Keratinocyte growth factor for the treatment of the acute respiratory distress syndrome (KARE): a randomised, double-blind, placebo-controlled phase 2 trial

McAuley, D. F., Cross, LJ. M., Hamid, U., Gardner, E., Elborn, J. S., Cullen, K. M., ... O'Kane, C. M. (2017). Keratinocyte growth factor for the treatment of the acute respiratory distress syndrome (KARE): a randomised, double-blind, placebo-controlled phase 2 trial. DOI: 10.1016/S2213-2600(17)30171-6

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Survival

Log−rank: p= 0.006

⋯ KGF
— Placebo

Probability of Survival

Time in days

No. at risk at interval

KGF	29	20	18	17	17
Placebo	31	30	27	27	26

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Time to unassisted breathing (survivors only)

Log−rank: p= 0.006

· · · KGF

— Placebo

No. at risk at interval

KGF	15	15	6	4	3	2	1
Placebo	27	19	4	1	1	1	0

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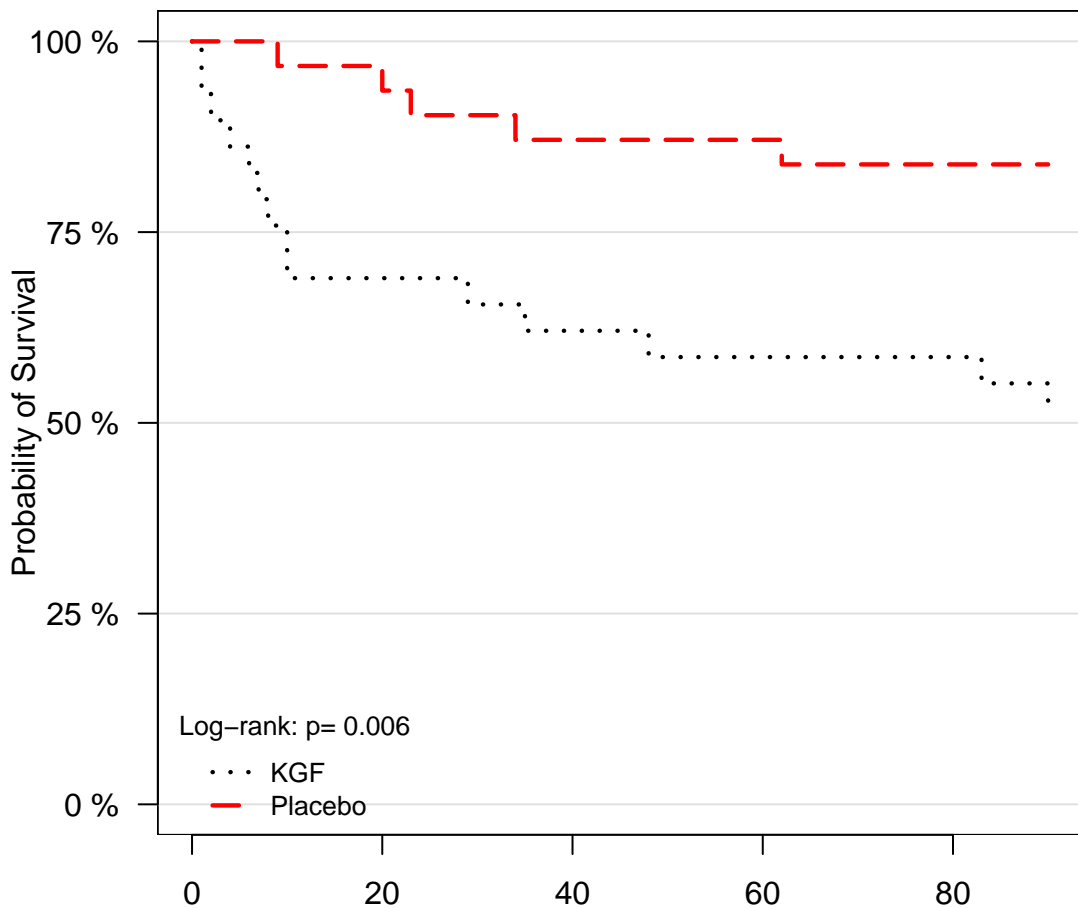## Oxygenation index Baseline
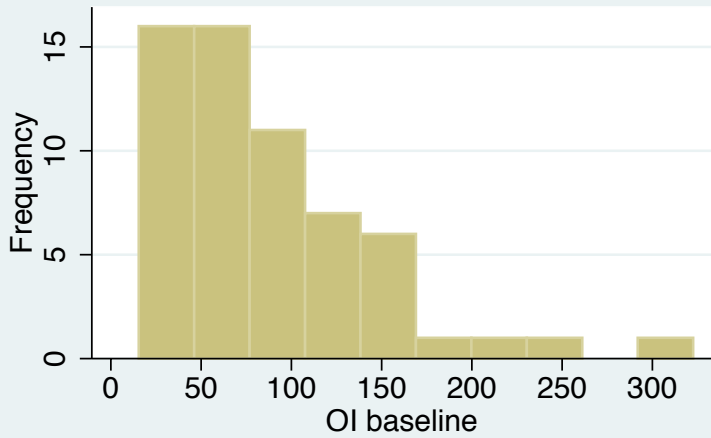
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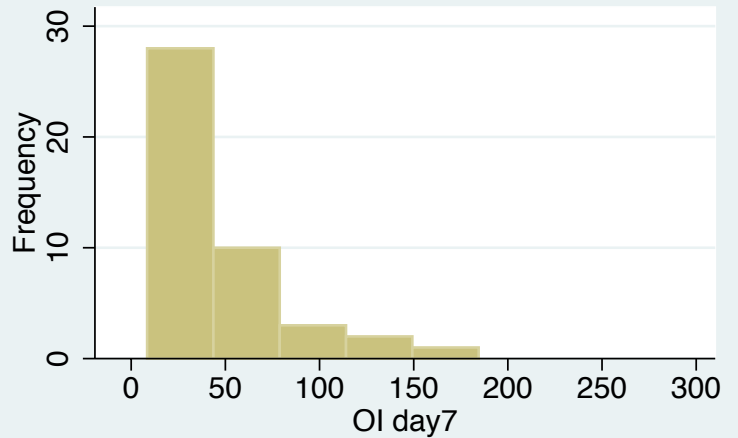
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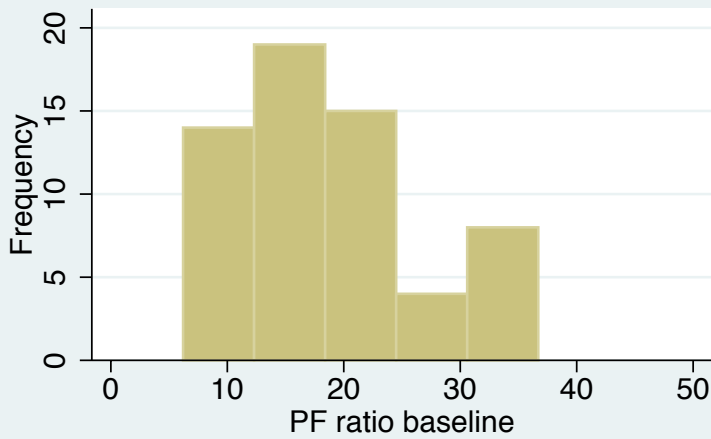
PaO2/FiO2 ratio Day 7

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Protocol

Keratinocyte growth factor in <u>A</u>cute lung injury to <u>RE</u>duce pulmonary dysfunction – a randomised placebo controlled trial (KARE)

Acronym:	KARE
Protocol Number:	10089DMCA-CS
Version Number / Date:	3.0 30 Nov 2012
EudraCT Number:	2010-021186-70
REC Reference Number:	10/NIR02/32
ISRCTN Number:	ISRCTN95690673
Health and Social Care R&D Office Reference Number:	EAT/4210/09
List of Amendments to Date:	1 amendment Protocol 2 to Protocol 2.1 to include the ISRCTN number and new EudraCT number
Chief Investigator:	Prof Danny McAuley Professor / Consultant Centre for Infection and Immunity Queen's University, Belfast Microbiology Building Grosvenor Road Belfast, BT12 6BN

PROTOCOL AUTHORISATION
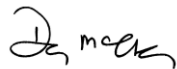
Full Protocol Title: Keratinocyte growth factor in Acute lung injury to REduce pulmonary dysfunction – a randomised placebo controlled trial (KARE)

Protocol Number: 10089DMCA-CS

Version Number / Date: 3.0 / 30 Nov 2012
                                   2.1 / 08 Oct 2010

A review of the protocol has been completed and is understood and approved by the following:

Danny McAuley   30 Nov 2012
Chief Investigator   Chief Investigator Signature   Date dd/mm/yyyy

TRIAL TEAM

Chief Investigator:	Professor Danny McAuley
Co-Investigators:	Dr L J Mark Cross
	Dr Cecilia O'Kane
	Professor Stuart Elborn
	Professor Michael Matthay
Clinical Trials Unit (CTU):	Clinical Research Support Centre Education & Research Centre, The Royal Hospitals Grosvenor Road, Belfast, N. Ireland, BT12 6BA
Trial Statistician:	Mike Stevenson, Senior Biostatistician Clinical Research Support Centre
Trial Data Manager:	Data Manager, Clinical Research Support Centre.
Study Sponsor:	Professor Ian Young, Associate Medical Director for Research, Belfast Health and Social Care Trust, Royal Hospitals, Grosvenor Road, Belfast, BT12 6BA.

Background Information

Acute lung injury (ALI) is a common devastating clinical syndrome characterised by life-threatening respiratory failure requiring mechanical ventilation and multiple organ failure and are a major cause of morbidity and mortality. ALI occurs in response to a variety of insults, such as trauma and severe sepsis. It affects all age groups; has a high mortality of up to 30-50% (Brun-Buisson, Minelli et al. 2004; Rubenfeld, Caldwell et al. 2005) and causes a long-term reduction in quality of life for survivors (Dowdy, Eid et al. 2006). ALI has significant resource implications, prolonging intensive care unit (ICU) and hospital stay, and requiring rehabilitation in the community (Rossi, Simini et al. 2006). The cost per ICU bed-day exceeds £1800 and delivery of critical care to patients with ALI accounts for a significant proportion of ICU capacity. Based on available data, in the UK and Ireland it is estimated that up to 45000 cases of ALI occur, with an estimated 13000-22000 deaths per year in patients with ALI (Brun-Buisson, Minelli et al. 2004; Rubenfeld, Caldwell et al. 2005; 2008). Only 54% of survivors are able to return to work 12 months after hospital discharge (Cheung, Tansey et al. 2006). The high incidence, mortality, long-term consequences and high economic costs mean that ALI is an extremely important problem.

Mechanisms of ALI

The pathogenesis of ALI involves pulmonary recruitment of macrophages and neutrophils. These cells are in an activated state characterised by upregulated expression of cell surface adhesion molecules, excessive cytokine production (tumour necrosis factor alpha (TNFα), interleukin (IL)-1β, IL-6, IL-8 and IL-10) and extracellular release of biologically active cytotoxic proteases including neutrophil elastase (NE) and matrix metalloproteinases (MMPs) (Ware and Matthay 2000). The resulting injury to alveolar epithelium and endothelium, which can be detected biochemically, determines the severity of lung injury (Modelska, Pittet et al. 1999). The damage to the alveolar barrier is central to the development of non-cardiogenic pulmonary oedema resulting in acute respiratory failure with refractory hypoxia and the requirement for mechanical ventilation. We have also demonstrated that increased pulmonary oedema is associated with reduced survival in patients with ALI (Craig, Duffy et al. 2009). In ALI patients with a markedly elevated dead space fraction has been identified as an independent predictor of mortality [21]. A significant fraction of the elevation in pulmonary dead space fraction is probably explained by lung vascular injury mediated via this pro-inflammatory cascade.

