

For patients withdrawing from the study, a sample should be taken for neutralising antibodies (see Section 7.2.11.1).

7.2.9 Long-term Follow-up Period: D90 and D180

The study has a long-term follow-up period, with assessments at 3 (D90) and 6 months (D180) after the first dose of study drug was administered. The D90 assessment can be either a visit or telephone contact. For both D90 and D180 follow-up, there is a time window of +14 days.

- Check patient survival
- Recording of AEs if the Investigator considers there is a causal relationship with the study drug
- Report as SAE if the patient died.
- Confirm whether patient is in the ICU/in hospital
- Check whether the patient is on ventilation
- At D180 only: EQ-5D-3L, FEV_1 and 6MWT (including ECG).

If it is not possible for the patient to attend a physical visit, as much information as possible, including QoL, should be obtained by phone.

7.2.10 Extended Long-term Follow-up: D360

The study has an extended long-term follow-up period with an assessment at 12 months (D360) after the first dose of study drug was administered. There is a time window of ± 14 days for this visit.

- Check patient survival
- Recording of AEs if the Investigator considers there is a causal relationship with the study drug
- Report as SAE if the patient died
- Confirm whether patient is in the ICU/in hospital

- Check whether the patient is on ventilation
- EQ-5D-3L, FEV_1 and 6MWT (including ECG).

If it is not possible for the patient to attend a physical visit, as much information as possible, including QoL, should be obtained by phone.

7.2.11 Early Termination Visit

Patients who discontinue early from the study should, if possible, have an early termination visit. The procedures will vary depending on whether the patient withdrew on or before D28 (see Section 7.2.11.1) or after D28 (see Section 7.2.11.2). This visit should take place as soon as possible after it was learned that the patient will not be able to complete follow-up (see also Section 4.4).

7.2.11.1 Withdrawal Prior to D28

For patients withdrawing on or before D28, procedures for the last day in the ICU should be followed. In addition, a sample should be taken for neutralising antibodies.

In the case of an ongoing AE, appropriate safety evaluations and/or additional tests may be performed at any time when clinically indicated at the discretion of the Investigator, until resolution or D28, whichever is first.

Any ongoing AEs should be followed up to resolution or D28, whichever is the sooner.

If the patient refuses to have any of the above assessments, or if the patient is lost to follow-up, then this should be noted in the e-CRF.

If a patient is lost to follow-up, wherever possible, mortality data will be sought from alternative sources, such as the patient's local physician or available national databases and all data obtained reported as SAEs.

7.2.11.2 Withdrawal after D28

If a patient withdraws after D28, no further follow-up will be attempted. However, if a patient is lost to follow-up, wherever possible, mortality data will be sought from alternative sources, such as the patient's local physician or available national databases and all data obtained reported as SAEs.

8   STATISTICAL METHODS

The statistical considerations summarised in this section outline the plan for data analysis of this study.

Before unblinding/database lock, a separate Statistical Analysis Plan (SAP) will be finalised, providing detailed methods for the analyses outlined below. After the clinical parts of the study have been completed, a blinded review of the data will be undertaken according to the SAP. This review will make decisions regarding: the handling of missing data and data for withdrawn patients; the handling of countries recruiting only a small number of patients in the statistical analyses that include country effects; and the definition of the Per-Protocol Set (PPS) at the patient level.

Any deviations from the planned analyses will be described and justified in the final integrated study report.

8.1   Study Patients

8.1.1   Disposition of Patients

Disposition and reasons for discontinuation will be summarised for all patients together with study drug exposure and study duration by treatment group.

The number and percentage of patients entering and completing each phase of the study will be presented by treatment group. Reasons for withdrawal pre- and post-randomisation will also be summarised.

The disposition of patients will also include information on the number and percentage of patients who:

- completed study drug and follow-up
- withdrew from study drug but completed follow-up
- withdrew from study drug and from follow-up

8.1.2   Protocol Deviations

Deviations from the protocol including violations of inclusion/exclusion criteria will be classified as "minor" or "major" in conjunction with the Sponsor and Medical Monitor. Major deviations from the protocol will lead to the exclusion of that patient from the PPS. Deviations will be defined prior to unblinding.

8.1.3   Analysis Sets

The following analysis sets will be defined for statistical analysis:

The **Full analysis set (FAS)** will consist of all randomised and treated patients. The primary efficacy analyses will be based on this data set. Patients will be included in the analysis according to the treatment to which they were randomised.

The **Per-Protocol Set** (**PPS**) will consist of patients in the FAS excluding patients with major protocol violations. A list of major protocol violations relevant for excluding data from the PPS will be detailed in the SAP. The precise definition of the PPS at the patient level will be identified at the blinded data review meeting.

Statistical analyses for the primary and secondary endpoints will be performed on both the FAS and PPS.

The **safety set** will consist of all patients who receive at least one dose of study drug. All safety and tolerability analyses will be based on this analysis set. A patient who

receives the wrong treatment according to their randomisation will be analysed for safety and tolerability in the treatment group corresponding to the treatment received.

Statistical analyses for the primary and secondary endpoints will be performed on both the FAS and PPS. The primary efficacy endpoint analysis will be based on the FAS; a secondary analysis will also be performed based on the PPS to assess the sensitivity of the analysis to the choice of analysis set. All safety analyses will be based on the safety set. Demographic and baseline characteristics will be evaluated for the FAS.

8.2   General Considerations

All statistical tests will be two sided and will be performed at the significance level of 0.05, unless otherwise stated.

Continuous data will be summarised by treatment group using descriptive statistics (number, mean, median, standard deviation [SD], minimum and maximum). Categorical data will be summarised by treatment group using frequency tables (frequencies and percentages).

8.2.1   Analysis and Data Conventions

8.2.1.1   Definition of Baseline

The baseline assessment, where relevant, will be the latest available valid pre-dose assessment.

8.2.1.2   Visit Windows

Assessments made outside of protocol-mandated windows will be displayed according to the e-CRF assessment recorded by the Investigator.

8.2.1.3   Unscheduled Assessments

Extra assessments (laboratory data or vital signs associated with non-protocol clinical visits or obtained in the course of investigating or managing AEs) will be included in listings, but not summaries. If more than one laboratory value is available for a given visit, the first valid observation will be used in summaries and all observations will be presented in listings. It is noted that invalid laboratory data will not be used (from haemolysed samples, mishandled samples, quantity not sufficient, or other conditions that would render values invalid).

8.2.1.4   Missing Data Handling

In general, data will not be imputed for the primary efficacy analysis or the safety analysis. For other efficacy analyses, where relevant, imputations will use the last observation carried forward method for patients in the analysis. Additional details will be given in the SAP.

Treatment by country interactions will be investigated and if any country has too few patients, a pooling strategy will be defined at the blinded data review, prior to unblinding.

The approach to multiplicity testing for the key secondary endpoints is explained in Section 8.5.2.1.1.

8.3 Demographics, Medical History, Baseline Characteristics and Concomitant Medications

Baseline assessments will consist of those assessments carried out in the screening period and those carried out prior to the first dose of study drug on D1.

Demographic and baseline characteristics will be summarised by treatment group and overall. No formal statistical comparisons will be undertaken to compare treatment groups for any of these parameters.

Demographic data, medical history, concomitant disease and concomitant medication will be summarised by descriptive statistics (number, mean, SD, median, minimum and maximum) or frequency tables, overall and stratified by treatment.

A medication given prior to the first injection of study drug will be classified as a prior medication. A medication given with or after the first injection of study drug will be classified as concomitant. Prior medications continuing during the study will be labelled accordingly in the listings.

8.4 Treatment Compliance

As the study drug is administered directly to the patient in the ICU, treatment compliance will not need to be measured.

8.5 Efficacy Analyses

8.5.1 Primary Efficacy Analysis

8.5.1.1 Hypotheses to Be Tested

The following hypotheses will be tested:

- Null hypothesis H_0: the value of VFDsurv in the treatment group is equal to that in the placebo group

- Alternative hypothesis H_A: the value of VFDsurv in the treatment group is not equal to that in the placebo group

8.5.1.2 Statistical Methods

The primary composite endpoint includes death and days free of mechanical ventilation within 28 days among survivors. VFDs to D28 is defined as the number of calendar days during which the patient is ventilator-free including two UAB days to D28, assuming that a patient survives for at least two consecutive calendar days after initiating UAB.

For example: If a patient initiates UAB in the afternoon of D16 and survives to D28 that patient would be assigned a VFD value of 12. If a patient initiates UAB in the afternoon of D16 but dies in the afternoon of D25 they would be assigned a VFD value of 9. If a patient survives for more than 48 hours after initiating UAB but then requires assisted breathing (for any reason) before D28, the VFD value would be the total number of UAB days before D28 unless a period of assisted breathing was less than 24 hours and the purpose of assisted breathing was a surgical procedure. Patients who die without initiating UAB will be assigned a VFD value of 0 and patients who require more than 28 days of mechanical ventilation will also be assigned a VFD value of 0.

The non-parametric analysis of the primary composite endpoint VFDsurv is based on a scoring scheme with patients who do better getting a higher score. All patients who die before 28 days will be assigned a VFDsurv score of -1. For those patients who survive to D28 the VFDsurv score will be equal to the VFD value calculated according to the above definition.

The statistical analysis for group comparison of the primary endpoint will then be based on the van Elteren test adjusting for country, ARDS severity and key baseline characteristics. [Ref: Van Elteren 1960] The statistical methodology with regard to the scoring scheme is as set down in Finkelstein and Schoenfeld. [Ref: Finkelstein et al 1999] The primary analysis of this endpoint will be based on the FAS, with a sensitivity analysis based on the PPS providing supportive information. Each of these analyses will be undertaken at the two-sided significance level of 0.05.

8.5.1.3   Subgroup Analyses

The primary endpoint will be summarised by country, treatment severity and key baseline characteristics. These analyses will be fully defined in the SAP.

8.5.2   Secondary Efficacy Analyses

8.5.2.1   All-cause Mortality

As confirmatory measures of efficacy, the following endpoints will be analysed:

- Mortality up to D28, up to D90 (3 months) and up to D180 (6 months)
- Mortality in ICU up to D28
- Mortality in hospital up to D28

The primary analysis of the binary mortality endpoints will be based on the FAS and will be undertaken using logistic regression adjusting for country, ARDS severity and key baseline characteristics. This analysis will be repeated using the PPS as a sensitivity analysis. Each of these analyses will be summarised using the observed mortality rates for the two treatment groups, a 95% confidence interval (CI) for the difference in these rates, an adjusted odds ratio and a 95% CI for the adjusted odds ratio. A two-sided significance level of 0.05 will be used for each of these endpoints.

There will also be an evaluation of the homogeneity of the treatment effect by investigating treatment by country, treatment by ARDS severity, and treatment by baseline factor interactions in the logistic model. Data for these efficacy endpoints will also be presented for key predefined subgroups of patients together with 95% CIs for the difference in those rates to supplement the investigation of homogeneity.

ICU mortality and hospital mortality will be analysed in the same way as overall mortality.

8.5.2.1.1   Control of Multiplicity

Multiplicity will be controlled across the key secondary endpoints using a pre-specified hierarchy. The pre-specified hierarchy is:

1. All-cause mortality at D28
2. All-cause mortality at D90
3. All-cause mortality at D180
4. Mortality in ICU at D28
5. Mortality in hospital at D28

Statistical significance will be declared down to the first non-significant endpoint (based on a two-sided significance level of 0.05) in this hierarchy. All p-values at and below this level will be considered as providing supportive information only.

VFDsurv will also be considered as a secondary endpoint, although no formal p-value comparisons will be undertaken for that endpoint as patients who die will be excluded from such an analysis.

8.5.2.2 Days Free of Organ Failure, Renal Support, Vasoactive Support, ICU Care and Days in Hospital

Organ failure-free days are defined as the number of days in the first 28 days after the first dose of study drug that the patient is alive and free of organ failure with a SOFA score of zero.

ICU-free days are defined as the number of days from the time of ICU discharge to D28, assuming survival for at least two consecutive calendar days after ICU discharge and continued stay outside the ICU setting to D28. If a patient returns to the ICU and subsequently needs ICU admission to D28, ICU-free days will be counted from the end of the last period of ICU discharge to D28. A period of ICU admission lasting less than 24 hours in relation to a surgical procedure will not count in the calculation of ICU-free days. If a patient was in the ICU at D27 or dies prior to D28, ICU-free days will be zero. Patients transferred to another hospital or healthcare facility will be followed to D28 to assess this endpoint.

The statistical methods to be used for these secondary efficacy endpoints will be detailed in the SAP.

8.5.2.3 Immunogenicity

The presence of neutralising antibodies to IFN beta-1a at D28 will be summarised by treatment groups in terms of counts.

8.5.2.4 Myxovirus Resistance Protein A

Details of the statistical analysis of MxA will be provided in the SAP.

8.5.2.5 Quality of Life, Respiratory and Neurological Functioning

Health-related QoL, respiratory functioning (FEV_1) and neurological functioning (6MWT) will be assessed at D180 (6 months) and included in the Clinical Study Report Addendum. Extended long-term follow-up data will also be gathered at D360 and submitted as an Addendum to the report (see Section 8.8.4).

The EQ-5D-3L data will be analysed overall. Summary statistics will also be provided in relation to the five individual domains. Further details will be provided in the SAP.

These data will only be available for survivors at D180. Analyses of these parameters will be based on the subset of patients who survive to that time point and not on the FAS. Only summary statistics and 95% CIs for treatment differences (for descriptive purposes) will be calculated.

8.6 Safety Analyses

These summaries will be based on the safety set.

8.6.1 Adverse Events

AEs and SAEs will be coded using the current version of Medical Dictionary for Regulatory Activities (MedDRA) and will be classified by system organ classes and preferred terms. In the event that intensity or relationship of an AE to the study drug is missing, a worst-case scenario will prevail (severe in intensity or probably related). Only TEAEs (AEs that occur after the first study drug administration, that were not pre-existing or that increased in intensity) will be included in the summary tables. Counting will be performed for patients and events separately. Patients experiencing the same event more than once will have that event counted once within the corresponding system organ class and with a unique preferred term. All AEs will be included in the data listings. Listings of SAEs, of AEs leading to study drug withdrawal and of AEs leading to death will also be provided.

8.6.2 Vital Signs

Vital signs data (BP, HR and temperature) will be summarised using descriptive statistics (mean, median, SD, minimum and maximum) by treatment group separately at D1 and daily to D28. The maximum change from baseline over the 28-day follow-up period will be calculated for each patient and summarised.

8.6.3 Clinical Laboratory and Urinalysis Variables

Descriptive statistics (mean, median, SD, minimum and maximum) for biochemistry and haematology variables will be obtained and tabulated by treatment group pre-dose on D1 and daily to D28. Number and percentage will be obtained for each category for urinalysis and tabulated by treatment group at the same time points.

Shift tables (within, below and above the normal range) will also be provided for each parameter in relation to the maximum change from baseline from D1 over the complete 28-day follow-up period.

8.6.4 Physical Examination

The incidence of physical examination findings will be summarised by treatment group.

8.6.5 Independent Data Monitoring Committee

An IDMC will be established for the study to monitor safety (see Section 9.8). No formal interim analyses will be performed.

8.7 Pharmacoeconomics

The expected incremental cost-effectiveness will be calculated for intravenous FP-1201-lyo compared with standard care in the treatment of patients with moderate or severe ARDS admitted to ICU in the study-related countries. Criteria that will be measured at D28 as part of the cost-effectiveness analyses in this study are:

- Days free of organ failure (D28 or last day in ICU if patient has left the ICU earlier than D28, or at withdrawal)
- Days free of renal support
- Days free of vasoactive support
- Days free of mechanical ventilation
- Number of ICU-free days

- Number of days in hospital

Details on the statistical analysis of these criteria and their role in the economic evaluation will be provided in the SAP.

8.8   Exploratory Analyses

8.8.1   Composite Endpoint at 90 Days

The D90 composite VFDsurv endpoint includes death and days free of mechanical ventilation within 90 days among survivors. VFDsurv to D90 is defined as the number of calendar days after initiating UAB to D90, using the same assumptions as for the D28 analysis.

8.8.2   Gas Exchange Ratio

The PaO_2/FiO_2 ratio will be used as an indicator of lung function. Moderate ARDS is defined as PaO_2/FiO_2 >100 mmHg and ≤200 mmHg with PEEP ≥5 cmH_2O; severe ARDS is defined as PaO_2/FiO_2 ≤100 mmHg with PEEP ≥5 cmH_2O.

The ordered categorical endpoint is defined as improvement (from severe to moderate/mild; from moderate to mild ARDS), no change, or worsening (from moderate to severe/death; from severe to death) in terms of gas exchange (PaO_2/FiO_2 ratio) from baseline to D28. The data analysed for this endpoint will be:

- % of patients improving from severe to moderate ARDS or from moderate to mild ARDS
- % of patients worsening from moderate to severe ARDS/death or from severe ARDS to death

Details on the statistical analysis of change in lung function will be provided in the SAP.

8.8.3   CD73 and Potential Inflammatory Markers; Pharmacogenetic Analysis

Details on the statistical analysis of CD73 and PIM biomarkers will be provided in the SAP.

No statistical analysis is planned for the pharmacogenetic investigation.

8.8.4   Extended Follow-up

Details of the statistical analysis of data from the long-term and extended follow-up (D180 and D360) of 6-month and 12-month mortality rates, EQ-5D-3L, 6MWT, and FEV_1 will be detailed in a separate SAP (as they will be reported separately as addendums to the Clinical Study Report).

8.9   Interim Analyses

No interim analyses are planned.

8.10   Determination of Sample Size

For 90% power and a two-sided Mann–Whitney U-test at the significance level of 0.05, a total of 272 patients are required based on the following assumptions:

- mortality rate of 30% in the control group and 15% in the FP-1201-lyo group at D28
- 20% of patients survive but with zero ventilator-free days (VFDs) in the control group
- A mean difference (FP-1201-lyo minus control) of 3.0 days in mean ventilator-free days where patients who die are assigned a score of 0

However, assuming 5% of patients (16) drop-out and a further 4% (12) of the remaining patients will not be evaluable for the efficacy analysis, the study will randomise 300 patients to build in some flexibility around the assumptions listed above.

PASS software (Version 11; NCSS LLC, Kaysville, UT, USA) was used to calculate the required sample size for this study. Table 6 gives values for power based on this sample size and alternative values for the mortality rates in the two treatment groups with a total sample size of 300 (including 5% drop-outs and a further 4% non-evaluable patients). These calculations are based on the construction of two multinomial distributions according to the above assumptions and the associated non-parametric comparison of those distributions.

Table 6   Values for Power for Various Mortality Rates per Treatment Group (n=300)

Mortality Exptl. group a_1	Mortality Control group a_2	VFDsurv=0[2] Exptl. group b_1	VFDsurv=0[1] Control group b_2	VFDsurv=y Exptl. Group d_1	VFDsurv=y Control Group d_2	Difference in mean VFD[3] (days) c	Power	Total sample size N
15%	*30%*	*7%*	*20%*	*2.8%*	*1.8%*	*4.0*	*90%, 80%*	*194, 144*
18%	*30%*	*4%*	*20%*	*2.8%*	*1.8%*	*4.0*	*90%, 80%*	*224, 164*
15%	*30%*	*11%*	*20%*	*2.7%*	*1.8%*	*3.5*	*90%, 80%*	*238, 180*
18%	*30%*	*8%*	*20%*	*2.7%*	*1.8%*	*3.5*	*90%, 80%*	*270, 206*
15%	*30%*	*14%*	*20%*	*2.5%*	*1.8%*	*3.0*	*90%, 80%*	*284, 226*
18%	*30%*	*11%*	*20%*	*2.5%*	*1.8%*	*3.0*	90%, *80%*	340, *248*
15%	*30%*	*18%*	*20%*	*2.4%*	*1.8%*	*2.5*	90%, *80%*	374, *262*
18%	30%	15%	20%	2.4%	1.8%	2.5	90%, 80%	438, 330

[1]*VFDsurv=0 is the number of patients alive at Day 28 but on mechanical ventilation for the whole 28-day period.*

[2]*Calculated value.*

[3]*This difference in mean VFD is calculated with deaths within 28 days given a value of 0 for VFD.*

[4]*Assumes 4% non-evaluable rate.*

Abbreviations: Exptl=experimental; VFD=ventilator-free day; VFDsurv=composite endpoint including any-cause death at D28 and days free of mechanical ventilation within 28 days among survivors.

9   ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

9.1   Data Quality Assurance

The Sponsor or Sponsor's designee will conduct a site visit to each study centre to verify the qualifications of each Investigator, inspect the site facilities and inform the Investigator of their responsibilities and the procedures for ensuring adequate and correct documentation.

The Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study participant. All information recorded in the e-CRF for this study must be consistent with the patients' source documentation (i.e., medical records).

9.1.1   Database Management and Quality Control

All study-related data generated by site personnel will be captured electronically at each study centre using the e-CRF.

Central laboratory assays (e.g., PD, genetic) will be managed by the central laboratories and results will be transferred for inclusion in the study analysis database.

Once the e-CRF clinical data have been submitted, corrections to the data fields will be captured in an audit trail. The reason for change, the name of the person who performed the change, and the time and date will be logged to provide an audit trail.

The specific procedures to be used for data entry and query resolution using the e-CRF will be provided to study sites in CRF completion instructions. In addition, site personnel will receive training on the e-CRF. Once the source data verification is complete and all queries are closed, Data Management will freeze the e-CRF page.

9.2   Case Report Forms and Source Documentation

All data obtained during this study must be promptly entered in the e-CRF. All source documents from which e-CRF entries are derived should be placed in the patient's medical records. Measurements for which source documents are usually available include laboratory assessments, CT scans and X-rays.

Data that will be entered directly into the e-CRF (i.e., for which there is no prior written or electronic record) are considered to be source data.

The e-CRF entries for each patient will be checked against source documents at the study site by the CRA.

9.2.1   Data Collection

The Investigators (and appropriately authorised staff) will be given access to an online web-based electronic data-capture system that is compliant with US Food and Drug Administration Title 21 Code of Federal Regulations Part 11. This system is specifically designed for the collection of clinical data in electronic format. Access rights to the electronic data-capture system will be carefully controlled and configured according to each individual's role throughout the study. Only the Investigator and authorised staff will be able to enter and correct data in the e-CRF.

The e-CRF should be completed for each patient included in the study and should reflect the latest observations on the patients participating in the study. Therefore, the e-CRF is to be completed as soon as possible during or immediately after the patient's visit or assessment. The Investigator must verify that all data entries in the e-CRF are

accurate and correct. If some assessments cannot be done, or if certain information is unavailable, not applicable or unknown, the Investigator should indicate this in the e-CRF.

Computerised data-check programs and manual checks will identify any data discrepancies for resolution. Corresponding queries will be generated in the system and the site will be informed online about new issues to be resolved. All discrepancies must be resolved online directly by the Investigator or by staff authorised to do this by Delegation of Authority.

After completion, the Investigator will be required to electronically sign off the clinical data.

9.3   Access to Source Data

During the study, the CRO site CRA will make regular site visits to review protocol compliance, conduct source data verification by comparing e-CRF entries and individual patient's medical records, assess drug accountability and management, assess laboratory procedures and ensure that the study is being conducted according to pertinent regulatory and protocol requirements. The review of medical records will be performed in a manner that ensures patient confidentiality is maintained.

Source data verification will be required to monitor the progress of the study. Moreover, regulatory authorities in certain countries, IECs and/or the Sponsor's Clinical Quality Assurance Group or designee may wish to carry out such source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical confidentiality. The Investigator must assure the CRO and the Sponsor that they will provide the necessary support at all times.

9.4   Archiving Study Records

According to ICH guidelines, essential documents should be retained for a minimum of 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. However, these documents should be retained for a longer period if required by the applicable legal requirements.

9.5   Good Clinical Practice

The procedures set out in this study protocol are designed to ensure that the Sponsor and Investigator abide by the principles of the Good Clinical Practice guidelines of the ICH and the Declaration of Helsinki. [Ref: Declaration of Helsinki 2013] The study will also be carried out in keeping with local legal requirements.

9.6   Informed Consent

The Investigator at each investigational site is responsible for ensuring that written informed consent for study participation is given by each patient or their legal representative prior to collection of study data and administration of the study drug. Unless the study site is in a country where consent can only be obtained from the patient but can be waived and obtained retrospectively, according to local IEC rules, the Investigator will not undertake any investigation specifically required only for the

clinical study until valid consent has been obtained. If consent has not been obtained, a patient cannot be randomised into the study.