In experimental and clinical studies, we and others have shown resolution of pulmonary oedema and improved outcome in ALI is associated with improved alveolar epithelial function (Ware and Matthay 2001; McAuley, Frank et al. 2004; Perkins, McAuley et al. 2006; Perkins, Gao et al. 2008), suggesting that a strategy to accelerate epithelial repair in ALI may be beneficial

Evidence for Keratinocyte Growth Factor (KGF) as a novel therapy in ALI

Keratinocyte Growth Factor (KGF) is a 28 kDa heparin-binding member of the fibroblast growth factor family (FGF-7). KGF specifically binds to the KGF receptor, which is a splice variant of the FGF receptor 2, which is expressed only in epithelial tissues. KGF acts as a paracrine mediator of proliferation, differentiation and upregulation of cytoprotective mechanisms in epithelial cells including alveolar type 2 cells, hepatocytes, gastrointestinal epithelial cells, transitional urothelial cells and keratinocytes.

KGF is known to modulate several mechanisms recognised to be important in alveolar epithelial repair. KGF;

1. promotes epithelial proliferation which is critical to the restoration of alveolar architecture.

2. promotes epithelial migration, differentiation and epithelial wound healing (William McKeown, Hyland et al. 2003; Spielberger, Stiff et al. 2004; Shannon, McKeown et al. 2006).
3. increases surfactant protein production.
4. downregulates pro-inflammatory cytokines and enhances endothelial cell resistance to injury and alters MMP release (Gillis, Savla et al. 1999).
5. reduces alveolar capillary permeability, pulmonary oedema and improves survival in animal models of ALI (Ware and Matthay 2002).
6. has anti-apoptotic effects via the Akt pathway (Ray P, Devaux Y et al. 2003)

Recombinant human KGF (palifermin) is a 140 amino acid protein with a molecular weight of 16.3 kilodaltons (kDa) and differs from endogenous KGF in that the first 23 N-terminal amino acid residues have been to deleted to improve protein stability. Palifermin is produced by recombinant DNA technology in Escherichia coli (Blijlevens and Sonis 2007).

Palifermin is already used to decrease the incidence, duration and severity of oral mucositis in patients with malignancies associated with chemo and/or radiotherapy (Blijlevens and Sonis 2007). The recommended dosage for palifermin is 60 micrograms/kg/day administered as an intravenous bolus and is licensed for a duration of 6 days (British National Fomularly 2010). The efficacy of KGF for accelerating epithelial repair in a non-pulmonary location in patients with severe dermal epithelial injury enhances interest in its potential use to treat epithelial injury in ALI.

Recently novel data in an *ex vivo* human model of LPS-induced lung injury found that treatment with KGF improved lung endothelial and epithelial barrier function and enhanced the rate of alveolar fluid clearance, hence reducing alveolar oedema (Lee, Fang et al. 2009). Preliminary data from a double-blind, placebo-controlled study investigating KGF in a model of acute lung inflammation induced by inhaled lipopolysaccharide (LPS) in healthy human volunteers found pre-treatment with KGF 60 micrograms/kg/day for 3 days was associated with increased bronchoalveolar lavage fluid (BALF) surfactant protein-D (SP-D), a type 2 alveolar epithelial cell biomarker. Increased BALF SP-D has been associated with improved outcome in ALI. Interestingly this was also associated with an increase in BALF interleukin-6 (IL-6) (personal communication; D McAuley). Finally, we have recently reported the use of KGF as a salvage therapy resulting in unexpected survival in a patient with ALI in the setting of a paraquat overdose with a high predicted mortality (Browne G, Bhavsar M et al. 2010).

Therefore the accumulation of evidence from the literature of in vitro, animal and human models as well as clinical studies support KGF as a potential therapy for patients with ALI. We postulate that KGF may improve alveolar epithelial/endothelial barrier dysfunction and therefore KGF may improve pulmonary dysfunction in ALI.

Risks to patients from study drug

Keratinocyte growth factor (palifermin) can cause recognized side effects, the most common of which is rash which resolves upon withdrawal of treatment.

Palifermin (5 μg/kg to 250 μg/kg) has been administered intravenously in a range of phase 1 clinical trials to healthy volunteers with a wide age range. KGF was well tolerated and safely administered with no serious adverse events (SAE) in this dose range. All adverse events (AE) were transient and resolved without intervention and no subject discontinued because of an AE (Zia-Amirhosseini, Salfi et al. 2006). The AE profiling was graded as mild to moderate. Study treatment AEs that occurred with a frequency greater than 10% are shown below (Zia-Amirhosseini, Salfi et al. 2006).

Adverse event (WHOART preferred term), n (%)	60 µg/kg (n = 16)	90 µg/kg (n = 15)	120 µg/kg (n = 8)	160 µg/kg (n = 8)	210 µg/kg (n = 8)	250 µg/kg (n = 8)	All palifermin doses (n = 63)	Placebo (n = 16)
Access pain	6 (38)	8 (53)	2 (25)	0 (0)	4 (50)	1 (13)	21 (33)	5 (31)
Headache	4 (25)	5 (33)	2 (25)	4 (50)	3 (38)	2 (25)	20 (32)	4 (25)
Elevated amylase	4 (25)	5 (33)	3 (38)	1 (13)	0 (0)	4 (50)	17 (27)	1 (6)
Erythema	0 (0)	3 (20)	0 (0)	0 (0)	6 (75)	7 (88)	16 (25)	4 (25)
Injection site erythema	5 (31)	2 (13)	2 (25)	1 (13)	2 (25)	1 (13)	13 (21)	0 (0)
Anemia	2 (13)	3 (20)	0 (0)	3 (38)	1 (13)	1 (13)	10 (16)	3 (19)
Dermatitis contact	4 (25)	1 (7)	3 (38)	1 (13)	1 (13)	0 (0)	10 (16)	3 (19)
Warm sensation	0 (0)	1 (7)	0 (0)	1 (13)	5 (63)	3 (38)	10 (16)	0 (0)
Enzyme abnormality (elevated lipase)	3 (19)	1 (7)	1 (13)	2 (25)	0 (0)	1 (13)	8 (13)	0 (0)
Pain back	1 (6)	1 (7)	0 (0)	3 (38)	2 (25)	1 (13)	8 (13)	2 (13)
Stomatitis	0 (0)	0 (0)	1 (13)	0 (0)	3 (38)	3 (38)	7 (11)	0 (0)
Access erythema	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)	5 (63)	6 (10)	4 (25)
Dizziness	3 (19)	1 (7)	0 (0)	1 (13)	1 (13)	0 (0)	6 (10)	0 (0)
Hypercholesterolemia	0 (0)	2 (13)	1 (13)	1 (13)	2 (25)	0 (0)	6 (10)	3 (19)
Pain abdominal	2 (13)	1 (7)	1 (13)	0 (0)	1 (13)	1 (13)	6 (10)	1 (6)
Taste perversion	0 (0)	0 (0)	1 (13)	2 (25)	0 (0)	3 (38)	6 (10)	0 (0)

WHOART, World Health Organization Adverse Reaction Terminology.

In a randomized placebo controlled study (n=212) of palifermin in patients to prevent oral mucositis (106 randomized to palifermin and 106 to placebo), the documented side effects were rash, fever, taste disturbance, discoloration of tongue, itching, altered touch sensation and peri-anal discomfort. Most of the adverse events were consistent with the action of palifermin on the epithelium (e.g., rash, pruritus, erythema, paresthesia, mouth and tongue disorders, and taste alteration). All the side effects are transient and were mild to moderate in severity (occurring approximately three days after the third dose of palifermin and lasting approximately three days), and not a cause for the discontinuation of study drug (Spielberger, Stiff et al. 2004). Palifermin was associated with a transient and asymptomatic increase in serum amylase and lipase levels. No correlation between abdominal pain or other clinical symptoms and increases in amylase or lipase was observed (Zia-Amirhosseini, Salfi et al. 2006). No cases of pancreatitis have been reported from the patients with haematological malignancies and fractionation of the increased amylase demonstrated that this was predominantly salivary amylase in origin (Summary of Product Characteristics for Kepivance 2011). The pharmacodynamic effect of palifermin has been demonstrated by studying the effect on Ki67 staining in human buccal mucosal epithelial cells demonstrating that KGF can cause epithelial proliferation with maximal effect at 72 hours (Zia-Amirhosseini, Salfi et al. 2006).

In an open label study the effect of renal function (normal eGFR to requiring haemodialysis) on the pharmacokinetics of palifermin has been investigated and there was no requirement for dose adjustment in patients with renal dysfunction (Gillespie B, Zia-Amirhosseini P et al. 2006).

The recommended licensed dosage for palifermin for oral mucositis is 60 micrograms/kg/day for 6 days (British National Fomularly 2010). There is no evidence of accumulation of study drug over this time period.

Treatment will be discontinued if a serious adverse event occurs. The mean half-life ranges from 4.5 - 6 hours (Zia-Amirhosseini, Salfi et al. 2006). Overall it has been proven to be a well tolerated drug.

Rationale for the KGF dose and duration

Data from preclinical models

There are a variety of animal models using KGF between 5 to 10 mg/kg in which beneficial effects have been demonstrated (Mason CM, Guery BP et al. 1996; Sugahara K, Iyama K et al. 1998; Ware and Matthay 2002).

<u>Data from human subjects</u>
Palifermin is indicated for the treatment of oral mucositis in patients with haematological malignancies receiving myeloablative radiochemotherapy . The recommended licensed dosage for palifermin is 60 micrograms/kg/day for 6 days (British National Fomularly 2010).

The dose and duration of treatment for this study are based on this normal therapeutic dose for treatment of oral mucositis. Futhermore, as the median duration of mechanical ventilation in patients with ALI is 6 days (Rubenfeld, Caldwell et al. 2005) a 6 day duration of treatment is appropriate for the treatment of patients with ALI.

Therefore the choice of dose and duration of palifermin to be used in this study has extensively been used previously in both healthy volunteers and patients, with only transient minimum AEs and is effective at augmenting epithelial repair.

There are no effective pharmacological therapies for ALI
The Cochrane systematic review of pharmacological treatments that included 22 studies of 14 different drugs concluded that "effective pharmacotherapy for ALI is extremely limited, with insufficient evidence to support any specific intervention" (Adhikari, Burns et al. 2004).

The National Heart, Lung and Blood Institute Working Group considered the future research directions in ALI in 2002 and concluded that clinical trials underpinned by mechanistic investigations were essential to develop new therapies for ALI (Matthay, Zimmerman et al. 2003).

Hypothesis
The hypothesis is that treatment with palifermin will improve surrogate clinical outcomes in adult patients with ALI and is safe.

Trial objective
The objectives of the trials are
1) To conduct a randomised, double-blind, placebo-controlled phase 2 trial of palifermin for the treatment of ALI and
2) To study the biological mechanisms of palifermin on pulmonary and systemic neutrophil function and inflammation; alveolar epithelial and endothelial function protease:antiprotease balance and lung extracellular matrix degradation and turnover

Trial design
Prospective, randomised, double-blind, placebo-controlled phase 2 , clinical study of palifermin in patients with ALI.

The trial is summarised in Figure 1.

Figure 1. Trial schematic diagram

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┌─────────────────────────────────────────────────────────────────────────┐
│                         Daily screening in ICU                            │
│                 Does the patient have a diagnosis of ALI?                  │
│  (Acute onset AND PaO₂/FiO₂ ratio ≤ 40kPa AND bilateral infiltrates on CXR│
│        AND no evidence of left atrial hypertension AND ventilated)         │
└─────────────────────────────────────────────────────────────────────────┘
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Daily screening in ICU
Does the patient have a diagnosis of ALI?
(Acute onset AND PaO_2/FiO_2 ratio ≤ 40kPa AND bilateral infiltrates on CXR AND no evidence of left atrial hypertension AND ventilated)

Patients with ALI assessed for eligibility

Excluded
Failure to fulfil inclusion and exclusion criteria

Consent obtained from the Per LR or Prof LR

Excluded
Consent declined

Within 72 hours of onset of ALI

Randomised to KARE study
N=60

Placebo
N=30

Palifermin
N=30

Data collection
Pulmonary and non-pulmonary organ function
ICU and hospital outcomes
Safety
Blood, BAL and urine samples

When patient has regained capacity they will be asked for consent to continue follow-up

Patients who refuse to continue will be withdrawn

Trial site
ICUs at the Belfast Health and Social Care Trust (BHSCT) and University Hospital of Southampton, NHS Foundation Trust.
.

Population
Patients will be prospectively screened daily to see if they fulfil the eligibility criteria for recruitment to the study. All patients with ALI will be entered into a screening log. If the patient is not recruited the reason will be recorded. Routinely collected fully anonymised patient demographics will also be collected on these patients. This will allow comparison to identify that the study population is representative of the overall cohort of patients. The research group have an established and effective system in place to screen and identify patients with ALI.

Patients will be eligible to participate in the study if they fulfil the following criteria:

Inclusion criteria:
ALI (Bernard, Artigas et al. 1994) as defined by acute onset of:
  a.   hypoxic respiratory failure (PaO2/FiO2 ≤ 40 kPa)
  b.   bilateral infiltrates on chest X-ray consistent with pulmonary oedema.
  c.   no clinical evidence of left atrial hypertension or if measured, a pulmonary arterial occlusion pressure (PAOP) less than or equal to 18 mmHg.
  d.   requirement for positive pressure mechanical ventilation via an endotracheal tube or tracheostomy.

All ALI criteria (a-d above) must occur within the same 24-hour period. The onset of ALI is when the last ALI criterion is met. Patients must be enrolled within 72 hours of ALI onset

Exclusion criteria:
  1.   Age < 18 years
  2.   More than 72 hours from the onset of ALI
  3.   Pregnancy
  4.   Participation in a clinical trial of an investigational medicinal product within 30 days
  5.   Consent declined
  6.   Current treatment with KGF
  7.   Known hypersensitivity to palifermin or Escherichia coli derived proteins
  8.   Previous adverse reaction to palifermin.
  9.   History of active malignancy excluding haematological malignancies.
  10.   Chronic liver disease with Child-Pugh score greater than 12.

Patients with ALI for more than 72 hours are excluded to evaluate the effects of KGF early in the course of ALI when alveolar damage is implicated in the development of ALI. Patients <18 years old are excluded because of limited clinical trial data with KGF in subjects younger than 18 years.

Trial Intervention
Patients will be randomised to Palifermin 60 μg/kg or normal saline placebo daily as a bolus intravenous injection for up to 6 days. Administration will not occur through an intravenous line that has been flushed with heparin. The intravenous line will be flushed with normal saline prior to and after study drug administration. The first dose of study drug will be administered within 4 hours of randomisation and subsequent doses will be at 10 am daily starting on the following calendar day.

Study drug termination criteria
Study drug will be continued until one of the following conditions is met, whichever comes first:

1. 6 days after randomisation (maximum treatment period)
2. 2 days following discontinuation of assisted ventilation
   - Unassisted breathing is defined as extubated with supplemental oxygen or room air; or open T-tube breathing; or tracheostomy mask breathing; or CPAP ≤5 cm H_2O without pressure support. Patients receiving pressure support via non-invasive ventilation will be defined as receiving assisted ventilation.
3. Study drug related adverse event (AE)
4. Discharge from Critical Care environment
5. Death
6. Discontinuation of active medical treatment
7. Patient or relative request for withdrawal of patient from the study
8. Decision by the attending clinician that the study drug should be discontinued on safety grounds

Outcome Measures
As this is a phase 2 clinical study, several outcomes will be evaluated to determine whether treatment with palifermin shows efficacy for important surrogate clinical and biologic outcomes.

Primary outcome measure
The primary endpoint of this clinical study is to evaluate the efficacy of palifermin to improve oxygenation index (OI) at day 7 or the last available OI prior to patient discontinuation from the study. OI is a physiological index of the severity of ALI and measures both impaired oxygenation and the amount of mechanical ventilation delivered. We and others have shown OI is independently predictive of mortality in patients with ALI (Fort, Farmer et al. 1997; Seeley, McAuley et al. 2008). We have chosen day 7 as we expect this time interval will minimise the competing effects of death and extubation, while allowing a sufficient time interval for a biological effect to occur.
OI is calculated as (mean airway pressure (cm H_2O) x FiO_2 x 100) ÷ PaO_2 (kPa). These simple measurements are easily and routinely collected as part of standard ventilator practice.

Secondary Outcome measures
1. Oxygenation index (OI) at days 3 and 14
2. Physiological indices of acute lung injury, as measured by respiratory compliance (Crs), and P/F ratio at days 3, 7 and 14

3. Change in sequential organ failure assessment (SOFA) score from baseline to day 7 and 14
4. Safety and tolerability as assessed by the occurrence of AEs and Suspected Unexpected Serious Reactions (SUSARs).

Although the duration of ventilation and ICU stay as well as ICU and hospital mortality and 28-day mortality will also be documented, these important clinical outcomes are not included as major outcome measures as the study is not adequately powered to assess these outcomes.

The secondary objective of the study is to measure the biological effects of KGF on:
1. Systemic and pulmonary neutrophil function
2. Systemic and pulmonary inflammatory response
3. Systemic and pulmonary epithelial and endothelial function
4. Systemic and pulmonary protease and anti-protease balance
5. Pulmonary extracellular matrix (ECM) degradation and turnover

Trial procedures
Informed consent procedure
The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. The Principal Investigator is responsible for ensuring that informed consent for trial participation is given by each patient or a legal representative. An appropriately trained doctor or nurse may take consent. Appropriate signatures and dates must be obtained on the informed consent documentation prior to collection of trial data and administration of the trial drug. If no consent is given a patient cannot be randomised into the trial.
The incapacitating nature of the condition precludes obtaining prospective informed consent from participants. In this situation informed consent will be sought from a Personal Legal Representative or Professional Legal representative.

Personal Legal Representative consent
Informed consent will be sought from the patient's 'Personal Legal Representative' (PerLR) who may be a relative, partner or close friend. The PerLR will be informed about the trial by the responsible clinician or a member of the research team and provided with a copy of the Covering Statement for the PerLR with an attached Participant Information Sheet (PIS) and asked to give an opinion as to whether the patient would object to taking part in such medical research. If the PerLR decides that the patient would have no objection to participating in the trial they will be asked to sign two copies of the PerLR Consent Form which will then be countersigned by the person taking consent. The PerLR will retain one copy of the signed Consent Form. The second copy will be photocopied and the photocopy placed in the patients' medical records whilst the original will be retained in the Trial Site File.

Professional Legal Representative consent
If the patient is unable to give informed consent and no PerLR is available, a doctor who is not connected with the conduct of the trial may act as a Professional Legal Representative (ProfLR). The doctor will be informed about the trial by the responsible clinician or a member of the research team and given a copy of the PIS. If the doctor decides that the patient is suitable for entry into the trial they will be asked to sign two copies of the Professional Legal Representative Consent Form. The doctor will retain one copy of the signed Consent Form. The second copy will be

photocopied and the photocopy placed in the patient's medical records; the original will be retained in the Trial Site File.

Retrospective patient consent
Patients will be informed of their participation in the trial by the responsible clinician or a member of the research team once they regain capacity to understand the details of the trial. The responsible clinician or a member of the research team will discuss the study with the patient and the patient will be given a copy of the PIS to keep. The patient will be asked for consent to participate in the trial and to sign two copies of the Consent to Continue Form which will then be countersigned by the person taking consent. The patient will retain one copy of the signed Consent Form. The second copy will be photocopied and the photocopy placed in the patients' medical records whilst the original will be retained in the Trial Site File.
Where consent to continue is not obtained, consent from the legal representative will remain valid. If the patient refuses consent, data collected about the patient will not be entered into the analysis.

Withdrawal of consent
Patients may withdraw or be withdrawn (by PerLR or ProfLR) from the trial at any time without prejudice. Data recorded up to the point of withdrawal will be included in the trial analysis. If a patient or PerLR requests termination of the trial drug during the treatment period, the drug will be stopped but the patient will continue to be followed-up as part of the trial. If a patient or a PerLR withdraws consent during trial treatment, the trial drug will be stopped but permission will be sought to access medical records for data related to the trial. If a patient or PerLR wishes to withdraw from the trial after completion of trial treatment, permission to access medical records for trial data will be sought.

Similar consent mechanisms have been used successfully in other trials in similar populations (Harvey, Harrison et al. 2005).

Patient registration and randomisation procedure
After informed consent, the researcher will contact the clinical trials pharmacist who will allocate a unique Subject Number to the patient and randomise the subject in a 1:1 ratio to the designated treatment group. Randomisation will be stratified by presence of severe sepsis requiring vasopressors. The clinical trials pharmacist will dispense the trial drugs. The researcher will then contact the CTU and register the randomised patient.

Study drug will be dispensed as per local Pharmacy guidelines. If the total 6 doses of study drug for each patient will be dispensed by pharmacy they will be stored in a secure temperature monitored fridge. This will only be accessed by trained senior nursing staff of the ICU. The study drug will be reconstituted and administered intravenously by an appropriately trained ICU clinical staff independent of the clinical trial according to local guidelines and therefore ensuring blinding for the clinical trial staff. The clinical staff who administers the study drug will not be involved in any of the study specific assessments. Any omission of study drug will be recorded in the Case Report Form (CRF) to monitor treatment compliance.

Drug accountability
The clinical trials pharmacist will be responsible for maintaining records of the study drug dispensed to patients in ICU including dates, quantity, lot number and expiry date. Drug administration will be recorded on the patient's prescription chart. At the end of the treatment period any remaining unused drug will be returned to the hospital pharmacy. Destruction of trial medication will be in accordance with Pharmacy Department SOPs and hospital waste management policy. A record of destruction will be maintained

Standardised management
All patients will receive standardised management with regards to feeding, antibiotic policy, fluid management and weaning. Patients will be managed using a standardised ventilation protocol aiming for tidal volumes of 6 ml/kg ideal body weight.
The influence of other treatments provided to critically ill patients will be minimised as the study will be undertaken in a single centre where standardised care is delivered based on evidence based local guidelines.

Assessments
For routinely collected clinical data the NHS record will be the source document and for study specific clinical measurements the CRF will be the source document.

Day 1 (baseline) data
The following will be recorded;
Patient demographics
- Date of birth, gender, height, weight and underlying aetiology of ALI will be documented at baseline. Concurrent medications will also be documented.
The Acute Physiology And Chronic Health Evaluation score II (APACHE II)
Simplified Acute Physiology Score II (SAPS II)
Oxygenation index
P/F ratio
Respiratory compliance (Crs)
Pulmonary dead space
Sequential organ failure assessment (SOFA) score
Haemodynamic variables
- Pulse, blood pressure, inotrope use
Ventilation parameters
- Mode of ventilation, Positive End Expiratory Pressure (PEEP), plateau pressure, peak pressure, tidal volume, and arterial blood gas (ABG) measurements
Renal replacement therapy (RRT)
- The reason for being on RRT will also be documented.
Clinical laboratory assessments: renal and liver function, haematological parameters, coagulation parameters, C reactive protein and amylase
Study drug administration

Blood, BAL and urine will be taken at baseline prior to study drug administration (day 1) from all patients.

Sampling procedures are outlined below.

Daily data
All daily measurements will be recorded and collected between 10am and 12midday.