Consent will be sought from the patients themselves, if this is possible. However, it is recognised that most patients will be unable to give written informed consent themselves due to alteration in their level of consciousness caused by therapeutic sedation. Written informed consent will be sought from a PerLR or PrfLR. In some countries, according to IEC rules, consent can only obtained from the patient. In these countries, if the patient is unable to give consent, their relatives and the patient's own family doctor will be informed about the study. Consent from the patient will be obtained as soon as the patient is able to understand the study and sign the consent form. As this is an international study, the appropriate guidance and law will be followed for each country.

If a protocol amendment is required, the ICD may need to be revised to reflect those changes. If the ICD is revised, it must be reviewed and approved by the appropriate IEC, and signed by all patients (or their representatives) subsequently enrolled in the study as well as those currently enrolled in the study.

9.6.1   Consent Procedure

The person giving consent will be informed about the study by the Investigator or by a member of the research team to whom the Investigator has delegated the consent process.

To provide all the necessary information, clinical study information and ICDs are quite lengthy. For this study, dependent on country specific regulations, an abbreviated initial section of the ICD will provide a short summary of key information, followed by the detailed information for those interested in receiving further information.

The patient, PerLR or PrfLR must be given adequate time to consider their decision about entering the study. However, the requirement to initiate study treatment within 48 hours of moderate to severe ARDS diagnosis will of necessity be a consideration.

In all cases, the patient, PerLR or PrfLR will be asked to sign two copies of the ICD, which will then be countersigned by the Investigator or the member of the study team to whom obtaining consent has been delegated. One copy of the document will be retained by the person giving consent (patient, PerLR or PrfLR). The second copy will be photocopied. The photocopy will be placed in the patient's medical records and the original will be retained in the Investigator Site File.

A similar procedure will be followed for retrospective patient informed consent, which will be an additional section at the end of the ICD (see Section 9.6.5).

The terms of the consent and when it was obtained must be documented in the e-CRF system.

The patient, PerLR or PrfLR will also be asked to give separate consent for a genetic sample to be taken. Consent for genetic sampling is not a prerequisite for study participation.

9.6.2   Patient Consent

Whenever possible, informed consent will be obtained from the patient. Once the patient has been informed about the study, the patient will be given a copy of the ICD. After being given an adequate amount of time to consider the information, if the patient decides to enter the study, they will be asked to sign two copies of the ICD.

9.6.3   Personal Legal Representative Consent

If the patient is unable to give consent then informed consent will be sought from the patient's PerLR. The PerLR may be a relative, partner or close friend. Any country-specific guidance on who may act as a PerLR will be followed.

Once the PerLR has been informed about the study, the PerLR will be given a copy of the ICD and will be asked to give an opinion as to whether the patient would object to taking part in the study.

After being given an adequate amount of time to consider the information, if the PerLR decides that the patient would have no objection to participating in the study then the PerLR will be asked to sign two copies of the ICD.

9.6.4   Professional Legal Representative Consent

If the patient is unable to give consent and no PerLR is available then a doctor at the investigational site who is not connected with the conduct of the study may act as a PrfLR. Any country-specific guidance on who may act as a PrfLR will be followed.

After being informed about the study, the PrfLR will be given a copy of the ICD. If the PrfLR decides that the patient is suitable for entry into the study then the PrfLR will be asked to sign two copies of the ICD.

9.6.5   Retrospective Patient Informed Consent

Patients for whom consent was given by a PerLR or a PrfLR will be informed of their participation in the study by the Investigator or by a member of the research team to whom the Investigator has delegated the consent process. This process will take place once the patient has regained the capacity to understand the details of the study. The timing of this process will thus vary between patients. However, every attempt to obtain retrospective consent must be made when the patient's condition is appropriate. If a patient's condition has precluded the re-consent process occurring by D360 (12 months), then no further attempts will be made to re-consent the patient.

Once informed, patients will be given an adequate amount of time to consider their decision to consent to continued participation in the study and to sign the re-consent section of the ICD.

If the patient does not give retrospective consent then the patient will be asked if the data collected to that time point can be analysed. If the patient will not allow any of their data to be used, then the data collected from the patient to that time point will not be entered into the analyses and no further data will be collected; the patient will be considered as withdrawn and the reason for withdrawal will be 'retrospective consent not given'.

9.6.6   Withdrawal of Consent

Patients may withdraw or be withdrawn (by the PerLR or PrfLR) from the study at any time, for any reason and such a decision will not affect the ongoing care given to the patient. Data recorded up to the point of withdrawal will be included in the study analyses, unless consent for any use of the data has also been withdrawn. In this case, data entered in the e-CRF would not be deleted, but would not be used in subsequent analyses or reports.

If a patient or PerLR/PrfLR requests termination of the administration of study drug during the treatment period, then the administration of study drug will be stopped but the patient will continue in the study and all follow-up assessments will be performed.

If a patient or a PerLR/PrfLR withdraws consent during the treatment period then the administration of study drug will be stopped and no further active study assessments will be performed from that point on. However, permission will be sought to access the patient's medical records to obtain data relevant to the study (e.g., mortality, VFDs, etc.).

If a patient or a PerLR/PrfLR withdraws consent after the treatment period then no further active study assessments will be performed from that point on. However, permission will be sought to access the patient's medical records to obtain data relevant to the study (e.g., mortality, VFDs, etc.).

9.7   Protocol Approval and Amendment

For the study to start, all required documentation must be approved by the IEC and Competent Authority, in accordance with local legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before the first patient is enrolled in the study.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IEC/competent authority approval prior to implementation (if appropriate).

Administrative changes (not affecting the patient benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

9.8   Independent Data Monitoring Committee

An IDMC will be established for the study. The IDMC and associated parties (CRO Pharmacovigilance, Data management, Sponsor as well as the CRO for IDMC management) will function under the terms of an IDMC Charter. The IDMC will comprise four members, including one independent biostatistician and three senior clinicians with significant experience in ARDS and who are not involved in the study. The duty of this IDMC is to protect the safety interests of patients and all others who may possibly be exposed to the study drug and to make recommendations to the Sponsor. The IDMC will review ongoing safety data in an unblinded manner. According to the current IDMC Charter, meetings are scheduled to take place after the data has been received for the last patient of approximately 30, 60, 200 and 300 patients who either have completed 14 days in the study following their first dose of study medication or have been withdrawn for any reason (including death). The IDMC will not review efficacy data, other than how it relates to the safety of the therapy. From each meeting, the IDMC will make a blinded recommendation to the Sponsor regarding the study to continue without change, modify study or enrolment to be placed on hold, or study termination. The sponsor is obligated to inform the study sites, Ethics Committees and Competent Authorities of the IDMC recommendations according to country specific requirements

9.9   Steering Committee

In addition to the IDMC, a separate and blinded Steering Committee of respected and experienced critical care personnel representing all participating countries will be established for this study. Members of the Steering Committee may also be

Investigators or Sub-investigators. Face-to-face meetings will be held as determined by need. Routine business will be conducted by email, post or teleconferencing.

The Steering Committee will provide advice and support to the study in matters concerning:

- Communicating with national sites (e.g., in the instance of recruitment or quality issues)
- Oversight of the progress of the study
- Informing and advising on all aspects of the study
- Review and advice on any proposal for a co-enrolment study

9.10 Duration of the Study

For an individual patient, the maximum duration of the study will be up to 376 days, including up to 48 hours for screening, 6 days' treatment and follow-up at 6 and 12 months (D360 with a ±14-day window).

The study will close when all patients have completed the 12-month extended follow-up visit.

9.11 Premature Termination of the Study

The Sponsor reserves the right to stop the study at any time on the basis of new information regarding safety or efficacy (e.g., discovery of an unexpected, significant or unacceptable risk to the patients enrolled in the study), or if study progress is unsatisfactory (e.g., failure to enrol patients at an acceptable rate), or for other valid reasons (e.g., Sponsor decides to suspend or discontinue development of the drug). After such a decision is made, the Investigator must inform all on-study patients within 1 week. All delivered study materials must be collected and all e-CRF pages completed to the extent possible.

9.12 Confidentiality

All study findings and documents will be regarded as confidential. The Investigator and members of their research team must not disclose any information without prior written approval from the Sponsor.

The anonymity of participating patients must be maintained. Patients will be identified on the e-CRF and other documents submitted to the CRO by their patient number and/or birth date, not by name. Documents that identify the patient must not to be submitted to the CRO (e.g., the ICD) and must be maintained in confidence by the Investigator.

9.13 Other Ethical and Regulatory Issues

If a significant safety issue is identified, either from an individual case report or review of aggregate data, then the Sponsor will issue prompt notification to all parties – regulatory authorities, Investigators and IEC/ EC.

A significant safety issue is one that has a significant impact on the course of the clinical study or programme (including the potential for suspension of the development programme or amendments to protocols) or warrants immediate update of informed consent.

9.14 Publication Policy

Any manuscript, abstract or other publication or presentation of results or information arising in connection with the study (including any ancillary study involving study patients) must be prepared in conjunction with the study Sponsor and must be submitted to the Sponsor for review and comment at least 45 days prior to submission for publication or presentation. No single centre or groups of centres may publish individually. The Sponsor's comments on the proposed publication shall be considered in good faith by the authors. The Sponsor may delay such submission by a maximum of 90 days if it reasonably believes that publication of results may compromise its intellectual property rights or may insist that such information or data is removed from the proposed publication. Publication of the results will not include confidential information without the permission of the Sponsor.

The original e-CRF pages and all data generated during the study under this protocol will become the property of the Sponsor.

The Sponsor may announce quality-assured summary data in order to comply with the requirements of financial regulatory authorities, while ensuring so far as possible that such announcements will not compromise the Investigators' ability to publish the data in appropriate scientific forums.

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11   APPENDIX 1: GUIDANCE ON SUPPORTIVE CARE MEASURES

11.1   Guidance on Supportive Care Measures

Abbreviations used in Appendix 1

APRV	Airway pressure release ventilation
ARDS	Acute respiratory distress syndrome
CPAP	Continuous positive airway pressure
CVP	Central Venous Pressure
e-CRF	Electronic Case Report Form
FiO_2	Fraction of inspired oxygen
HFOV	High-frequency oscillatory ventilation
ICU	Intensive Care Unit
PaO_2	Arterial partial pressure of oxygen
PBW	Predicted body weight
PEEP	Positive end expiratory pressure
Pplat	Inspiratory plateau pressure
PS	Pressure support
SBT	Spontaneous breathing trial
SpO_2	Peripheral capillary oxygen saturation
Vt	Tidal volume

1. **Introduction**

   Patients treated in the FPCLI002 study will be managed with supportive care measures according to best practice and in line with guidance agreed locally by each intensive care unit (ICU) to ensure full buy-in by the ICU team.

   - Each participating ICU must be in agreement with the use of a lung-protective ventilation strategy incorporating a low tidal volume (Vt) strategy for ventilation based on predicted body weight (PBW).

   - Each participating ICU must give due consideration to the study guidance detailed below on all supportive measures.

2. **Prohibited Medications and Procedures**

   There are no prohibited concomitant medications or procedures.

   The guidance detailed below on nutritional support, nitric oxide use, corticosteroid use, glucose management, neuromuscular blockers use and sedation should be followed.

3. **Ventilation Management**

   Investigative sites must use a lung-protective ventilation approach incorporating a low Vt strategy based on PBW in line with the ARDS Network publication.[Ref: The Acute Respiratory Distress Syndrome Network 2000] The goal for ventilation of a patient with acute

respiratory distress syndrome (ARDS) is to maintain adequate gas exchange while minimising ventilator-induced lung injury and barotrauma. The ARDS Network conclusions reported in 2000 should be followed and are part of the ventilation strategy set out below under ventilator procedures.

Non-conventional ventilation: Given the two recently published studies on high-frequency oscillatory ventilation (HFOV) (Oscillate [Ref: Ferguson N D. et al 2013] and OSCAR [Ref: Young D et al 2013; the OSCAR Study Group]) mechanical ventilation using HFOV is discouraged as a first-line ventilation strategy but is allowed as a non-conventional ventilation strategy. However it should be noted that when using HFOV it is not possible to record some ventilation parameters as detailed in the study assessments. All the possible parameters should be recorded.

3.1   Ventilator Procedures

ICUs should have a ventilator policy and this should be followed. In the absence of such a policy, the ARDS Network lung-protective lower Vt strategy is to be used in this study. This strategy was associated with lower mortality rates in three ARDS Network studies (ARMA [Ref: ARDS Network 1998], ALVEOLI [Ref: The National Heart, Lung and Blood Institute ARDS Clinical Trials Network 2004], and FACTT [Ref: The National Heart, Lung and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network 2006]) and has been widely adopted. This strategy is detailed below:

- Ventilation mode: Although volume-assist control mode was used in the ARDS Network study, any mode of ventilation capable of delivering the prescribed Vt (6 mL/kg PBW ±2 mL/kg) within the pressure limitation (plateau pressure limitation ≤30 cm of water) may be used, provided the Vt and airway pressure targets are monitored and adjusted appropriately.