Oxygenation index
P/F ratio
Respiratory compliance (Crs)
Pulmonary dead space
Sequential organ failure assessment (SOFA) score
Haemodynamic variables
Ventilation parameters
Need for renal replacement therapy (RRT)
Clinical laboratory assessments: renal and liver function, haematological parameters, coagulation parameters, C reactive protein and amylase
Diuretic use
Overall fluid balance including blood product transfusions, FFP, red cell, platelets
ICU acquired infection
- The incidence of ICU acquired infection will be recorded according to standardised definitions.
Study drug administration
Adverse event assessment

The duration of ventilation and ICU stay as well as ICU and hospital mortality will also be recorded. 28-day mortality will also be documented.

Day 4, 7 and 14
BAL will be taken on day 4

Blood, and urine will be taken on day 4 and 7, 14

Sampling procedures
Blood and urine sampling
Twenty-five ml of blood will be collected by trained study staff and processed according to standard procedures as previously described (Shyamsundar, McKeown et al. 2009).
In addition 10ml aliquots of urine will be collected by trained study staff.
Bronchoscopy and BAL
Bronchosopy and BAL will be undertaken and BAL fluid processed as previously described (Haslam and Baughman 1999; Shyamsundar, McKeown et al. 2009).
In keeping with standard recommendations, patients who are receiving more than 80% inspired oxygen or have a high positive end expiratory pressure (PEEP) of >15cm H2O will not undergo bronchoscopy and BAL. In addition if the ICU consultant has any concerns regarding safety the procedure will not be undertaken.
Participants will be closely monitored during and after bronchoscopy and BAL.
Participants will receive sedation and analgesia (to prevent discomfort) as part of standard care. Bronchoscopy and BAL can be associated with transient oxygen desaturation. Patients will be pre-oxygenated. Predefined stopping criteria are

established and If oxygen saturation, as measured by pulse oximetry falls to <93% bronchoscopy and BAL will be stopped.

Samples will be labelled with the patient's unique Subject Number.
All samples will be stored at –70oC until analysis.

Samples will be stored beyond study completion. As new scientific data become available we will be able to use this resource of stored samples to investigate if this new data is relevant to ALI pending additional ethical approval.

Laboratory measurements
The following will be measured
Systemic and pulmonary neutrophil function including measurement of total and differential white cell count, MPO and neutrophil apoptosis
Systemic and pulmonary inflammatory response including measurement of CRP, cytokines (including TNFα, IL-1ra, IL-1β, IL-4, IL-6, IL-8, IL-10 and MCP-1), HO-1, adhesion and activation molecule expression, coagulation factors (including thrombin-anti-thrombin complex, tissue factor, protein C, thrombomodulin and plasminogen activator inhibitor-1) and RAGE ligands. In addition alveolar leukocytes cell lysates will be analysed for total and phosphorylated p38, ERK and JNK MAPKs, and STAT-1/-3 by western blot (Elkington, Emerson et al. 2005). Alveolar leukocytes cytoplasmic extracts will be probed by western blot for activated and total IκBα and β. Nuclear extracts will be analysed by NFκB and AP-1 transcription factor assays (Wickremasinghe, Thomas et al. 2004).
Systemic and pulmonary epithelial and endothelial function including measurement of cell specific biomarkers (such as RAGE, SP-D, Ang I/II and vWF), measurement of alveolar permeability assessed by measurement of plasma and BALF total protein, albumin and immunoglobulin and in-vitro wound repair assessment in response to BAL fluid from subjects treated with or without palifermin (Geiser, Jarreau et al. 2000). Systemic endothelial function will also be measured by spot urine albumin:creatinine ratio (ACR).
Systemic and pulmonary protease and anti-protease balance including measurement of MMPs (MMP-1,-2,-3,-7,-8,-9,-12,-13), serine proteases and anti-proteases. Pulmonary extracellular matrix (ECM) degradation and turnover as measured by urinary desmosine indexed to urine creatinine and procollagen peptide III.

Alveolar leucocytes will be isolated to study their function, including inflammatory mediator release alone and in co-culture experiments as well as response to anti-inflammatory agents in vitro. mRNA will be extracted and analysed for proteases and proteins modulating the inflammatory response.
BAL fluid will also be used to study the influence of KGF treatment upon inflammatory mediator release, interstitial matrix turnover and apoptosis of alveolar epithelial cells alone and in co-culture experiments with endothelial cells and fibroblasts. BAL fluid will also be investigated at baseline and on day 4 for both bacterial culture and for the presence of viruses.   A sample of BAL will also be sent for microbiological analysis to include assessment for anaerobic bacteria.

A summary of trial procedures is shown in Table 1.

Table 1. Trial Procedures

	Day 1	Day 2-3	Day 4	Day 5-6	Day 7	Day 8-13	Day 14
Eligibility assessment	X						
Informed consent	X						
Randomisation	X						
Baseline data	X						
Daily data		X	X	X	X	X	X
Study drug administration	X	X	X	X			
Adverse events	X	X	X	X	X	X	X
BAL sampling	X		X				
Blood and urine sampling	X		X		X		X

Data Management

Data collection and recording
All data for an individual patient will be collected by the Chief Investigator or their delegated nominees and recorded in the CRF.

Patient identification in the CRF will be through their unique Subject Number allocated at the time of randomisation and initials. Data will be collected from the time the patient is considered for entry into the trial through to their discharge from hospital.

Submitted data will be reviewed for completeness and entered onto a secure, backed-up custom database. Due care will be taken to ensure data safety and integrity, and compliance with the Data Protection Act 1998.

Training Issues
To ensure accurate, complete and reliable data, the CTU will do the following:

- Be available for consultation with the trial personnel by mail, telephone and/or fax.
- Review and evaluate CRF data, detect errors in data collection and request data verification.

Data Storage
All essential documentation and trial records will be stored by the Chief Investigator in conformance with the applicable regulatory requirements and access to stored information will be restricted to authorised personnel.

Archiving
Trial documentation and data will be archived for at least 15 years after completion of the trial in keeping with the applicable regulatory requirements.

Pharmacovigilance
Definition of Adverse Events
The EU Clinical Trials Directive 2001/20 provides the definitions given in Table 2.

Table 2. Terms and Definitions for Adverse Events

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response in a participant to an investigational medicinal product, which is related to any dose administered to that participant.
Unexpected Adverse Reaction (UAR)	An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in the Summary of Product Characteristics (SPC) for that product (for products with a marketing authorisation)
Serious Adverse Event (SAE) Serious Adverse Reaction (SAR) Suspected Unexpected Serious Adverse Reaction (SUSAR)	Respectively, any adverse event, adverse reaction or unexpected adverse reaction that: • results in death • is life-threatening • requires hospitalisation or prolongation of existing hospitalisation* • results in persistent or significant disability or incapacity • consists of a congenital anomaly or birth defect is any other important medical event(s) that carries a real, not hypothetical, risk of one of the outcomes above and suspected transmission via a medicinal product of an infectious agent.

Reporting Requirements
Adverse Event (AE) Reporting
The following AE will be recorded on the AE page in the CRF:
•   Moderate or severe rash requiring discontinuation of study drug. In previous clinical studies with Palifermin there have been very infrequent discontinuations related to Palifermin induced dermatological reactions, but this will be monitored for in a daily basis to ensure volunteer safety.
• Other AE requiring discontinuation of study drug

These events will be included as part of the safety analysis for the trial and do not need to be reported separately to the CTU.

Serious Adverse Events (SAEs)

SAEs that occur between trial entry and up to 28 days after completion of the study drug will be reported.

All serious adverse events will be recorded in the adverse event report page in the CRF. The severity grade (mild, moderate, severe), duration (start and end dates) and relationship to the study drug will be recorded in keeping with regulatory requirements.

The investigator must record any serious adverse events as defined above in the CRF, regardless of relationship to study drug as determined by the investigator. The investigator should attempt, if possible, to establish a diagnosis based on the subject's signs and symptoms. When a diagnosis for the reported signs or symptoms is known, the investigator should report the diagnosis as the adverse event, rather than reporting the individual symptoms.

Suspected Unexpected Serious Adverse Reactions (SUSARs)

Suspected Unexpected Serious Adverse Reactions (SUSARs) are SAEs that are unexpected i.e. their nature or severity is not consistent with the Summary of Product Characteristics and are considered to be caused by the study drug.

These should be reported using the SAE form in the patient's CRF and must be reported by the Chief Investigator within 24 hours of becoming aware of them. To do this, the SAE form should be completed and faxed to the CTU. The Chief Investigator's assessment of causality of SAEs (i.e. their relationship to trial treatment) will be reported on the SAE form. Subsequently, the Chief Investigator will be required to submit a full report on the resolution of the event. SUSARs will be recorded and reported in line with UK statutory requirements for clinical trials involving Investigational Medicinal Products (IMP) and copied to Swedish Orphan Biovitrum AB. The Chief Investigator is responsible for reporting adverse events to the sponsor, Research Ethics Committee, MHRA and Swedish Orphan Biovitrum AB within required timelines.

All adverse events should be treated appropriately. Treatment may include one or more of the following: no action taken (i.e., further observation only); non-drug therapy given; discontinuation of study drug; subject's hospitalization prolonged. The action taken to treat the adverse event will be recorded in the CRF. Once an adverse event is detected, it should be followed until its resolution or stabilized, or the events are otherwise explained and the outcome recorded in the CRF. Adverse events are included in an annual report to the sponsor, Research Ethics Committee and MHRA.

End of Trial

The trial will end when 60 patients have been recruited and completed 28-day follow-up.

The trial will be stopped prematurely if:
- Mandated by the Ethics Committee
- Mandated by the MHRA
- Mandated by the sponsor eg following recommendations from the DMEC
- Funding for the trial ceases

The Research Ethics Committee that originally gave a favourable opinion of the trial and the MHRA that issued the Clinical Trial Authorisation will be notified in writing if the trial has been concluded or terminated early.

Statistical considerations
Sample Size
The primary outcome measure will be the difference in OI between the palifermin and placebo treated groups at day 7. We have chosen day 7 as we expect this time interval will minimise the competing effects of death and extubation, while at the same time allowing a sufficient time interval for a biological effect to occur. Based on our data from a recently completed clinical trial in ALI, the mean (standard deviation; SD) OI at day 7 in patients with ALI is 62 (51) cmH2O/kPa (Craig, Duffy et al in press.). A sample size of 56 subjects (28 in each group) will have 80% power at a two-tailed significance level of 0.05 to detect a clinically significant difference of 39 cmH2O/kPa in OI between groups. In a previous phase 2 study of similar size, we have found that an intervention can demonstrate a change in OI of a similar magnitude (Craig, Duffy et al in press.) confirming a treatment effect of this size can be achieved.

Although we anticipate few withdrawals or loss to follow-up we have allowed for this in the sample size calculation. In our previous single centre study of simvastatin in ALI there were no withdrawals. In a multi-centre UK study of pulmonary artery catheters in ICU patients (PAC-Man), no patients were lost to follow up, and only 2.4% withdrew consent after recovering competency (Harvey, Harrison et al. 2005). Therefore a drop-out rate of 5% has been estimated and the study will require a total of 60 patients (30 in each group).

Using the sample size of 60 patients determined from the primary outcome measure, the differences in the secondary outcomes at day 7 (Craig, Duffy et al in press.), that can be detected between the groups are presented in table 3. Data are mean (SD). All calculations assume 80% power at a two-tailed significance level of 0.05.

Table 3.

Outcome	Value in patients with ALI	Detectable effect size
Crs (ml/cm H20)	57.8 (36.5)	27.8
SOFA score	7.2 (4.2)	3.2

Data Analysis
Standard approaches will be used to detect patterns in missing data. Analyses will be on an intention-to-treat basis. A single analysis is planned at the end of the trial. A P value of 0.05 will be considered as significant.

For continuously distributed outcomes, differences between groups will be tested using independent samples t-tests, analysis of variance (ANOVA) and analysis of covariance (ANCOVA) with transformations of variables to Normality if appropriate, or non-parametric equivalents. Chi-squared tests (or Fisher's Exact tests) will be used for categorical variables.

Correlations between changes in the biological markers measured and physiological and clinical outcomes will assessed by appropriate graphical and statistical methods including Chi-square and Pearson's correlation coefficient.

A detailed Statistical Analysis Plan will be written before the end of the trial.

Regulations, ethics and governance
The trial will comply with the principles, requirements and standards set out in the Research Governance Framework and The Medicines for Human Use (Clinical Trials) Regulations 2004 and subsequent amendments.

Sponsorship

The BHSCT will act as sponsor.

Regulatory approvals

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Approval from a Research Ethics Committee and a Clinical Trial Authorisation is needed before the start of the trial.

The trial will registered with the International Standard Randomised Controlled Trial Number register.

The trial will be registered with the Northern Ireland Clinical Research Network NICRN for Critical Care Clinical Research Portfolio. Accrual data on patient recruitment will be forwarded to the NICRN Co-ordinating Centre on a monthly basis from the CTU.

Ethical considerations

The vulnerability of this study group is fully appreciated and every effort will be undertaken to protect their safety and well-being. In line with The Medicines For Human Use (Clinical Trials) Regulations 2004 and subsequent amendments and to comply with the Research Governance Framework, consenting processes are standardised and a robust SOP for consenting participants will be adhered to.

Patient Confidentiality

Patient confidentiality will be maintained at every stage and compliance with the Data Protection Act (1998).

Good Clinical Practice

The trial will be carried out in accordance with the principles of the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines (www.ich.org).

Trial Monitoring

Site monitoring will be directed by the sponsor according to the study risk assessment. Site visits will be performed on a regular basis to ensure that all regulatory requirements are met and to monitor the quality of the data collected. Site monitoring visits will involve source data verification where applicable.

Indemnity

The BHSCT will provide indemnity for any negligent harm caused to patients.

Funding

The study is funded by Health and Social Care R&D Office. Palifermin is provided as a gift by Swedish Orphan Biovitrum AB.

Safety and well being of study participants

Participant safety and well-being are protected by implementation of the sponsors SOPs as set out in the Research Governance Framework and The Medicines for Human Use (Clinical Trials) Regulations 2004. As sponsor the BHSCT requires all research to be managed through a dedicated Research Management System. Systems are in place to ensure that all investigators are able to demonstrate that they are qualified by education, training or experience to fulfil their roles and those systems and procedures are in place which can assure the quality of every aspect of the trial.

Safety of investigators

The University and the Trust have Health and Safety Policies applicable to all employees. All personnel should also ensure they adhere to any other Health and Safety regulations relating to their area of work. The Chief investigator will ensure that all personnel have been trained appropriately to undertake their specific tasks.
As the study fits closely to standard practice, there are few risks identified which are hazardous to the investigators. The study team will complete GCP and consent training prior to start up.

Trial management

The Clinical Research Support Centre (CRSC) CTU will be the Trial Co-ordinating Centre.

Trial management group (TMG)

The TMG will consist of the Chief Investigator, co-investigators and staff from the CTU. The Chief Investigator will have overall responsibility for the conduct of the study. Dr Mark Cross will be responsible on a day-to-day basis for the trial. Regular meetings of the TMG will be held to discuss and solve problems and monitor progress. The Chairman of CritPaL (Barry Williams) will advise the TMG and will represent the patient's perspective ensuring that the trial remains considerate of the needs of the patients and their families.
The Chief Investigator will take responsibility for the need to change the protocol for any reason, reviewing relevant information from other sources and considering recommendations from the DMEC.

Data Monitoring and Ethics Committee (DMEC)

A DMEC will be appointed. The committee will be independent of the study team and will comprise an intensive care clinician and a clinician with experience in undertaking clinical trial. The DMEC will meet to agree conduct and remit. The DMEC will meet after the first 5 patients have been enrolled into the study and meet every 6 months thereafter. In the event of an occurrence of an unexpected severe adverse reaction an additional unplanned DMEC meeting will be convened. As this is a phase 2 trial, an interim analysis of efficacy is not planned although this issue can be discussed by the DMEC as required. The DMEC will function primarily as a check for safety, reviewing adverse events. They will report any issues pertaining to safety to the Chief Investigator. It will be the responsibility of the Chief Investigator to inform the sponsor who will take appropriate action to halt the trial if concerns exist about patient safety.

Trial schedule

Investigative site preparation: August 2010

Planned recruitment period: October 2010 – May 20113
Planned completion of last patient: Mar 2013
Biochemical analysis: Mar 2013 – May 2013
Analysis and writing up of results: May 2013 – July 2013

This timeline is based on our previous successfully completed phase 2 study in ALI (Craig, Duffy et al in press.).

Dissemination
The trial will be reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (www.consort-statement.org).
Dissemination will be achieved in several ways: (1) the findings will be presented at national and international meetings with open access abstracts on-line e.g. the American Thoracic Society annual meeting; and (2) in accordance with the open access policies proposed by the leading research funding bodies we aim to publish the findings in high quality peer-reviewed open access (via Pubmed) journals. This will secure a searchable compendium of these publications and make the results readily accessible to the public, health care professionals and scientists.
Due to limited resources, it will be not be possible to provide each surviving patient with a personal copy of the results of the trial. However a lay person's summary of the principal findings of the results will be sent to all patients involved in the study at their request. In addition a lay person's summary will be sent to local and national patient support and liaison groups (e.g. CritPaL). Where appropriate, research details will also be posted on institutional websites available to the general public. In addition, the most significant results will be communicated to the public through press releases.

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Table S1. Mode of ventilation and adjunctive therapy

Placebo	Baseline ventilation	Ventilation Day 7	Adjunctive Therapy
	SIMV	Extubated	None
	SIMV	PS	None
	SIMV	PS	None
	SIMV	PS	NMB
	PS	Extubated	None
	PS	PS	None
	PS	BIPAP	iNO and NMB
	SIMV	PS	NMB
	SIMV	PS	None
	PS	Extubated	None
	PS	PS	None
	SIMV	Deceased	None
	PS	PS	None
	PS	Extubated	None
	BIPAP	PS	NMB
	PS	Extubated	None
	SIMV	PS	None
	PS	PS	None
	SIMV	SIMV	NMB
	SIMV	PS	None
	PS	Extubated	None
	SIMV	SIMV	iNO and NMB
	PS	Extubated	Prone position and NMB
	PS	Extubated	None
	SIMV	PS	None
	SIMV	Extubated	None
	PS	PS	None
	SIMV	PS	None
	SIMV	Extubated	None
	SIMV	PS	None
	SIMV	PS	None
KGF	**Baseline ventilation**	**Ventilation Day 7**	**Adjunctive Therapy**
	SIMV	SIMV	None
	SIMV	PS	None
	PS	Deceased	None
	SIMV	PS	None
	SIMV	PS	None
	SIMV	SIMV	None
	SIMV	PS	None
	SIMV	Deceased	None
	PS	SIMV	None
	PS	PS	None
	PS	PS	None
	SIMV	Deceased	None
	SIMV	PS	NMB
	BIPAP	SIMV	None
	SIMV	PS	None
	PS	PS	NMB
	SIMV	SIMV	NMB
	PS	PS	None
	SIMV	PS	None
	SIMV	SIMV	None
	SIMV	PS	NMB
	SIMV	SIMV	None
	SIMV	SIMV	NMB
	PS	CPAP	None
	PS	PS	NMB
	PS	PS	None
	SIMV	PS	None
	PS	PS	None
	SIMV	Deceased	iNO

SIMV=Synchronized intermittent mandatory ventilation. PS= Pressure support ventilation.
CPAP=Continuous positive airway pressure. NMB=Neuromuscular Blockade. iNO=inhaled nitric
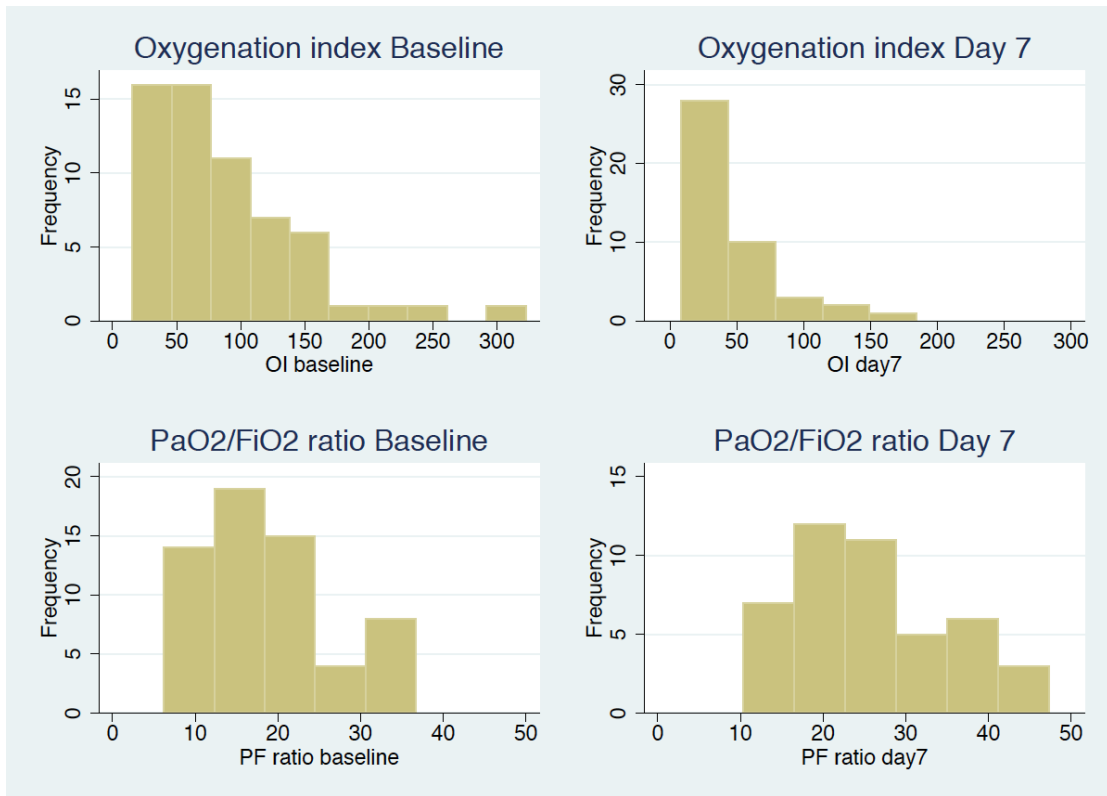oxide. Prone=prone position.

Table S2. Mean airway pressure

	KGF	Placebo	Difference (95% CI)	p-value
Day 3	10.8 ± 4.4 (n=26)	10.3 ± 4.3 (n=30)	0.54 (-1.80 to 2.89)	0.65
Day 7	10.0 ± 3.8 (n=23)	10.0 ± 4.6 (n=21)	0.00 (-2.58 to 2.58)	1.00
Day 14	11.2 ± 4.2 (n=11)	9.0 ± 4.6 (n=5)	2.18 (-2.78 to 7.14)	0.36

Table S3. Classification of AEs.

	KGF	Placebo
Cardiac disorders	2	0
Dermatology/Skin	0	1
Gastrointestinal disorders	1	0
General disorders (including multi-organ failure)	5	4
Hepatobiliary disorders	1	0
Infections and infestations	2	0
Nervous system disorders	2	0
Respiratory - thoracic and mediastinal disorders	1	0

Figure S1: Distribution histograms for OI and PaO$_2$/FiO$_2$ ratio at baseline and day 7.