- During airway pressure release ventilation (APRV), Vt is defined as the sum of the volume that results from the ventilator pressure release and an estimation of the average spontaneous Vt.

- Measurement and recording of inspiratory plateau pressure (Pplat) should be according to the ICU routine at the investigational site. Measurement and recording at intervals of at least 4 hours and after changes in Vt and positive end expiratory pressure (PEEP) is recommended.

- If Pplat is ≥30 cmH$_2$O, then Vt should be reduced to 5 mL/kg PBW and then to 4 mL/kg PBW if necessary to decrease Pplat to ≤30 cmH$_2$O.

- If "severe dyspnoea" is seen (> 3 double breaths per minute or the airway pressure remains at or below the PEEP level during inspiration), then the Vt should be raised to 7 or 8 mL/kg PBW if Pplat remains <30 cmH$_2$O. If Pplat exceeds 30 cmH$_2$O on 7 or 8 mL/kg PBW, then a lower Vt should be used and increased sedation should be considered.

- If a pH < 7.15 is observed, Vt may be raised and the Pplat limit suspended (not required).

- The oxygenation target is: arterial partial pressure of oxygen (PaO$_2$) of 7.33–10.67 kPa or peripheral capillary oxygen saturation (SpO$_2$) of 88–95%.

- The minimum PEEP that should be used is 5 cmH$_2$O.

- Within 5 minutes of consistent measurements that are below the oxygenation target range, the fraction of inspired oxygen (FiO$_2$) or PEEP should be adjusted upwards.

- Within 30 minutes of consistent measurements above the oxygenation target range, the FiO_2 or PEEP should be adjusted downwards.

- In the absence of an ICU policy, Investigators should implement the strategy for PEEP as described in EXPRESS [Ref: Mercat A et al 2008] or the ALVEOLI 2004 [Ref: The National Heart, Lung and Blood Institute ARDS Clinical Trials Network 2004] as long as Vt and plateau airway pressures are maintained within target.

- It is recommended to raise the ventilator respiratory rate incrementally up to 35 breaths per minute (maximum set rate) if a pH <7.30 is observed.

- No specific rules are set in this study about the ratio of the duration of inspiration to the duration of expiration (inspiration: expiration ratio) settings.

- The use of bicarbonate is allowed in this study (it is neither encouraged nor discouraged) if a pH ≤7.30 is observed.

3.2   Extra-corporeal Membrane Oxygenation

The use of extra-corporeal membrane oxygenation is allowed as a rescue therapy and its use should be recorded in the study e-CRF.

3.3   Weaning Procedures

Where there is an ICU weaning policy on ventilation this should be followed. In the absence of a unit policy the guidelines below should be followed.

- Patients should be assessed for the following weaning readiness criteria each day between 06:00 and 12:00. This assessment will NOT be performed within the first 24 hours after randomisation.

    a) PaO_2/FiO_2 >300 after 1 hour with PEEP <10 and FiO_2 <0.5

    b) Values of both PEEP and FiO_2 ≤ values from previous day

    c) The patient is not receiving neuromuscular blocking agents

    d) The patient is exhibiting inspiratory efforts

    e) Systolic arterial pressure ≥90 mmHg without vasopressor support (≤5 µg/kg/min dopamine or dobutamine will not be considered vasopressor support in this context)

- If criteria a–e are met, application of respiratory management as per protocol will be terminated and, in the treatment group, extra-corporeal carbon dioxide removal will be interrupted and vascular catheters removed. Clinicians will then be free to use any ventilation protocol they consider clinically appropriate.

- A spontaneous breathing trial (SBT) procedure and assessment for unassisted breathing will be performed. The SBT will take place over up to 120 minutes of spontaneous breathing with FiO_2 <0.5 using any of the following approaches:

    a) Pressure support (PS) <5 cmH_2O, PEEP <5 cmH_2O

    b) Continuous positive airway pressure (CPAP) <5 cmH_2O

    c) T-piece

    d) Tracheotomy mask

- Criteria for reporting a SBT as "**successful**" are:

    a) SpO_2 ≥90% and/or PaO_2 ≥60 mmHg

    b) Respiratory rate ≤35/min

    c) pH ≥7.30

    d) No respiratory distress (defined as ≥2 of the following):

     o   Heart rate ≥120% of the rate at 06:00 (≤5 min at ≥120% may be tolerated)

     o   Marked use of accessory muscles

     o   Abdominal paradox

     o   Diaphoresis

     o   Marked subjective dyspnoea

If any of criteria a–d are not met, the SBT will be reported as "**unsuccessful**" and previous ventilator settings will be initiated or PS ≥10 cmH$_2$O with PEEP and FiO$_2$ = previous settings. The patient should be reassessed for weaning the following day.

If all criteria a–d are met for the last 30 minutes of the trial, the SBT will be reported as "**successful**" and ventilation support will be removed.

- Patients will be reported as **"ventilator free"** after two consecutive calendar days of **"unassisted breathing"**. **"Unassisted breathing"** will be defined as any of the following:

    a)   Spontaneously breathing with face mask, nasal prong oxygen or room air

    b)   T-piece breathing

    c)   Tracheostomy mask breathing

    d)   CPAP ≤5 without PS or intermittent mandatory ventilation assistance

    e)   Use of CPAP or bi-level positive airway pressure solely for sleep apnoea management

4. Fluid Management

Fluid management will be unrestricted during episodes of shock. However, in patients not in shock, a conservative fluid approach should be adopted. If the ICU has a fluid balance policy for ventilated patients, this should be followed. If no policy exists, those patients not in shock should be managed with a conservative fluid management approach represented by a simplification of the algorithm utilised in the ARDS Network FACTT study [Ref: The National Heart, Lung and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network 2006] (see below).

4.1   Conservative Fluid Management Algorithm

This fluid management protocol captures the primary positive outcome of the FACTT (protocol 2001 [Ref: ARDS Network 2001] and report 2006 [Ref: The National Heart, Lung and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network 2006]) study on increasing ventilator-free days. If not already being used, this conservative fluid management approach should be initiated within 4 hours of study drug treatment being initiated and should be continued until the patient achieves unassisted breathing or D7, whichever occurs first.

Fluid administration will be recorded daily while the patient is in ICU and will be noted in the study e-CRF as being positive, neutral or negative.

This conservative fluid management protocol states that for a patient:

    a)   Discontinue maintenance fluids.

    b)   Continue medications and nutrition.

    c)   Manage electrolytes and blood products as per usual practice.

    d)   For shock, use any combination of fluid boluses (see footnote b of Table 8) and vasopressor(s) to achieve a mean arterial pressure of 60 mmHg (or higher if clinically indicated) as quickly as possible.

Vasopressors should then be stopped as quickly as can be tolerated beginning 4 hours after blood pressure has stabilised.

e) Withhold diuretic therapy in renal failure if feasible and until 12 hours after the last fluid bolus or vasopressor was given. Renal failure is defined as: dialysis dependence, OR oliguria with serum creatinine >3 mg/dL (265 μmol/L), OR serum creatinine 0–3 mg/dL (265 μmol/L) with urinary indices indicative of acute kidney injury

Table 7   Conservative Fluid Management Algorithm

CVP (recommended), cmH_2O	PAOP (optional), cmH_2O	MAP ≥60 mmHg AND off vasopressors for ≥12 hours	
		Average urine output <0.5 mL/kg/h (PBW)	Average urine output ≥0.5 mL/kg/h (PBW)
>8	>12	Furosemide[a] Reassess in 1 hour	Furosemide[a] Reassess in 4 hours
4–8	8–12	Give fluid bolus as fast as possible[b] Reassess in 1 hour	
<4	<8		No intervention Reassess in 4 hours

[a] *Recommended furosemide dosing to begin with 20 mg bolus or 3 mg/h infusion or last known effective dose. Double each subsequent dose until goal achieved (oliguria reversal or intravascular pressure target) or maximum infusion rate of 24 mg/h or 160 mg bolus reached. Do not exceed 620 mg/d. Also, if patient has heart failure, consider treatment with dobutamine.*

[b] *Recommended fluid bolus= 15 mL/kg (PBW) crystalloid (round to nearest 250 mL) or 1 unit packed red cells or 25 g albumin.*

Abbreviations: CVP=central venous pressure; MAP=mean arterial pressure; PAOP=pulmonary artery occlusion pressure; PBW=predicted body weight.

5. Body Positioning

The use of prone positioning is recommended in severe ARDS (PaO_2/FiO_2 <150 mmHg) as per the PROSEVA study [Ref: PROSEVA Study Group 2013] and if used should be recorded in the e-CRF.

6. Nutritional Support

Nutritional support should be initiated as early as possible (total parenteral nutrition or enteral nutrition).

7. Nitric Oxide

The use of nitric oxide is not recommended; if used, this should be recorded in the study e-CRF.

8. Corticosteroids

The use of corticosteroids to treat ARDS or sepsis should be avoided; if used, this should be recorded in the study e-CRF.

9. Glucose Management

Maintaining blood glucose levels in a reasonable range is important. Each investigational site will use its own ICU protocol for glucose and insulin

administration. In the absence of a locally agreed ICU best practice protocol, the target should be to maintain blood glucose at 8–10 mMol/L (144–180 mg/dL) as per the control limb of the NICE-SUGAR study. [Ref: NICE-SUGAR Study Investigators 2009]

10. Neuromuscular Blockers

In line with the results of the ACURASYS study, [Ref: Papazian L et al 2010 for the ACURASYS study investigators] neuromuscular blockers can be given to patients with ARDS when their PaO_2/FiO_2 ratio is <120 with a PEEP ≥5 cmH_2O. When given, neuromuscular blockers should be administered for a maximum of 48 hours. Otherwise, use neuromuscular blockers as clinically indicated and record in the e-CRF.

11. Sedation

The minimum sedation possible will be given; that is, no more sedation than the patient needs. Daily interruption of sedation to permit a detailed assessment of the patient should be considered as part of a weaning protocol, dependent on the individual patient's condition.

12. Prevention of Pneumonia

Efforts to prevent ventilator-associated pneumonia, bacterial or pneumonia cause unspecified, and new nosocomial lung infections should be made by:

    a) Administration of a chlorhexidine oral rinse and chlorhexidine gel paste, as this can reduce the oral bacterial load. Several studies have shown that oral decontamination is an effective method of reducing ventilator-associated pneumonia

    b) Raising the head of the patient's bed: elevation of the head of the bed to 30° is supported as a preventive strategy that lowers the risk of aspiration

13. Deep Vein Thrombosis: Thrombosis Prevention

Investigators should follow their local guidance for prevention of deep vein thrombosis.

14. Stress Ulcer Prevention

Investigators should follow their local guidance for prevention of stress ulcers.

11.2 Reference List

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12   APPENDIX 2: GUIDELINES FOR THE SIX-MINUTE WALK TEST

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EUROPEAN RESPIRATORY *journal*

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every breath counts

An official European Respiratory Society/American Thoracic Society technical standard: field walking tests in chronic respiratory disease

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ABSTRACT   Field walking tests are commonly employed to evaluate exercise capacity, assess prognosis
and evaluate treatment response in chronic respiratory diseases. In recent years, there has been a wealth of
new literature pertinent to the conduct of the 6-min walk test (6MWT), and a growing evidence base
describing the incremental and endurance shuttle walk tests (ISWT and ESWT, respectively). The aim of
this document is to describe the standard operating procedures for the 6MWT, ISWT and ESWT, which can
be consistently employed by clinicians and researchers.

   The Technical Standard was developed by a multidisciplinary and international group of clinicians and
researchers with expertise in the application of field walking tests. The procedures are underpinned by a
concurrent systematic review of literature relevant to measurement properties and test conduct in adults
with chronic respiratory disease.

   Current data confirm that the 6MWT, ISWT and ESWT are valid, reliable and responsive to change with
some interventions. However, results are sensitive to small changes in methodology. It is important that two
tests are conducted for the 6MWT and ISWT.

   This Technical Standard for field walking tests reflects current evidence regarding procedures that should
be used to achieve robust results.