Keratinocyte growth factor for the treatment of the acute respiratory distress syndrome (KARE): a randomised, double-blind, placebo-controlled phase 2 trial

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Keratinocyte Growth Factor for the treatment of the Acute Respiratory Distress Syndrome: a randomised clinical trial (The KARE trial).

Daniel F McAuley MD*[1,2,3], LJ Mark Cross PhD[1,2], Umar Hamid MD[1,2], Evie Gardner MSc[3], J Stuart Elborn MD*[1], Kathy M Cullen PhD[1,2], Ahilanandan Dushianthan PhD[4,5,6], Michael PW Grocott MD*[4,5,6], Michael A Matthay MD*[7], Cecilia M O'Kane PhD[1]

*full professor

[1]Centre for Experimental Medicine, The Queen's University of Belfast, Health Sciences Building, 97 Lisburn Road, Belfast, BT9 7BL, N Ireland

[2]Royal Victoria Hospital, Belfast Health and Social Care Trust, Grosvenor Road, Belfast, BT12 6BA, N Ireland

[3]Northern Ireland Clinical Trials Unit, Royal Victoria Hospital, Belfast Health and Social Care Trust, Grosvenor Road, Belfast, BT12 6BA, N Ireland

[4]Anaesthesia and Critical Care Research Unit CE-93, MP-24, E-level, Centre Block University Hospital Southampton NHS Foundation Trust, Southampton, SO16 6YD, UK

[5]Critical Care Research Area, Southampton NIHR Respiratory Biomedical Research Unit, University Hospital Southampton NHS Foundation Trust, Southampton, SO16 6YD, UK

[6]Integrative Physiology and Critical Illness Group, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, SO16 6YD, UK

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[7]Departments of Medicine and Anesthesia, Cardiovascular Research Institute, University of California, San Francisco, 505 Parnassus Ave, M-917, San Francisco, CA 94143-0624, California, USA

Corresponding Author:

Professor Daniel Francis McAuley

Centre for Experimental Medicine,

The Queen's University of Belfast,

97 Lisburn Road,

Belfast, BT9 7BL,

Northern Ireland

Telephone number +44 2890972144

Fax number +44 2890972671

Email   d.f.mcauley@qub.ac.uk

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Summary

Background. Data from in vitro, animal and human lung injury models suggest that keratinocyte growth factor (KGF) may be beneficial in the acute respiratory distress syndrome (ARDS). The objective of this trial was to investigate the effect of KGF in patients with ARDS.

Methods. This was a prospective, randomised, double-blind, allocation concealed placebo-controlled phase 2 trial conducted in two intensive care units, involving patients fulfilling the American-European Consensus Conference Definition of ARDS. Patients were randomised in a 1:1 ratio to receive either KGF (Palifermin 60 μg/kg) or placebo (0·9% sodium chloride solution) daily for a maximum of six days. Patients and investigators were blinded. The primary endpoint was oxygenation index (OI) at day 7. Secondary outcomes included respiratory compliance (Crs) and PaO_2/FiO_2 ratio, sequential organ failure assessment (SOFA) score and safety. Duration of ventilation, length of stay and mortality were recorded. The trial was registered with International Standard Randomised Controlled Trial Registry, number ISRCTN95690673.

Findings. The trial recruited 60 patients with 29 patients receiving KGF and 31 patients placebo. There was no significant difference between the two groups in OI at day 7 (the primary outcome) or in any other secondary physiological outcomes. The KGF group, compared to placebo, had fewer median ventilator-free days (1 [0-17] versus 20 [13-22] days; difference -8 days, 95% CI -17- -2; P < 0·001), a longer median duration of ventilation in survivors (16 [13-30] versus 11 [8-16] days; difference 6 days, 95% CI 2-14; P = 0·002) and a higher mortality at 28 days (31·0% versus 9·7%; risk ratio 3·2 95% CI 1·0-10·7; P = 0·054).

Interpretation. KGF did not improve physiological or clinical outcomes in ARDS and may be harmful.

Research in context

Evidence before this study

We searched PubMed from January 1, 2000 to December 31, 2016 for non-animal, clinical studies with MeSH terms or synonyms for "acute respiratory distress syndrome", and "Keratinocyte Growth Factor" in the title/abstract. Our search only identified one study in a model of lung injury induced by inhaled endotoxin in healthy human volunteers, which found KGF improved biomarkers of epithelial repair and resolution of injury.

Added value of this study

This randomised phase 2 clinical trial of KGF in patients with ARDS found no difference in the primary and secondary physiologic outcomes, however ventilator free days over 28 days were reduced and mortality was higher at 28 days in the KGF treated patients.

Implications of all the available evidence

KGF is not beneficial in the treatment of ARDS and may make clinical outcomes worse. KGF should not be used to treat patients who have ARDS.

Background

The acute respiratory distress syndrome (ARDS) is a common clinical syndrome

characterised by life-threatening respiratory failure requiring mechanical ventilation

and multiple organ failure and is a major cause of morbidity and mortality.[1,2]

The pathogenesis of ARDS involves pulmonary neutrophil- and macrophage-driven

inflammation,[3–5] and injury to the alveolar epithelium and endothelium.[2] The

resolution of pulmonary oedema and improved outcome in ARDS is associated with

enhanced alveolar epithelial function, suggesting that a strategy to accelerate

epithelial repair in ARDS may be beneficial.[6] There is currently no effective

pharmacological therapy which targets the underlying mechanisms implicated in the

development of ARDS.[7] The National Heart, Lung and Blood Institute Working Group

considered the future research directions in ARDS and highlighted the lung

epithelium as an important target for new therapies for ARDS.[8]

Keratinocyte Growth Factor (KGF) modulates several mechanisms recognised to be

important in alveolar epithelial repair[9] and therefore may be a potential therapeutic

intervention in ARDS. Recombinant human KGF (palifermin) is a 23 N-terminal amino

acid truncated version of KGF. Palifermin is used for the treatment of oral mucositis in

patients with malignancies associated with chemo and/or radiotherapy and has been

shown to decrease the incidence, duration and severity of oral mucositis.[10] This

evidence of epithelial repair has led to interest in its potential use to treat epithelial

injury in ARDS. Beneficial effects of KGF have been demonstrated in a variety of

animal models of lung injury,[11] in a human *ex vivo* lung perfusion model of endotoxin-

induced lung injury,[12] and in a model of lung injury induced by inhaled endotoxin in

healthy human volunteers.[13] Therefore the available *in vitro*, animal and human

model data, as well as clinical studies in mucositis, support KGF as a potential

therapy for patients with ARDS. The aim of the current trial was to test the hypothesis that KGF may augment epithelial repair and therefore improve pulmonary dysfunction in ARDS.

Methods

KARE was a randomised, double-blind, allocation concealed, placebo-controlled clinical trial undertaken in 2 adult general intensive care units (ICUs) both of which were based in a University Hospital setting in the United Kingdom.

The trial was approved by a national research ethics committee (10/NIR02/32) and the MHRA (CTA number 32485/0021/001-0001 and EudraCT Number: 2010-021186-70) and research governance departments at each site in the UK. The trial was registered on the International Standard Randomised Controlled Trial Registry (ISRCTN95690673). The trial was sponsored by the Belfast Health and Social Care Trust and was coordinated by the Northern Ireland Clinical Trials Unit (CTU). The trial design has been published in detail previously[14] and the trial protocol is available in the online supplement. The trial is reported in line with the Consolidated Standards of Reporting Trials (CONSORT) guidelines.[15] Additional mechanistic outcomes are listed in the study protocol but are not reported in this paper.

Patients

Patients were eligible if they were intubated and mechanically ventilated and had a partial pressure of arterial oxygen to fractional inspired oxygen concentration (PaO_2/FIO_2) ratio of ≤ 40 kPa, if bilateral pulmonary infiltrates consistent with pulmonary oedema were present on chest radiograph, and if there was no evidence of

left atrial hypertension as defined by the American-European Consensus Conference Definition of acute lung injury/ARDS.[16]

The exclusion criteria were presence of ARDS for more than 72 hours, current treatment with KGF, known hypersensitivity to palifermin or Escherichia coli derived proteins (palifermin is produced in an *Escherichia coli* based protein production system therefore hypersensitivity to Escherichia coli derived protein is an exclusion criteria), previous adverse reaction to palifermin, imminent withdrawal of medical treatment, chronic liver disease (Child-Pugh score greater than 12), history of active malignancy excluding haematological malignancies, age < 18 years, pregnancy, enrolment in another clinical trial of an investigational medicinal product within the previous 30 days and inability to obtain informed consent. The trial protocol was amended to permit enrolment of patients within a 72 hour window of the onset of ARDS from an original enrolment window of 48 hours, and also enrolment of patients with pancreatitis and haematological malignancies. Patients or their representatives provided written informed consent.

Randomisation and masking

Swedish Orphan Biovitrum AB supplied the palifermin for the trial.

Patients were randomised in a 1:1 ratio to receive an IV bolus of either KGF (palifermin, 60 µg/kg) or placebo (0.9% sodium chloride solution) daily for a maximum of six days. The dose and duration of treatment for this study were based on the normal therapeutic dose for treatment of oral mucositis. The recommended licensed dosage for palifermin is 60 micrograms/kg/day for 6 days.[17] Futhermore, the median duration of mechanical ventilation in patients with ARDS was 5-6 days.[18]

The randomisation schedule was computer generated by the trial biostatistician. Randomization was by variable block size stratified by site and the presence of severe sepsis requiring vasopressors. An independent clinical trials pharmacist allocated the subject to the designated treatment group. Patients and investigators were blinded.

Study drug was continued until 6 days after randomisation (maximum treatment period), 2 days following discontinuation of assisted ventilation, discharge from critical care, patient or relative request for withdrawal of patient from the trial, death or discontinuation of active medical treatment, study drug related serious adverse event (SAE) or decision by the attending physician that the study drug should be discontinued on safety grounds.

At enrolment, the patients' demographic characteristics, ventilatory and physiological variables and admission acute physiology and chronic health evaluation II (APACHE II) scores were recorded. The cause of ARDS was identified by the treating physician. For each day in the ICU, ventilatory and physiological variables were recorded. Daily ventilator parameters and corresponding arterial blood gas data were collected at a time point nearest to 08:00 to 10:00, or nearest to this time point. Mean airway pressure was recorded in patients receiving pressure support ventilation. Plateau pressure was not recorded in patients receiving pressure support ventilation. Clinical care was at local physician discretion and based on the local ICU guidelines including low tidal-volume ventilation.

Outcomes

The primary outcome was oxygenation index (OI) at day 7. OI is a physiological index of the severity of ARDS which measures both impaired oxygenation and

the amount of mechanical ventilation delivered. OI has been found to be independently predictive of mortality in patients with ARDS.[19] Secondary outcomes were OI at days 3 and 14, physiological indices of pulmonary function, as measured by respiratory compliance (Crs) and PaO_2/FiO_2 ratio at days 3, 7 and 14, as well as a change in the sequential organ failure assessment (SOFA) score from baseline to day 3, 7 and 14. Pulmonary dead space fraction space was initially included as a secondary outcome but as this specialised measurement could not be undertaken at the second site the protocol was amended to remove this as an outcome. Safety and tolerability was assessed by the occurrence of adverse events (AEs). Although ventilator free days, the duration of ventilation and ICU stay as well as ICU and hospital mortality and 28-day mortality were recorded, these clinical outcomes were not considered as outcome measures as the trial did not have power, as defined a priori in the study protocol, to assess these.

It was intended that events that were expected in this population would not be reported as serious adverse events (SAEs), however the approved protocol stated all SAEs that occurred would be reported, regardless of the underlying relationship to the underlying clinical condition. Subsequently all SAEs were reported and relationship to underlying clinical condition recorded.

Statistical Analysis

Sample size assumptions were based on previously published data [20]. Assuming a mean (standard deviation; SD) OI at day seven in patients with ARDS of 62 (51) cmH_2O/kPa,[20] a sample size of 56 subjects (28 in each group) would need to be enrolled to have 80% power at a two-tailed significance level of 0.05 to

detect a mean reduction of 39 cmH$_2$O/kPa in OI with KGF. The PAC-Man trial,[21] which recruited a similar critically ill population, had a dropout rate of 3% and therefore, a drop-out rate of 5% was estimated and the trial required a total of 60 patients.

Analyses were on an intention-to-treat basis. For OI, the primary analysis used the last available data prior to patient discontinuation from the trial if data was missing for a specified time point. In addition, the analysis for OI was repeated using only data available at the specified time point. Secondary outcomes were analysed using only data available at the specified time point. A single analysis was undertaken at the end of the trial with no interim analyses undertaken prior to the completion of the trial. A P value of 0.05 was considered as statistically significant and all tests were two-sided. For continuously distributed outcomes, differences between groups were tested using independent samples t-tests, analysis of variance (ANOVA) and analysis of covariance (ANCOVA) with transformations of variables to Normality if appropriate, or non-parametric equivalents. Chi-squared tests (or Fisher's Exact tests) were used for categorical variables. Censorship of data collection occurred at day 90 post randomisation.

Role of the funding source

Results

Patients were recruited from 23rd February 2011 until 26th February 2014. The

trial completed when the planned 60 patients were recruited and 28-day follow-

up was completed. Of the 368 patients who were assessed for eligibility, 60

(16%) underwent randomisation. All patients received their treatment as

allocated and were analysed for the primary outcome (Figure 1).

The baseline characteristics of the patients at randomisation were similar in the

two study groups except higher Positive end-expiratory pressure (PEEP) and OI

and a lower PaO_2/FiO_2 ratio in the placebo group. The main causes of ARDS

were pneumonia, aspiration and sepsis (Table 1). Patients received study drug

for a mean (±SD) of 5·1 ± 1·5 days in the KGF group and 5·5 ± 0·9 days in the

placebo group (P = 0·21). The most common reasons for discontinuation of study

drug were completion of the treatment course and death (Table 2). The modes of

ventilation at baseline and day 7 and the adjunctive therapy received are given in

Table e1.

Outcomes

The primary outcome was oxygenation index which was not different between

the KGF and placebo groups at day 7 (62·3±57·8 versus 43·1±33·5, mean

difference (95% CI) of 19·2 (-5·6 to 44·0); P = 0·13). OI was significantly lower in

the placebo group at day 14 in the primary analysis (59·4±58·4 versus

30·1±24·2, mean difference (95% CI) of 29·3 (5·6 to 53·0); P = 0·02), however

when only data available at the pre-specified time point was analysed there was

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no difference between the groups (Table 3). There was no difference in Crs, PaO_2/FiO_2 ratio or SOFA score between the groups up to day 14 (Table 3). There were significantly fewer ventilator-free days in the KGF compared to the placebo group (1 [0-17] versus 20 [13-22] days; median difference -8 days [95% CI -17- -2]; P < 0·001). Mortality in the KGF group was higher at 28 days. A total of 9 patients (31·0%) in the KGF group and 3 patients (9·7%) in the placebo group died by day 28 (risk ratio 3·2 [95% CI 1·0 – 10·7]; P = 0·054). Mortality in the KGF group was also higher at 90 days (44·8% versus 16·1%; risk ratio 2·8 [95% CI 1·1 – 6·8]; P = 0·02). ICU and hospital mortality were also higher in the KGF group. Mortality was numerically greater in the KGF group at one year but did not reach statistical significance (P=0·06) (Table 4).

For survivors only to day 90 (n=42), the duration of ventilation was increased in the KGF group (16 [13-30] versus 11 [8-16] days; P = 0·002). In addition, in the KGF group compared to the placebo group the duration of ICU stay (22 [14-34] versus 12 [10-19] days; P = 0·001) and hospital stay (Table 4) were also increased

From randomization to day 90, the probability of survival (online supplement Figure e1a) or breathing without assistance (online supplement Figure e1b) was higher in the placebo group.

The causes of death in the 9 patients who died by 28 days in the KGF group versus the 3 patients in the placebo group are listed in Table 5. Three of the

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deaths by day 28 in the KGF group were related to central nervous system disorders including hydrocephalus, hypoxic brain injury and cerebral haematoma.

Nineteen patients experienced adverse events (AEs) including 16 serious adverse events (SAEs). All of the serious adverse events were assessed by the chief investigator and an independent ICU physician as being due to the patient's underlying medical condition and unrelated to study drug. Overall adverse events (AEs) related to study drug were significantly more common in the KGF group (Table 6). Both AEs assessed as related to the study drug were due to pyrexia. The classification of AEs is provided in the online supplement (Table e2).

Discussion

In this double-blind, randomised, placebo-controlled clinical trial in patients with ARDS, there was no difference in the primary physiologic outcome of oxygenation index. However, ventilator free days over 28 days were reduced and mortality at 28 days was higher in the KGF treated patients. These findings are in contrast to the pre-clinical and animal data as well as data from clinically relevant human models of ARDS[11–13] which supported the potential for KGF as a therapy for ARDS.

There are several issues which need to be considered in interpreting the results of this trial. First, the clinical outcomes of mortality and duration of ventilation were not pre-specified endpoints; therefore the findings of the adverse effect of KGF on clinical outcomes in this trial must be interpreted with caution. However, there was also no evidence to support a beneficial effect on physiological outcomes, including the primary outcome OI at day 7. At day 14, OI was lower in

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the placebo group when the last available data point was carried forward if data were missing. This effect was lost when only available data were analyzed. A survivor bias in the placebo group may explain this discrepant finding. As shown in Table 5, three of the deaths in the KGF group were related to central nervous system disorders which are unlikely to be attributable to KGF. Finally, the placebo group had a lower than expected mortality in this trial (<10%). To put the unexpectedly low mortality in the placebo group in context, the mortality range from comparable single site and multi-centre studies as well as a large observational study in patients with ARDS was approximately 25-35%.[1,20,22] Therefore, the difference in mortality may relate, at least in part, to unexpectedly low mortality in the placebo group. The low mortality in the placebo group was unexpected in the setting of a randomised trial stratified for severe sepsis requiring vasopressors. At baseline patients in the placebo group had a higher PEEP and oxygenation index and a lower PaO_2/FiO_2 ratio but if anything this would indicate the placebo group may have been sicker at baseline and therefore unlikely to explain either the low placebo mortality or the differences in outcome. The trial was undertaken at only 2 sites which may limit the robustness of our findings, although a general population of critically ill adult patients was recruited.

A potential limitation of the study is that the measurement of mean airway pressure while a patient is receiving pressure support ventilation may be less accurate due to the fact it includes the undetermined negative pleural pressure associated with the patient's own respiratory effort. However regardless this does not modify the finding of worse clinical outcomes associated with KGF.

It is however possible that KGF caused harm. There are several potential

reasons for this. It may be related to the dosage regimen used in this trial. In a

nickel-induced murine model of lung injury, over-expression of KGF for 3 days

prior to injury was protective while in contrast, KGF caused pulmonary

inflammation and lung injury after 7 days or longer.[23] Although the dosage

regimen was chosen based on the treatment regimen to treat chemo-radiation

induced mucositis,[10] the interval between the doses of palifermin was shorter

which may have been potentially harmful.[24] We recruited a heterogenous cohort

of patients with ARDS due to any aetiology in an attempt to ensure that our

findings would be applicable to the overall population of patients with ARDS.

However, it is possible that the response to KGF may be dependent on the

aetiology of ARDS. In support of this concept, while in the majority of murine

studies KGF is beneficial in experimental lung injury, in a murine model of lung

injury induced by influenza A, KGF caused increased mortality associated with

increased viral load and pulmonary inflammation.[25] In our clinical trial no patients

had a diagnosis of influenza, but it is possible that the specific aetiological factor

driving ARDS predicts a different response to KGF. Differential effects of

treatment may depend in part on the combination of biologic and clinical factors

in ARDS, as shown in two retrospective studies using ARDSnet trial data.[26,27]

Most of the previous pre-clinical studies used KGF as a preventive measure to

mitigate the degree of lung injury.[11,13] It is possible that KGF receptors may not

be expressed on the target alveolar epithelial cells in patients in whom the

epithelium is injured and therefore cannot mediate a therapeutic effect.

Furthermore the effects of KGF on alveolar epithelial cell hyperplasia and

proliferation may be different following injury than in the setting of pre-treatment

models. Another potential factor is the route of administration in this trial. Most of the animal studies utilised intra-tracheal instillation or inhalation of KGF[11] which directly targets the injured lung epithelium allowing a lower dose to be used with less "off-target " effects or systemic toxicity. We chose not to use local pulmonary delivery since in ARDS this not result in adequate KGF reaching oedema-filled or atelectatic regions of the injured lung. Given the targeted pulmonary delivery in many pre-clinical studies, systemic administration may have been effective. In a model of lung injury induced by inhaled endotoxin in healthy human volunteers, systemic administration of KGF at the dosage used in the current study was associated with evidence of alveolar epithelial repair and improved macrophage phagocytosis.[13] While this healthy volunteers study informed the design of the current trial, in critically ill patients with ARDS with altered drug metabolism, systemic administration at this dose may not have been able to improve alveolar epithelial and macrophage function.

Finally, there is a possibility that KGF is already maximally expressed in the setting of ARDS and additional exogenous KGF is not associated with any additional beneficial effects.

In conclusion this trial found that KGF was not beneficial in terms of physiological outcomes in ARDS and may make clinical outcomes worse, and therefore it is recommended that KGF is not used to treat patients who have ARDS.

Authors' contribution

DFM, COK and MAM conceived and designed the trial. DFM and COK obtained the funding for and managed the trial. All authors made a substantial contribution to the protocol development. EG was the study statistician and analysed the data. DM wrote the first draft of the manuscript and all authors have contributed to the writing of, reviewed and approved this final version of the manuscript.

Conflict of interest statement

Dr. McAuley reports a grant from the Northern Ireland Public Health Agency Research and Development Division for the conduct of the study. Outside the submitted work, Dr McAuley reports personal fees from consultancy for GlaxoSmithKline, SOBI, Peptinnovate, Boehringer Ingelheim and Bayer, Outside the submitted work, his institution has received funds from grants from the UK NIHR and others and from GlaxoSmithKline for Dr McAuley undertaking bronchoscopy as part of a clinical trial. In addition, Dr. McAuley has a patent issued to his institution for a treatment for ARDS.