@ERSpublications
**Technical Standard document: standard operating procedures for the 6MWT, ISWT and ESWT in
chronic respiratory disease** http://ow.ly/Bq2B9

Overview

The aim of this Technical Standard is to document the standard operating procedures for the 6-min walk test (6MWT), incremental shuttle walk test (ISWT) and endurance shuttle walk test (ESWT) in adults with chronic respiratory disease. The testing procedures were developed by a multinational and multidisciplinary group of experts in field exercise testing, informed by a systematic review of the measurement properties and interpretation of the 6MWT, ISWT and ESWT in adults with chronic respiratory disease [1].

The key findings of the Technical Standard are as follows.

1) The 6-min walking distance (6MWD), ISWT and ESWT demonstrate good construct validity. Strong relationships with measures of exercise performance and physical activity support their conceptualisation as tests of functional exercise performance.

2) A lower 6MWD is strongly associated with increased risk of hospitalisation and mortality in people with chronic respiratory disease, with a small number of studies suggesting a similar relationship for the ISWT.

3) The 6MWD, ISWT and ESWT exhibit good test–retest reliability; however, there is strong evidence of a learning effect for the 6MWT and ISWT. Two tests should be performed when the 6MWT or ISWT are used to measure change over time.

4) The 6MWD, ISWT and ESWT are responsive to treatment effects in people with chronic respiratory disease; however, most studies have evaluated responsiveness to rehabilitation and fewer data are available to confirm responsiveness to other interventions.

5) The 6MWD and ISWT elicit a peak oxygen uptake (V'_{O_2peak}) that is similar to that during a cardiopulmonary exercise test (CPET). As a result, the contraindications and precautions for field testing should be consistent with those used for a CPET.

6) The 6MWD is very sensitive to variations in methodology, including use of encouragement, provision of supplemental oxygen, changes in track layout and length, and use of wheeled walkers. These factors should be held constant on repeat testing.

7) The 6MWD is the primary outcome of the 6MWT.

8) The lowest arterial oxygen saturation measured by pulse oximetry (S_{pO_2}) recorded during a 6MWT has emerged as an important marker of disease severity and prognosis; however, it may not be consistent with end-test S_{pO_2}. Continuous pulse oximetry is recommended during the 6MWT, to ensure that the lowest S_{pO_2} is recorded.

9) Available evidence suggests a minimal important difference (MID) of 30 m for the 6MWD in adults with chronic respiratory disease.

10) The primary outcome of the ISWT is distance, measured to the nearest 10 m.

11) The primary outcome of the ESWT is time, although distance has also been reported.

12) Application of reference equations for 6MWD or ISWT to an individual gives rise to substantial variation in predicted values. If reference values are to be used, an equation generated and verified in a local population should be applied where possible.

13) Testing procedures that are consistent across the 6MWT, ISWT and ESWT include test location and staffing, patient assessment and preparation, use of oxygen and medications, indications for test cessation, and quality assurance. These procedures are detailed in the Technical Standard.

Introduction

The 6MWT plays a key role in evaluating functional exercise capacity, assessing prognosis and evaluating response to treatment across a wide range of respiratory diseases. Since publication of the previous American Thoracic Society (ATS) statement on the 6MWT in 2002 [2], new information regarding the 6MWT has emerged in a range of areas, including methods of test performance and interpretation. This increased body of knowledge has significant implications for the good conduct of the 6MWT in individuals with chronic respiratory disease, in both research and clinical settings.

The last 10 years have also seen a growing body of evidence describing the uses and measurement properties of the ISWT and the ESWT. Given the increasing uptake of these field tests in clinical practice, the scope of the 2002 document has been expanded to include the ISWT and ESWT. While the use of other field tests has also been reported in chronic respiratory disease (*e.g.* timed up and go, sit to stand test, 4-m gait speed test), the committee considered that there were currently insufficient data to include them in this version of the Technical Standard.

The testing procedures outlined in this document are based on a systematic review of the literature describing the use and properties of these field walking tests in adults with chronic respiratory disease, including chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), cystic fibrosis (CF), bronchiectasis, asthma, pulmonary arterial hypertension (PAH) and pulmonary vascular disease [1]. As a consequence, these standards are not intended for application to children or to individuals who do not have chronic respiratory disease. This Technical Standard provides guidance on the practical aspects of conducting field walking tests in a safe, effective and reproducible manner. This document is not intended to provide guidance regarding when or in which patients these tests should be performed. The measurement properties of each field walking test are described individually. Standard operating procedures common to all three tests are outlined and those specific to each test are described. A brief comparison of the three tests is included. Finally, important differences between this document and the previous ATS statement on the 6MWT are highlighted.

Methods

An *ad hoc* Task Force was assembled to develop technical standards for the performance of the 6MWT, ISWT and ESWT based upon a systematic review of the evidence [1]. Co-chairs were selected by the Pulmonary Rehabilitation Assembly and Proficiency Standards Committee of the ATS and the Rehabilitation and Chronic Care Assembly and Allied Respiratory Professionals Assembly of the European Respiratory Society (ERS), then approved by the leadership of both societies. Members of the Task Force were selected by the co-chairs on the basis of their expertise in application of field walking tests in research and/or clinical practice. All potential conflicts of interest were disclosed and managed according to the policies and procedures for joint ATS/ERS projects. Drafts of this document were prepared by two members (Anne E. Holland and Sally J. Singh), with all authors providing comment and suggestions for the final document. The methods checklist is presented in table 1.

The systematic review which informs this document addressed seven questions relevant to the measurement properties, performance, clinical utility, reporting, monitoring, reference equations and interpretation for field walking tests in chronic respiratory disease. Full details are provided in a companion paper [1].

The 6MWT: measurement properties important to test conduct

The 6MWT is a self-paced test of walking capacity. Patients are asked to walk as far as possible in 6 min along a flat corridor. The distance in metres is recorded. Standardised instructions and encouragement are commonly given during the test [2].

Validity of 6MWD

There is a large body of data demonstrating construct and criterion validity for the 6MWD in individuals with chronic respiratory disease [1]. Relationships are strongest with measures of maximal exercise

TABLE 1 Methods checklist

	Yes	No
Panel assembly		
Included experts for relevant clinical and nonclinical disciplines	X	
Included individual who represents the views of patients and society at large		X
Included methodologist with appropriate expertise	X	
Literature review		
Performed in collaboration with a librarian	X	
Searched multiple electronic databases	X	
Reviewed reference lists of retrieved studies	X	
Evidence synthesis		
Applied pre-specified inclusion and exclusion criteria	X	
Evaluated included studies for sources of bias	X	
Explicitly summarised benefits and harms	X	
Used PRISMA1 to report systematic review		X
Used GRADE to describe quality of evidence		X
Generation of recommendations		
Used GRADE to rate the strength of recommendations		NA

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; GRADE: Grading of Recommendations Assessment, Development and Evaluation; NA: not applicable.

performance and physical activity (correlation coefficients 0.4–0.93). Relationships between 6MWD and respiratory function (forced expiratory volume in 1 s (FEV1), forced vital capacity and diffusing capacity of the lung for carbon monoxide (D_{LCO})) (correlation coefficients 0.31–0.55) or health-related quality of life (HRQoL) (correlation coefficients 0.03–0.65) are of weak to moderate strength across all disease groups examined.

Direct comparisons of the physiological demands of the 6MWT and CPET show that although measures of peak exercise performance (V'_{O_2peak} and heart rate (HR) peak) are similar between the tests, the 6MWT has substantially lower ventilatory requirements (peak carbon dioxide production, peak ventilation and respiratory exchange ratio) [3–8], which may contribute to its tolerability in adults with chronic respiratory disease. Overall, these data lend support to the conceptualisation of the 6MWT as a test of functional exercise capacity.

Reliability and learning effect for 6MWD

The 6MWD is a reliable measure in people with chronic respiratory disease, with excellent intra-class correlation coefficients (ICCs) (range 0.82–0.99) [1]. There are no discernible differences in reliability across groups with different chronic respiratory diseases. Despite its excellent reliability, there is strong evidence of a learning effect for the 6MWD when two or more tests are conducted. 13 studies in patients with COPD show a pooled mean improvement on the second 6MWT of 26.3 m [1]. This estimate does not change when including only the subgroup of studies where the two tests are conducted within 24 h (26.1 m). The largest study to address this issue (n=1514) reported a 95% confidence interval for the learning effect of 24–29 m [9]. There is some variability across individuals: the proportion of participants who walked further on the second 6MWT ranged from 50% to 87% across studies. Fewer data are available in other chronic respiratory diseases, although the available studies tend to support a learning effect across all chronic respiratory diseases [1]. Whether the learning effect is equally significant for individuals who have previously performed multiple 6MWTs is difficult to establish, as few data are available. If the tests are repeated 3 months later, the learning effect appears to persist in individuals with COPD; however, it may be smaller in magnitude [10].

The effect of learning on the 6MWD is large enough to be clinically important when the 6MWT is used to evaluate response to treatment or change over time. In these situations, two 6MWTs should be performed and the best 6MWD recorded. Use of two 6MWDs may also decrease the sample size requirements for clinical trials, due to reduced variability in the pre- and post-intervention measures [11]. Where the 6MWD is used as a one-off measure to stage disease or assess risk (e.g. likelihood of hospitalisation or mortality), the magnitude of the learning effect may be less important and one test may be sufficient. However, clinicians should be mindful of the learning effect if the 6MWD is approaching pre-defined thresholds on which treatment decisions may be based [12, 13]; in this situation, repeat testing should be considered. One test may also be sufficient for patients who have recently performed the test, where the learning effect is smaller (e.g. end of pulmonary rehabilitation) [10].

Relationship of 6MWD to clinical outcomes

The 6MWD has a strong relationship to important clinical outcomes in individuals with chronic respiratory disease. A shorter 6MWD was associated with increased mortality in 13 (93%) out of the 14 COPD studies reviewed [1]. Associations were less consistent in ILD (four (50%) out of eight studies) and PAH (six (66%) out of nine studies) [1]. There is less evidence for the association of 6MWD with hospitalisation, but all studies in which it was evaluated (n=3, COPD and ILD) found a statistically significant relationship [1]. It was outside the scope of this document to identify thresholds for 6MWD to categorise patients according to their risk for these outcomes.

Methodological factors affecting test performance

The 6MWD is highly sensitive to changes in methodology (table 2). Given the impact of encouragement on 6MWD [33] and the use of encouraged tests in the generation of reference equations [1], it is recommended that standardised phrases of encouragement are used (see later section on the testing protocol for 6MWT). Provision of supplemental oxygen [26–29], method for carrying the supplemental oxygen [31, 32] and use of wheeled walkers [20–25] also have an important impact on 6MWD. These factors must be kept constant on repeat testing. The 6MWD generated using an externally paced treadmill is substantially lower than in a hallway [14, 15], which is probably the result of the poor walking efficiency during treadmill walking in subjects unaccustomed to this activity. Externally paced treadmill testing is not recommended. Track layout and length may also affect performance on the test, especially when very short track lengths are used [19]; these factors should be kept constant where within-subject comparison of 6MWD on subsequent occasions

TABLE 2 Effect of methodological variations on 6-min walking distance (6MWD)

Variation in methodology	Studies n	First author [ref.]	Effect on 6MWD
Hallway *versus* treadmill	2	Stevens [14] de Almeida [15]	13–20% less on treadmill
Indoors *versus* outdoors	1	Brooks [16]	4 m (1%) more outside
Circular *versus* straight track	2	Bansal [17] Sciurba [18]	13–19 m (3–5%) more on circular track
Track length	2	Sciurba [18] Beekman [19]	No statistically significant difference in 6MWD from tracks of 15–121 m 50 m more on 30-m track compared to 10-m track
Wheeled walking aid *versus* no aid	6	Gupta [20] Honeyman [21] Probst [22] Roomi [23] Solway [24] Vaes [25]	Weighted mean 6.2% more with wheeled walker Range 2–46 m more with wheeled walker 83 m more with modern draisine compared to wheeled walker
With *versus* without oxygen	4	Davidson [26] Fujimoto [27] Rooyackers [28] Jolly [29]	12–59 m more with oxygen
Oxygen *versus* compressed air	2	Jolly [29] McDonald [30]	17–109 m more with oxygen
Carry oxygen *versus* oxygen in wheeled cart	1	Crisafulli [31]	23 m more with wheeled cart
Patient carries oxygen *versus* tester carries oxygen	1	Woodcock [32]	24 m *versus* 35 m improvement
Encouragement	1	Guyatt [33]	30.5 m more with encouragement
Instructions	1	Weir [34]	53 m further when asked to walk as ''fast'' as possible, rather than as ''far'' as possible

is required. For track lengths of >15 m, differences may be small enough such that tests on different track layouts can still be used for risk stratification [1]; however, more data are needed to confirm this.

Monitoring and reporting for 6MWT

Safety

Complications associated with the performance of the 6MWT are unusual. We were unable to find published reports of complications associated with performance of the 6MWT in clinical trials. Only two articles have specifically addressed the issue of complications during the 6MWT. In 741 patients attending an outpatient pulmonary rehabilitation programme who completed the 6MWT in accordance with a standardised protocol, including continuous monitoring of oxyhaemoglobin saturation (S_pO_2) and HR, adverse events were noted in 43 (6%) of patients [35]. The most common adverse event was oxygen desaturation <80%, upon which the test was terminated by the operator (35 out of 43); chest pain (one out of 43) and tachycardia (one out of 43) were also recorded. No long-term adverse sequelae from these events were reported. In the remaining tests the patients developed intolerable symptoms and the test was discontinued, which would be expected during the 6MWT and is not considered a complication. A second study in ILD (n=19) showed that desaturation to <80% occurred in 58% of participants during the 6MWT [36]. Concurrent ECG monitoring showed no clinically significant arrhythmias that needed treatment, although atrial tachycardia occurred in one case.

Measurements

The 6MWD is the primary outcome of the 6MWT, given its excellent reliability and validity, as well as its strong relationship to important clinical outcomes. The 6MWD should be reported for every test in metres or feet. However, other outcomes have been reported, such as S_pO_2, HR responses, symptoms of dyspnoea and fatigue and 6-min walk work (6MWD × body weight).

Oxyhaemoglobin saturation

Oxygen desaturation during a 6MWT provides information regarding exercise-induced desaturation, disease severity and disease progress. Exercise-induced desaturation is associated with impaired daily

physical activity, faster FEV1 decline and worse prognosis [37, 38], which supports its clinical importance. The 6MWT is more sensitive for identifying exercise-induced desaturation than cycle testing [39]. Measurements of S_{pO_2} during the 6MWT are reliable [1], provided that an adequate pulse signal is obtained. However, change in S_{pO_2} may be more variable in people with systemic sclerosis-associated ILD (SSc-ILD) than in other lung conditions (ICCs ranging 0.24–0.64 in SSc-ILD, compared to 0.80–0.97 in CF and COPD) [1], perhaps due to cutaneous involvement in SSc-ILD. The 6MWT may be safe without continuous monitoring of S_{pO_2} [36]. However, constant monitoring of S_{pO_2} during the 6MWT is needed to obtain an accurate measure of exercise-induced desaturation, as the lowest S_{pO_2} often does not occur at the end of the test [40, 41].

A number of novel desaturation indices have been proposed in patients with ILD, with the aim of improving the ability of the 6MWT to predict mortality. These include the distance–saturation product [42–45], the desaturation area [46] and the desaturation–distance ratio [47]. As only a small number of studies are available, the utility of these measures has not been confirmed and they are not routinely collected during the 6MWT.

Heart rate

The resting and end-test HR are often recorded during the 6MWT. Limited data are available regarding the reliability of HR measures during the 6MWT; however, change in HR recorded on the pulse oximeter appears reliable (ICCs 0.62–0.87) [9, 48]. No studies have compared the HR obtained from pulse oximetry to that obtained with other methods (*e.g.* frequency meter). Other measures of HR response include HR recovery (HRR), which is the reduction in HR with rest that occurs after the 6MWT is concluded. A reduced HRR during the first minute after the 6MWT has been associated with poor outcomes, including increased mortality, in ILD [49] and PAH [50, 51]. However, there is not a universally accepted cut-off for HRR that is applicable across chronic respiratory diseases.

Dyspnoea

Dyspnoea may be an important determinant of the 6MWD in patients with chronic respiratory disease [52–55], where it reflects both the physiology of exercise limitation [56, 57] and the impact of exercise limitation on daily life [58]. Dyspnoea scores collected during the 6MWT exhibit good reliability, with the modified Borg dyspnoea scale [59] (ICCs 0.59–0.92) showing greater reliability than the 15-count dyspnoea scale (ICC 0.66) and visual analogue scale for dyspnoea (coefficient of variation 0.22) [1]; however, few studies have investigated these latter measures.

Subjective fatigue

Fatigue is a common feature in patients with chronic respiratory diseases, both as a local muscle phenomenon and general fatigue. This is reflected during the 6MWT, where COPD patients experienced more fatigue compared to healthy elderly subjects [60]. Patient-reported fatigue is commonly measured at the beginning and end of the 6MWT using the Borg scale [59], which exhibits moderate reliability [9, 61]. Fatigue measured during the 6MWT using the Borg scale is associated with lower 6MWT, slower gait speed, more severe airflow obstruction, more dyspnoea on exertion and lower HRQoL [1]. Notably, low-frequency muscle fatigue in the quadriceps is seldom found with the 6MWT, although low-frequency fatigue in distal leg muscles has been reported to be greater in COPD patients compared with controls [62].

6-min walk work

The 6-min walk work is the product of 6MWD and body weight, which may provide a better estimation of the work required to perform the test than distance alone. The 6MWD × weight product correlates more strongly with D_{LCO} and V'_{O_2peak} than 6MWD alone [63, 64]. To date, no studies have investigated the sensitivity of 6MWD × weight product to change over time. Additional studies are needed to better characterise the utility of the 6-min walk work in adults with respiratory disease.

In summary, the 6MWD is the primary outcome of the 6MWT and should be recorded on every test. The S_{pO_2} and HR should be measured continuously during the 6MWT, to ensure that the lowest S_{pO_2} and the end-test HR are recorded. Care should be taken that obtaining these measures does not affect performance. Current data indicate that the 6MWT has an excellent safety profile when the test is stopped if S_{pO_2} falls to <80%; few data are available to define the safety profile if desaturation to <80% is permitted. Dyspnoea and subjective fatigue should be measured before and after the 6MWT.

Identifying meaningful change in 6MWD
Minimal important difference for the 6MWD

Over the past 10 years, a number of new estimates of the MID for 6MWD have been published. Our systematic review [1] identified 11 studies, in COPD (six studies), ILD (three studies) and PAH (two studies). The majority of MID estimates from these studies are based on distribution-based methods (using statistical properties of the measure), rather than anchor-based methods (where change in 6MWD is related to another clinically important marker of change). Most values were generated for individuals with COPD participating in a rehabilitation programme rather than pharmacotherapy. The median estimate across all studies was 30 m [1]. There is currently little evidence to suggest that the MID varies according to patient characteristics, including the type of chronic lung disease or the baseline 6MWD. Most MID estimates were generated using group mean data and are best used to interpret group mean changes in 6MWD [65–67]. However, studies that derived the MID using methodologies that are applicable to individuals reported similar MID estimates [68, 69]. More studies are required to explore the magnitude of important changes in 6MWD with different interventions and to establish the MID in other chronic respiratory diseases.

In summary, the available evidence suggests a MID of 30 m for adult patients with chronic respiratory disease. There is some variability across studies and methods to determine the MID; however, based on the large evidence base now available, we can be confident that the MID lies between 25 and 33 m.

Responsiveness of the 6MWD

Few studies have been explicitly designed to assess the responsiveness of 6MWD to treatment effects, but a large number of randomised trials provide insights in this area. We chose the Cochrane reviews on exercise interventions for COPD [70, 71] and ILD [72] and the Cochrane review on endothelin receptor antagonists in PAH [73] to investigate the responsiveness of 6MWD to interventions of known efficacy. The meta-analyses for 6MWD showed highly statistically significant improvements in 6MWD, with effects between 34 and 78 m and effect sizes between 0.38 and 1.07 [1]. The standardised response means (mean change/ standard deviation of change) ranged from 0.2 to 1.2 [1]. The 6MWD therefore appears to be responsive to treatment effects in patients with COPD, ILD and PAH. It should be noted that most of these values have been generated from rehabilitation-based studies.

Reference equations for 6MWD

The systematic review identified 16 published studies from 1998 to 2013 that included 6MWD prediction equations from healthy adults [1]. One additional study has been published since the systematic review [74]. These studies were conducted using a wide variety of populations and methodologies. Track lengths ranged from 20 to 50 m and the number of tests ranged from one to four. Application of these equations to an individual gives rise to substantial variation in the predicted distance.

Factors that may affect the 6MWD in healthy adults, and therefore the predicted distance, include methodology for 6MWT, percentage of peak HR achieved, height, age, sex, weight and perhaps race/ ethnicity. The utility of % predicted values in assessing clinically meaningful change in 6MWD over time has not been investigated.

Due to the wide variation in predicted 6MWD generated by different equations, reference equations generated and verified in a local population should be applied where possible. A summary of reference equations can be found in the accompanying systematic review [1].

The ISWT and ESWT: measurement properties important to test conduct
Validity of the ISWT and ESWT

The ISWT is an externally paced maximal exercise test; the speed of walking is controlled by a series of pre-recorded signals. The speed of walking increases until the participant can no longer continue. The maximum duration of the test is 20 min.

Given the progressive nature of the ISWT, a strong relationship with performance on laboratory-based tests might be anticipated. Seven studies in COPD and one in lung cancer show that the V'_{O_2peak}, estimated V'_{O_2peak}, peak work and distance measured on ISWT show moderate to strong correlations with measures of maximal exercise performance on CPET [1], with no difference in measured oxygen uptake (V'_{O_2}) between the tests [6, 75, 76]. Hill *et al.* [6] performed a minute-by-minute analysis, and both the ISWT and CPET demonstrated a linear response in V'_{O_2peak}, suggesting that the two tests provoke a similar cardiopulmonary response. In summary, the current literature suggests that the ISWT is a valid measure of cardiopulmonary exercise capacity in COPD and provokes a similar physiological response to a CPET. More evidence in other disease populations is required.

The ESWT is a derivative of the ISWT, where patients walk for as long as possible at a predetermined percentage of maximum walking performance as assessed by the ISWT [77]. To set the speed for the ESWT, the ISWT must have been completed previously. The ESWT has been shown to elicit similar end-test HR and dyspnoea responses compared to a treadmill endurance test at the same intensity [77].

Reliability and learning effect for ISWT

Only a handful of studies have reported the reliability of the ISWT, with most data in COPD. One study documented an ICC of 0.88 (95% CI 0.83–0.92) [78]. Another study reported an ICC of 0.89 [79]. These two studies suggest that the association between test–retest walk distances is strong, with the majority of variability being attributable to between-subject differences. More studies are needed to confirm these findings.

There is a small but statistically significant difference between the first two ISWTs performed (mean differences of 20 and 25 m for tests performed on the same day, and 23.5 m for tests performed on different days) [6, 80, 81]. The effect of learning on the ISWT is large enough to be clinically important when evaluating change over time. It is recommended that two ISWTs be performed and the best distance recorded. It remains to be established whether one test would be sufficient where the ISWT is used as a one-off measure to stage disease or assess risk (*e.g.* likelihood of hospitalisation or mortality). Whether one test is sufficient if the test has previously been conducted, similar to the 6MWT, is still unclear. It is common to repeat the test once after an intervention, but whether this underestimates the impact of the intervention remains to be established. The reliability of the test in chronic respiratory diseases other than COPD has not been explored.

Reliability and learning effect for ESWT

Three studies have examined the reliability of the ESWT, all in COPD [6, 81, 82]. The differences between tests repeated on the same day were generally small and statistically nonsignificant (pooled mean difference +26 s). Two tests do not appear to be necessary, although it is acknowledged that the number of studies is limited. Measurements of HR, S_pO_2 and modified Borg dyspnoea scale appear to repeat well during the test [1]. The reliability of the ESWT in other chronic respiratory diseases has not been examined.

Relationship of ISWT and ESWT to clinical outcomes

A small number of studies show that the ISWT is a significant predictor of survival and re-admission in people with COPD, with a lower distance predicting a greater risk of admission [83–85]. The relationship of ESWT to these outcomes is unknown.

Methodological factors affecting ISWT and ESWT performance

Unlike the 6MWT, the track for the ISWT and the ESWT is fixed, and alterations of the course have not been studied. The effect of encouragement has not been directly observed, but the external pacing of the tests may make it difficult to override the test protocol. The impact of supplemental oxygen is influenced by the mode of delivery. If patients are required to support a cylinder independently, oxygen supplementation confers little advantage in walking distance compared to air walking conditions [86], but if the oxygen (or heliox) is carried by a clinician/researcher, the benefit is magnified [87]. Accordingly, if supplemental oxygen is to be used during testing, the same approach should be taken for repeat testing, with the device and the flow rate remaining constant.