Outside the submitted work, Dr. Matthay reports grants from GlaxoSmithKline, and Amgen, personal fees from Boerhinger-Ingelheim, Bayer, Biogen, Cerus Therapeutics, Quark Pharmaceuticals and Thesan Inc. and other from Roche-Genentec,

Dr O'Kane reports a grant from the Northern Ireland Public Health Agency Research and Development Division for the conduct of the study. Outside the

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Figure 1: CONSORT diagram for screening, randomization and follow-up of the study participants.
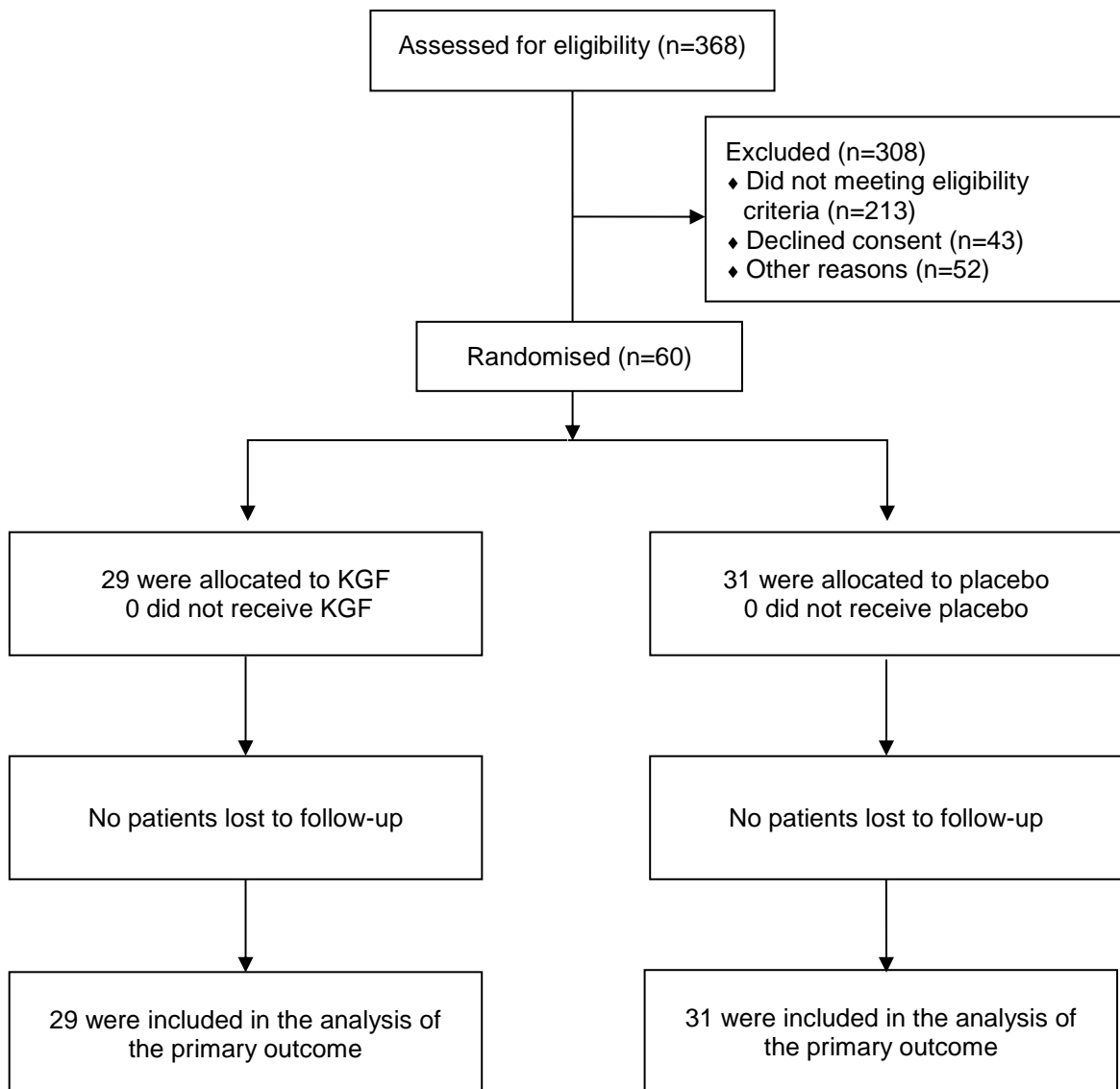
Table 1. Baseline demographic and clinical characterisitics

	KGF	Placebo
Age – year	55.6±17.5	61.0±15.4
Male sex – no. (%)	17 (58.6)	20 (64.5)
Sepsis requiring Vasopressors — no. (%)	14 (48.3)	15 (48.4)
Cause of ARDS† — no. (%)		
Smoke or toxin inhalation	1 (2.4)	0 (0)
Aspiration	10 (24.3)	7 (14.6)
Thoracic trauma	1 (2.4)	2 (4.2)
Pneumonia	10 (24.3)	18 (37.5)
Sepsis	11 (26.8)	16 (33.3)
Pancreatitis	0 (0)	2 (4.2)
Non-thoracic trauma	5 (12.2)	1 (2.1)
Other	3 (7.3)	2 (4.2)
APACHE II score	18.8±9.0	22.7±6.5
LIS score	2.0±0.6	2.2±0.6
Mean arterial pressure - mmHg	63.5±11.2	64.5±10.3
Tidal Volume at randomisation - ml/kg PBW	7.9±2.6	8.3±2.1
PEEP - cm H_2O*	7.3±2.2	8.5±2.4
Mean airway pressure - cm H_2O	12.2±4.2	13.9±4.1
Plateau pressure - cm H_2O	23.3±4.7	24.0±3.3
Oxygenation index - kPa*	72.3±51.6	103.2±60.2
PaO_2/FiO_2 ratio - kPa*	21.4±8.6	15.8±5.7
Compliance - ml/cm H_2O	40.1±16.3	43.9±15.8
SOFA score	9.5±4.0	8.9±3.1

*The baseline characteristics were similar in the study groups except for a higher PEEP (P=0.04), higher OI (P=0.04) and a lower PaO_2/FiO_2 ratio (P=0.006) in the placebo group.

† Patients may have had more than one cause of ARDS identified.

Other casuses of ARDS in the KGF group were blood transfusions, empyema and acute liver failure secondary to a possible drug reaction and in the placebo group were seizures due to possible encephalitis and pulmonary haemorrhage.

Mean ± SD for continuous data and number (%) for categorical data.

Table 2. Treatment after trial entry

	KGF	Placebo
Study drug given	29 (100)	31 (100)
No. of days on treatment	5.2 ± 1.5	5.8 ± 0.6
Reasons for termination of study drug		
6 days after randomisation	22 (75.9)	26 (83.9)
2 days following discontinuation of assisted ventilation	0 (0)	1 (3.2)
Study drug related AE	0 (0)	0 (0)
Discharge from Critical Care	0 (0)	3 (9.7)
Request from PerLR/ProfLR/patient	0 (0)	0 (0)
Discontinuation of active treatment	0 (0)	0 (0)
Decision by a physician on safety grounds	2 (6.9)	0 (0)
Death	4 (13.8)	0 (0)
Other	1 (3.4)	1 (3.2)
Protocol violations	0	0

Mean ± SD for continuous data and number (%) for categorical data

Table 3. Outcome measures

	KGF	Placebo	Difference (95% CI)	p-value
OI at day 7 or at the last available OI*	62.3±57.8 (n=29)	43.1±33.5 (n=31)	19.2 (-5.6 - 44.0)	0.13
OI at the last available OI* Day 3 Day 14	 66.9±55.0 (n=29) 59.4±58.4 (n=29)	 60.1±45.4 (n=31) 30.1±24.2 (n=31)	 6.8 (-19.2 - 32.8) 29.3 (5.6 - 53.0)	 0.60 0.02
OI# Day 3 Day 7 Day 14	 62.8±50.1 (n=26) 45.4±32.1 (n=23) 52.9±35.2 (n=11)	 60.9±45.9 (n=30) 48.6±38.6 (n=21) 43.3±37.2 (n=5)	 1.8 (-23.9 - 27.6) -3.2 (-24.8 - 18.3) 9.6 (-31.8 - 51.0)	 0.89 0.76 0.63
Respiratory compliance Day 3 Day 7 Day 14	 48.6±16.4 (n=16) 51.1±25.2 (n=14) 45.0±10.4 (n=6)	 53.5±28.8 (n=20) 65.1±15.4 (n=7) 77.5† (n=1)	 -4.8 (-21.3 - 11.6) -14.0 (-35.9 - 7.9)	 0.55 0.20
PaO_2/FiO_2 ratio# Day 3 Day 7 Day 14	 23.1±9.1 (n=26) 27.6±10.4 (n=23) 27.2±12.0 (n=11)	 20.3±6.0 (n=31) 24.6±7.6 (n=21) 21.3±9.0 (n=7)	 2.8 (-1.4 - 7.1) 3.0 (-2.6 - 8.6) 5.9 (-5.3 - 17.2)	 0.18 0.29 0.28
SOFA score Day 3 Day 7 Day 14	 7.6 ± 3.6 (n=22) 6.7 ± 3.0 (n=15) 6.9 ± 2.0 (n=10)	 7.8±4.0 (n=29) 7.9±4.1 (n=17) 5.9±2.8 (n=7)	 -0.2 (-2.4 - 2.0) -1.1 (-3.8 - 1.5) 1.0 (-1.4 - 3.5)	 0.83 0.38 0.39
Change in SOFA score Day 3 Day 7 Day 14	 -1.4±1.9 (n=21) -2.9±2.7 (n=14) -2.3±1.7 (n=9)	 -1.2±2.3 (n=29) -2.0±3.0 (n=17) -3.9±3.8 (n=7)	 -0.3 (-1.5 - 1.0) -0.9 (-3.0 - 1.3) 1.5 (-1.5 - 4.6)	 0.68 0.42 0.31

Mean ± SD
*Analysis used the last available data prior to patient discontinuation from the study if data was missing for a specified time point. Other outcomes were analysed using only data available at the specified time point.
†No SD as n=1
Distribution histograms for OI and PaO_2/FiO_2 ratio have been provided in figure e2 in the online supplement.
Mean airway pressure data (which was not a protocol specified outcome) is presented in Table e3.

Table 4. Clinical outcomes

	KGF n = 29	Placebo n = 31	Difference / Risk ratio* (95% CI)	p value
Ventilator free days to day 28	1 (0-17) [0-22]	20 (13-22) [0-25]	-8 (-17- -2.0)	<0.001
Duration of ventilation (days)#	16 (13-30) [11-90]	11 (8-16) [4-50]	6 (2-14)	0.002
ICU stay (days)#	22 (14-32) [12-90]	12 (10-19) [5-49]	9 (3-17)	0.001
Hospital length of stay (days)#	39 (30-67) [22-90]	23 (18-33) [12-84]	17 (7-33)	0.001
28 day mortality	9 (31.0)	3 (9.7)	3.2 (1.0-10.7)	0.054
90 day mortality	13 (44.8)	5 (16.1)	2.8 (1.1, 6.8)	0.02
ICU mortality	12 (41.4)	2 (6.5)	6.4 (1.6-26.2)	0.001
Hospital mortality	14 (48.3)	4 (12.9)	3.7 (1.4-10.1)	0.003
1 year mortality	15 (51.7)	8 (25.8)	2.0 (1.0-4.0)	0.06

*Median (interquartile range); [range] and difference (95% CI) presented for continuous variables; number (%) and risk ratio (95% CI) for all categorical variables. #survivors only censored at day 90 (n=15 KGF, n=27 placebo). Fishers's Exact test was used to compare categorical variables.

Table 5. Causes of death in patients who died by day 28.

Treatment Group	Cause of death
KGF	Multi-organ failure
KGF	Obstructive hydrocephalus and sepsis
KGF	Hypoxic brain injury
KGF	Sepsis and multi-organ failure
KGF	Sepsis and multi-organ failure
KGF	Cerebral haematoma
KGF	Myocardial infarction and ruptured aortic aneurysm and metastatic pancreatic cancer diagnosed during ICU admission
KGF	Systemic fungal infection
KGF	COPD and hypercapnic respiratory failure
Placebo	Ruptured aortic aneurysm with multi-organ failure
Placebo	Sepsis and multi-organ failure
Placebo	Multi-organ failure, pulmonary hemorrhage, pneumocystis pneumonia

Table 6. Safety outcomes

	KGF	Placebo	OR (95% CI)	p-value
Total AEs – no.	14	5	4.9 (1.3 - 20.3)	0.008
AEs related to study drug – no.	2	0	-	
Total SAEs – no.	12	4	4.8 (1.2 - 23.0)	0.01
SAEs related to study drug – no.	0	0	-	
SAEs related to study drug and unexpected – no.	0	0	-	