Monitoring and reporting for ISWT and ESWT

Safety

Adverse events have not been directly studied or reported in association with either the ISWT or the ESWT. We were unable to find any literature describing complications of conducting the shuttle tests. To date, there is no clear guidance on the value of monitoring S_pO_2 and the level to which this may fall in any individual has yet to be described. However, consensus suggests that the ISWT and the ESWT should be discontinued if S_pO_2 falls below 80%. The test has been reported in patients with cardiac disease [88–90] with no adverse events; to date the test has not been used for patients with PAH.

Measurements

The primary outcome of the ISWT is distance, reported as an accumulation of 10-m lengths. The minimum distance is 0 m if patients fail to complete the first 10 m, and the maximum is 1020 m. The ESWT, like all endurance tests, is reported as time (minutes and seconds); although it can be expressed as distance completed, this is less commonly reported for endurance tests in the literature. The speed that the participant walks is chosen from 16 available speeds (1.78–6.00 km·h^{-1}), with the speed calculated from the

performance on the ISWT. Additional outcomes have been reported; these include S_{pO_2}, HR responses, dyspnoea, fatigue and the reason for terminating the test.

Identifying meaningful change in ISWT and ESWT

Minimal important difference for the ISWT

The MID for the ISWT has been described in one paper that was specifically designed to assess this threshold [91] and confirmed within a second paper [92]. Both studies used a similar approach, with a preference-based anchor method using rehabilitation as the intervention. The original paper described that a change of 47.5 m (approximately five shuttles) was associated with feeling "slightly better", while a change of 78.7 m (approximately eight shuttles) was associated with the next rating ("better"). The authors concluded that the MID for the ISWT was 47.5 m. More studies are required, exploring different interventions and different approaches, to describe the MID in COPD. The MID for other chronic respiratory diseases has not been explored.

Minimal important difference for the ESWT

The MID for the ESWT in patients receiving pulmonary rehabilitation or bronchodilation has been estimated using a distribution-based approach and anchor-based method (change in ESWT time and distance was related to patients' perception of change from baseline) [93]. Bronchodilation data indicated that a change of 65 s (95% CI 45–85 s) or 85 m (95% CI 60–115 m), representing 13–15% of baseline, was associated with a one-point change on the Likert rating scale and thus likely to be perceivable to patients. An estimate of the MID could not be obtained from the pulmonary rehabilitation data, although preliminary data suggest this is in the region of 180 s [93]. It is possible that the MID may be context or intervention specific, although to date this has not been shown for 6MWD.

Responsiveness of the shuttle walk tests

Despite the ISWT being employed in a number of studies, only eight studies were explicitly designed to evaluate the responsiveness to treatment of the ISWT and/or ESWT [86, 94–100]. These studies suggest that the ISWT and ESWT are both responsive. The standardised response means (mean change/standard deviation of change) range from 0.72 to 1.55 for the ISWT and from 0.52 to 1.27 for the ESWT [1].

Reference equations for ISWT and ESWT

Three papers have described reference values for the ISWT, two from South America [101, 102] and one from the UK [103]. There is a need for more data, including diversity in the country of origin. Existing data suggest that age, sex and body mass index are important variables. Data from a single centre in the UK [1] included measures of strength (quadriceps maximum voluntary contraction) and physical activity (Duke Physical Activity Status Index and a physical activity monitor), in an attempt to further explain the variability; however, these additions did not improve the reference equation.

To date there has been no attempt to identify reference equations for the ESWT.

Selecting a field walking test

It is beyond the scope of this Technical Standard to recommend one field test over another. The systematic review underpinning this document did not address the question of the relative merits of each test. However, some general considerations that may influence test selection are outlined here.

Field walking tests may be performed to identify the individual's exercise capacity (peak exercise capacity, functional exercise capacity or endurance), factors limiting exercise performance (dyspnoea, subjective fatigue, musculoskeletal limitations) and often their response to an intervention. The field tests can also be used to identify a threshold to predict survival and the likelihood of a hospital readmission, with a more extensive body of evidence reporting use of 6MWD rather than ISWT or ESWT for this purpose [1]. Prescription of exercise intensity for pulmonary rehabilitation, using the established principles of exercise training, may also be a consideration. The uses of the 6MWT and the ISWT/ESWT have been reported in all of these circumstances [1]. Practical considerations include whether sufficient space is available. If the test of choice is the 6MWT then the test should be conducted as recommended along a course at least 30 m in length; if this space is not available then consideration should be given to using the ISWT/ESWT. Both the 6MWT and ISWT require two tests to be performed prior to an intervention [1] and thus the time taken to conduct each test is comparable.

The 6MWT and ISWT/ESWT offer quite different protocols: the 6MWT is self-paced, and the shuttle tests are externally paced. Although both tests generally provoke a V'_{O_2} similar to that seen on a CPET [6, 75, 104–106], the precise pattern of response may be different for the externally paced tests (ISWT/ESWT) compared to a self-paced test (6MWT). The response of the ISWT mirrors the physiological response

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TABLE 3 Equipment required for conduct of field walking tests
At least one chair, positioned at one end of the walking course
A validated scale to measure dyspnoea and subjective fatigue
Sphygmomanometer for blood pressure measurement
Pulse oximeter
Stopwatch
Pre-measured marks along the track/corridor
Access to oxygen and telephone in case of an emergency
An emergency plan
Portable supplemental oxygen if required by patient to perform exercise test
Clipboard with reporting sheet and pen

observed in an incremental laboratory-based test, with an incremental increase in V'_{O_2} over time, whereas the self-paced 6MWT shows a steady-state V'_{O_2} profile after the third minute [3, 75, 104]. Because of the similarity in response of the ISWT and CPET, prescribing an exercise regimen as a percentage of peak performance on a field walking test may be easier with the ISWT than the 6MWT, because of the incremental nature of the test. Responses to the ESWT show a significantly more rapid rise in V'_{O_2} and ventilation than during the 6MWT, but similar end-test values [95]. The V'_{O_2peak} on ESWT does not differ significantly when compared to the ISWT and 6MWT [6]. The physiological responses to each test are reported in more detail in the systematic review [1].

The comparative sensitivity of field walking tests has been examined in small studies that have looked at rehabilitation or bronchodilation as the intervention, predominantly in COPD. PEPIN et al. [95] observed an enhanced response in the ESWT compared to the 6MWT after an acute administration of a bronchodilator, with standardised response means of 0.66 and 0.42, respectively. Similarly, EATON et al. [98] reported that, after a course of pulmonary rehabilitation, the magnitude of the change was proportionally greater on the ESWT than the 6MWT (92% versus 17%). The ESWT has also proven to be more responsive than the 6MWT in a randomised trial of an exercise intervention for COPD [107]. These results suggest that the ESWT may be more sensitive to change than the 6MWT, following bronchodilation or pulmonary rehabilitation.

Given the differing protocols, physiological patterns of response, measurement properties and circumstances in which field walking tests are applied, the choice of test should be carefully considered. Regardless of the test selected, robust results can only be obtained with careful attention to testing procedures as described in the following sections.

Test procedures applicable to all field walking tests
Equipment
The equipment required to conduct a field walking test is listed in table 3.

Test location and staffing
The test should be conducted along a quiet course, physiotherapy gym or dedicated exercise testing room. A comfortable temperature is important and air conditioning should be available if needed. Testing should be performed in a location where a rapid, appropriate response to an emergency is possible. The appropriate location of a crash cart should be determined by the physician supervising the facility. Supplies that must be available include oxygen, sublingual nitroglycerine and salbutamol (metered dose inhaler or nebuliser). A telephone or other means of calling for help should be available in case of emergency.

The assessor performing the test should be certified in cardiopulmonary resuscitation with a minimum of Basic Life Support certification. Training, experience and certification in related healthcare fields are also desirable. A certified individual should be readily available to respond if needed. Physicians are not required to be present during all tests. The physician ordering the test or a supervising laboratory physician may decide whether physician attendance at a specific test is required.

Patient assessment
Given that all three field-based exercise tests can elicit a V'_{O_2peak} and peak HR that are similar to the CPET [1], absolute and relative contraindications for exercise testing should be consistent with recommendations for maximal exercise testing (table 4) [108]. Patients with any of these findings should be referred to the physician ordering or supervising the test for individual clinical assessment and a decision about the conduct of the test. Stable exertional angina is not an absolute contraindication for field walking tests, but patients with these symptoms should perform the test after using their anti-angina medication, and rescue

TABLE 4 Absolute and relative contraindications for field walking tests

Absolute	Relative
Acute myocardial infarction (3–5 days)	Left main coronary stenosis or its equivalent
Unstable angina	Moderate stenotic valvular heart disease
Uncontrolled arrhythmias causing symptoms or haemodynamic compromise	Severe untreated arterial hypertension at rest (200 mmHg systolic, 120 mmHg diastolic)
Syncope	Tachyarrhythmias or bradyarrhythmias
Active endocarditis	High-degree atrioventricular block
Acute myocarditis or pericarditis	Hypertrophic cardiomyopathy
Symptomatic severe aortic stenosis	Significant pulmonary hypertension
Uncontrolled heart failure	Advanced or complicated pregnancy
Acute pulmonary embolus or pulmonary infarction	Electrolyte abnormalities
Thrombosis of lower extremities	Orthopaedic impairment that prevents walking
Suspected dissecting aneurysm	
Uncontrolled asthma	
Pulmonary oedema	
Room air S_pO_2 at rest $\leqslant 85\%$[#]	
Acute respiratory failure	
Acute noncardiopulmonary disorder that may affect exercise performance or be aggravated by exercise (*i.e.* infection, renal failure, thyrotoxicosis)	
Mental impairment leading to inability to cooperate	

S_pO_2: arterial oxygen saturation measured by pulse oximetry. [#]: exercise patient with supplemental oxygen. Reproduced from [108] with permission from the publisher.

nitrate medication should be readily available. All comorbidities and medication use should be recorded prior to the test.

Patient preparation

Patients should wear comfortable clothing and appropriate shoes for walking. Patients should use their usual walking aids during the test and this should be documented on the assessment form. Patients should not have exercised vigorously within 2 h of beginning the test but should have taken their usual medications. All subsequent testing occasions should occur at about the same time of day to minimise intra-day variability, including variability in self-paced tests (6MWT) associated with bronchodilator use [109]. A warm-up is not permitted prior to commencing the test, neither is a shortened version of the test. If respiratory function tests are to be performed on the same day, this should occur prior to exercise testing, to avoid the confounding effects of exercise on these measures. The patients should then rest for at least 15 min before commencing an exercise test.

Use of oxygen

If a patient is on long-term oxygen therapy, oxygen should be given at their standard flow rate or as directed by a physician or a protocol. For any test where the outcome is distance, oxygen flow should be held constant throughout the test. If the purpose of the exercise test is to compare distance walked between tests, any subsequent test should be performed using the same oxygen conditions, in order to make a valid comparison between testing occasions. If oxygen supplementation is needed during the walks and serial tests are planned, then oxygen should be delivered in the same manner (flow rate and delivery device) for all subsequent walks. If the flow rate must be increased for subsequent visits due to worsening gas exchange, this should be noted on the worksheet and considered during interpretation of any changes in performance.

The type of oxygen delivery device should also be noted on the report: for instance, whether the subject carried liquid oxygen or pushed or pulled an oxygen tank, and whether the delivery was pulsed or continuous. Assessors should avoid transportation of the oxygen source where possible; however, if the subject is not able to control/carry/manage their own oxygen cylinder, the assessor should try to walk slightly behind the subject to avoid setting the walking pace. It should be clearly documented how the assessor has assisted with the transport of the oxygen, so any subsequent walk tests with the same subject can be performed in the same manner. Oxygen is not to be titrated during any of the tests where distance is a measured outcome. If oxygen titration is desired, this should be done during a separate test.

TABLE 5 Standardised instructions for the 6-min walk test
The aim of this test is to walk as far as possible for 6 minutes. You will walk along this hallway between the markers, as many times as you can in 6 minutes.
I will let you know as each minute goes past, and then at 6 minutes I will ask you to stop where you are. 6 minutes is a long time to walk, so you will be exerting yourself. You are permitted to slow down, to stop, and to rest as necessary, but please resume walking as soon as you are able.
Remember that the objective is to walk AS FAR AS POSSIBLE for 6 minutes, but don't run or jog.
Do you have any questions?

Medications

Patients should be instructed to take all usual medications. The type of medication, dose and number of hours it was taken before the test should be noted. Significant improvement in the distance walked or the dyspnoea scale after administration of bronchodilators has been demonstrated in patients with COPD [93–96], although this may be small and clinically insignificant for the 6MWT [95, 110].

Measurements

Patients should rest in a chair, located near the starting position, before the test starts. Absolute and relative contraindications should be checked for prior to test commencement (table 4). The following measurements should be obtained at rest: S_pO_2 and HR from pulse oximetry, baseline dyspnoea and fatigue using a reproducible scale, and systemic blood pressure, if not recently documented [108].

Immediately prior to the test

The assessor should provide standardised instructions, either verbally for the 6MWT (table 5) or from the ISWT or ESWT audio recording. The patient should be positioned at the starting line. For the 6MWT, the timer should be started as soon as the patient starts to walk. The recorded instructions of the ISWT and ESWT will prompt the individual to start.

A pulse oximeter should be used for continuous measurement of S_pO_2 and HR. The assessor should not "pace" the patient during the test, but should walk behind such that measures of nadir S_pO_2 and end-test HR can be recorded without influencing the patient's movement. The assessor should ensure that the probe is placed such that a quality signal is obtained.

Immediately on test cessation

The S_pO_2 and pulse rate should be recorded from the oximeter, and the patient should be asked to rate their dyspnoea and subjective fatigue on the standardised scale. In addition, it is important to understand the reason for test termination/limitation, so patients should be asked why they could not walk any further. It is common for patients to report either dyspnoea or leg fatigue as the primary reason for a restricted performance.

Reasons for the assessor to stop an exercise test

In some individuals, profound desaturation (S_pO_2 <80%) may occur during a walking-based exercise test. Use of a 6MWT protocol that directs test cessation when S_pO_2 falls to <80% is associated with an extremely low rate of adverse events [35]; this has not been reported for the ISWT or ESWT. Few data are available to determine the risk if the test is not stopped when S_pO_2 falls below 80%. Ceasing a test when S_pO_2 falls to <80% is also consistent with the recommendations for incremental exercise testing [108]. If S_pO_2 recovers to ⩾85% during the 6MWT, the patient may be asked to recommence walking.

Other reasons for test cessation include chest pain, intolerable dyspnoea, leg cramps, staggering, diaphoresis, and a pale or ashen appearance. If a test is stopped for any of these reasons, the patient should sit or lie supine as appropriate. The following should be obtained based on the judgment of the assessor: blood pressure, pulse rate, S_pO_2 and a physician evaluation. Oxygen should be administered as appropriate.

Test repetition

To establish a stable baseline for the 6MWT and ISWT so that change over time can be detected, two tests must be completed. These can be performed on the same day but there must be an interval between tests of at least 30 min and measures of HR and S_pO_2 must have returned to baseline prior to the second test.

Quality assurance

Consistency of testing procedures and conditions is critical to ensure that results are of good quality. It is important that all assessors are familiar with the test procedures, as the test requires clear processes to be followed.

TABLE 6 Standardised encouragement for the 6-min walk test	
1 min	You are doing well. You have 5 minutes to go.
2 min	Keep up the good work. You have 4 minutes to go.
3 min	You are doing well. You are halfway.
4 min	Keep up the good work. You have only 2 minutes left.
5 min	You are doing well. You have only 1 minute to go.
6 min	Please stop where you are.
If the patient stops during the test, every 30 s once S_pO_2 is $\geqslant$85%	Please resume walking whenever you feel able.

S_pO_2: arterial oxygen saturation measured by pulse oximetry.

For the ISWT and ESWT, the assessor must be able to walk at exactly the first speed of walking to pace the patient; this is particularly important for patients with a higher functional capacity where their natural speed of walking is much faster than the very slow pace required on the first level of the ISWT. All assessors should have their performance peer reviewed to ensure that performance of the tests is in accordance with the Technical Standard. Ideally, quality assurance testing should require the assessor to conduct the test on participants with a range of functional exercise capacity.

It must be ensured that the pulse oximeter is operating according to the manufacturer's instructions and that the track is accurately measured. The effects of any changes in testing scenario (*e.g.* location, equipment) should be evaluated to assess their effect on test outcomes. Any changes in procedures should be reviewed and approved by the programme director. Consistent documentation must be ensured for every test and a regular audit should be considered, to ensure that important procedures have been followed (*e.g.* recording of appropriate physiological signals at baseline and end-test, documentation of nadir S_pO_2, end-test HR and 6MWD, consistent use of oxygen, appropriate test repetition).

Testing protocol for 6MWT
Course
The 6MWT should be performed along a flat, straight course with a hard surface with little pedestrian traffic. It is recommended that the walking course be 30 m or more in length, to be consistent with the courses on which reference equations have been generated [1]. The ends of the course should be marked such that they are easily visible to patients.

Conduct
The patient should be encouraged every 60 s using the standard phrases (table 6). Other words of encouragement and other nonverbal prompts should not be used. If the patient stops walking during the test, the timer must not be stopped. The patient should be allowed to rest while sitting or standing, as they prefer. While the patient is stopped, standardised encouragement should be provided every 30 s (table 6). The time that the patient stopped and the time that walking is recommenced should be recorded.

Recording performance of the 6MWT
The primary outcome to be reported is 6MWD. The number of laps and any additional distance covered (the number of metres or feet in the final partial lap) should be recorded. The total distance walked is calculated, rounding to the nearest metre or foot. If the patient stopped during the test, the total time stopped, the number of stops and the average walking speed over the 6 min are also reported [111]. In patients who cannot walk for 6 min, this may provide alternative metrics for detecting change over time [111] and may facilitate exercise prescription [112]. It is optional to report the 6MWD as a percentage of predicted. If the % predicted 6MWD is reported, the reference equations used should be stated. Lowest S_pO_2, end-test HR and symptom scores obtained before and after the test should also be reported. A sample recording form can be found in the online supplementary material (table S1).

Testing protocol for ISWT
Course
The course is 10 m in length and is identified for the patient by two cones with an inset of 0.5 m from either end (fig. 1), thus avoiding abrupt changes in direction [113].

At the beginning of the test the instructions are played to the patient from an audio recording (table 7). Once the instructions have been played, and it is confirmed that the patient has understood the task, the

FIGURE 1 Course layout for the incremental and endurance shuttle walk tests. Cones are inset 0.5 m from either end to avoid abrupt changes in direction.
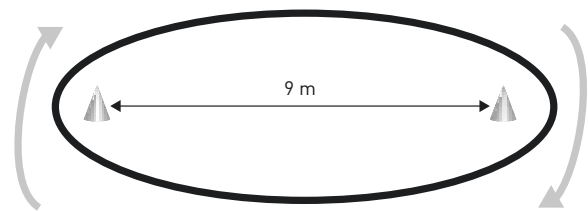
patient is positioned at one end of the course in preparation to start the test. The speed at which the patient should walk is directed by an audio signal and cannot be overridden. There is a triple bleep indicating the test has started: at this point the participant commences walking and the timer is activated.

Conduct of the test

It is important for the assessor to watch the patient but also to ensure they keep count of the number of lengths as the subject completes them, throughout the duration of the test. It is advisable to time the performance as an additional measure, to confirm manual recording of the number of shuttles completed. As the speed of walking increases every minute, indicated by a triple bleep [113], the patient should be advised: "You now need to increase your speed of walking." During the test, only one verbal cue can be used to encourage the patient to pick up their speed: "You need to increase your speed to keep up with the test."

Termination of the test

The test is terminated when either 1) the patient indicates that they are unable to continue, 2) the operator determines that the patient is not fit to continue, or 3) the operator assesses that the patient was unable to sustain the speed and cover the distance to the cone prior to the beep sounding [113].

Operator termination of the test

The operator will be required to terminate the test if the patient fails to reach the cone/marker in the time allowed [113]. This is defined as the patient being >0.5 m away from the cone when the bleep sounds on a second successive 10-m length. When the patient is just outside the 0.5-m marker they are advised to increase their speed of walking; if the patient fails to do so then the test is terminated and the distance recorded.

The test should be discontinued by the operator if SpO_2 falls below 80% as described in the ATS/American College of Chest Physicians (ACCP) statement on cardiopulmonary exercise testing [108].

TABLE 7 Incremental and endurance shuttle walk test instructions

Incremental shuttle walk test instructions

The object of the progressive shuttle walking test is to walk as long as possible, there and back along the 10-metre course, keeping to the speed indicated by the bleeps on the audio recording. You will hear these bleeps at regular intervals.

You should walk at a steady pace, aiming to turn around the cone at one end of the course when you hear the first bleep, and at the other end when you hear the next. At first, your walking speed will be very slow, but you will need to speed up at the end of each minute. Your aim should be to follow the set rhythm for as long as you can. Each single bleep signals the end of a shuttle and each triple bleep signals an increase in walking speed. You should stop walking only when you become too breathless to maintain the required speed or can no longer keep up with the set pace.

The test is maximal and progressive. In other words, it is easier at the start and harder at the end. The walking speed for the first minute is very slow. You have 20 seconds to complete each 10-metre shuttle, so don't go too fast. The test will start in 15 seconds, so get ready at the start now. Level one starts with a triple bleep after the 4-second countdown.

Endurance shuttle walk test instructions

Walking test level (1 to 16). The instructions below are repeated for all 16 levels.

The walking speed for the first 2 minutes is fairly slow, so don't go too fast. The test will start in 10 seconds so get ready at the start now. The test starts with a triple bleep after a 4-second countdown.

At the next triple bleep increase your walking speed.

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Participant termination of the test
The patient may terminate the test if they are unable to continue. In respiratory disease, the most common reason for terminating the test is excessive dyspnoea; however, other reasons may include fatigue (commonly leg fatigue) or pain (knee/hip/low back pain).

Recording performance of the test
The assessor should calculate the distance walked, in metres, including the last 10-m length that was completed, and record this on the form available (see online supplementary material table S2).

Testing protocol for ESWT
Course
The test is conducted along the same course as described for the ISWT (fig. 1) [113].

Conduct of the test
Unlike the ISWT, the ESWT is not incremental and is performed at a constant speed, but there is a warm-up period of ~1.5 min [77]. At this point, there are standardised instructions for the participant played from the audio recording, advising the individual that at the next bleep the speed of walking will increase (table 7). This is the speed at which the test is performed. It is important to pre-define the speed at which the test is going to be conducted. This can be calculated from the ISWT: the speed may be taken from a pre-defined percentage of peak performance on the ISWT (*e.g.* 70–85% estimated V'_{O_2peak}) [114] or a percentage of the peak speed achieved [115]. Once the instructions have been played to the patient, they should be directed to one end of the course. A triple bleep indicates that the test has started [77]. The initial stages of the test are at a slower speed and are a warm-up for the participant. After the warm-up period, the speed of walking increases; this is advised on the audio recording at the end of the warm-up period. The timer is started at the end of the warm-up period. Participants are then paced for the first two shuttles. During the test, only one verbal cue can be used to encourage the patient to pick up their speed: "You need to increase your speed to keep up with the test."

Termination of the ESWT
The procedure to terminate the test is as described for the ISWT [113].

Recording performance of the test
It is conventional for endurance performance to be recorded as time (in seconds). It is important that the speed of walking and time are recorded. A sample scoring sheet is included in the online supplementary material (table S2).

Key changes in this updated Technical Standard
Absolute and relative contraindications for field walking tests
Research published since 2002 has shown that, in adults with moderate chronic respiratory disease, the 6MWT and ISWT elicit a V'_{O_2peak} that is comparable to that on CPET [3–6, 8]. As a result, the absolute and relative contraindications for CPET have been adopted for field walking tests of exercise capacity.

Continuous measurement of S_{pO_2}
New data show both the importance of nadir S_{pO_2} during the 6MWT as a marker of prognosis [38, 46, 116–119] and that end-test S_{pO_2} frequently does not reflect this important value [40, 41]. These factors, together with improvements in pulse oximetry technology in managing motion artefact, have made it feasible and desirable to measure S_{pO_2} continuously during field walking tests.

6MWT repetition
There are now consistent and compelling data showing a learning effect for the 6MWD [1, 9]. As a result, it is recommended that two 6MWTs are performed when the test is used to measure change over time.

Addition of the ISWT and ESWT
The ISWT and ESWT have emerged as robust and useful tests of exercise capacity in chronic respiratory disease [1]. The standard operating procedures for these tests are described here for the first time.

Common testing procedures for field walking tests
Testing procedures, such as test staffing, patient assessment, patient preparation, use of oxygen and medications, and quality assurance, are now consistent across the three field walking tests described in this Technical Standard.

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