Statistical Analysis Plan of the Personalized Mechanical Ventilation Guided by UltraSound in Patients with Acute Respiratory Distress Syndrome (PEGASUS) study

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Version of protocol	Version 1.0

Signatures

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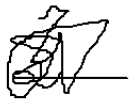
Signature	
Name Statistician	Lina Kramer
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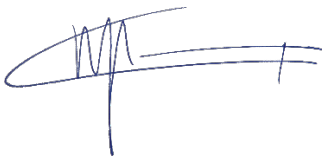
Signature	
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Date of signing	20-03-2026
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Inhoud

Background

Acute respiratory distress syndrome (ARDS) is a frequent cause of hypoxemic respiratory failure with a mortality rate of approximately 30%. Identifying ARDS subphenotypes based on “focal” or “non-focal” lung morphology has the potential to better target mechanical ventilation strategies of individual patients. However, classifying morphology through chest radiography or computed tomography is either inaccurate or impractical. Lung ultrasound (LUS) is a non-invasive bedside tool that can accurately distinguish “focal” from “non-focal” lung morphology. We hypothesize that LUS-guided personalized mechanical ventilation in ARDS patients leads to a reduction in 90-day mortality compared to conventional mechanical ventilation.

Methods

Design

The PEGASUS study is an investigator-initiated, multicenter, international, superiority RCT comparing personalized ventilation guided by lung ultrasound with the standard of care in ARDS patient admitted to the Intensive care unit (ICU). The protocol, with a detailed description of the study population, the interventions and follow–up plan of PEGASUS was published and registered on clinicaltrial.gov (ID: NCT05492344) prior to initiation of recruitment. The protocol was approved by the institutional ethics committee of the Amsterdam University Medical Center (ref: 2022.0148 - NL79110.018.21) and by the ethics committees of participating centers. A data safety monitoring board (DSMB) was installed before recruitment (1). Data management Plan, Trial Master File, and standard operating procedures are stored on the servers of the Amsterdam UMC.

Screening and eligibility criteria

Patients in participating ICUs were screened daily for eligibility. Patients were eligible if they met the Berlin criteria for ARDS (2) and were receiving mechanical ventilation. For eligible patients with exclusion criteria, the date and reason for exclusion were recorded. All exclusion criteria are detailed in the published protocol (1).

Randomization and blinding

Eligible patients are randomly allocated in a 1:1 ratio to the 'personalized' or the 'standard of care' strategy. The allocation sequence is computer–generated by an independent investigator using permuted blocks of different block sizes, with a maximum block size of eight and stratified per center. Randomization is performed by local investigators patient–by–patient employing a dedicated, password protected, SSL–encrypted website. Due to the nature of the intervention tested, blinding is not possible.

Outcomes

Primary outcome

The primary outcome is all cause mortality at day 90 after randomization in line with EMA recommendations for clinical investigations in ARDS (3).

Secondary outcomes

Definitions are described in **Table 1**:

- Ventilator free days at day 28 (VFD28) in days;
- Duration of ventilation in survivors in days;
- All-cause mortality at day 28;
- Absolute number of days on the ventilator until day 90 in days;
- ICU length of stay in days;
- Hospital length of stay in days;
- All-cause ICU mortality;
- All-cause hospital mortality;
- Change in clinical frailty score (4) between pre-admission and day 90;
- Incidence of pneumothorax;
- Incidence of ventilator–associated pneumonia (VAP);
- Need for rescue strategies (extracorporeal membrane oxygenation (ECMO), continues neuromuscular blockage, pressure release ventilation, dialysis, Inhaled vasodilators)

Cleaning and closing of the database

The database will be locked as soon as all data are entered and all discrepant or missing data are resolved, after all efforts are employed to complete the database, and we consider that the remaining issues cannot be fixed. At this step, the data will be reviewed before database locking. After that, the study database will be locked and exported for the statistical analysis. At this stage, permission for access to the database will be removed for all investigators, and the database is locked and archived.

Missing data

No or minimal losses to follow–up for the primary outcome is anticipated. If missing data is found for the primary outcome, we will perform a complete cases analysis. For all other data points, missing data will be imputed using multivariate imputation (MICE).

Sample size

A sample of 538 patients (269 per group) is needed to detect an absolute between-group difference in 90-day mortality of 10% in favor of the intervention group, assuming a 27% mortality in the control group (5), with a power of 80% at a two-tailed significance level of 0.047. In the sample size calculation, a preplanned interim analysis of the primary endpoint has been taken in account when 269 patients have completed the study (p-value threshold of 0.003).

Table 1 – Definitions

Ventilator–free days at day 28	Start time: day of randomization (the same as the day of intubation due to the strict time for inclusion). Timeframe: 28 days. Successful extubation: > 24 hours without reintubation in a 28–day survivor. Interval reintubation: counted from the day of the last successful extubation if there were repeated intubation episodes in the first 28 days. Non–invasive ventilation: not counted. Tracheostomy: same as above (> 24 hours off positive pressure ventilation). 28–day non–survivors: -1 ventilator–free days even if extubated in the period. Death after 28 days: censored and considered the duration of ventilation only.
Duration of ventilation in survivors	Duration, in days, between intubation and successfully extubation, defined as a patient breathing without invasive assistance of the mechanical ventilator for at least 24 consecutive hours. All relevant data will be taken into account and collected, including all additional periods of ventilation during the first 28 days. Only patients surviving the first 28 days will be considered.
28–day all-cause mortality	Any death occurring during the first 28 days after randomization.
Absolute number of days on the ventilator until day 90	Every day that a patient is connected to the ventilator is counted as a ventilator day.
ICU length of stay	The exact numbers of days spent on the ICU from randomization until day 90.
Hospital length of stay	Number of days from randomization till hospital discharge.
ICU mortality	Any death occurring during ICU stay.
Hospital mortality	Any death occurring during hospital stay.
Incidence of pneumothorax	Air in the pleural space with no vascular bed surrounding the visceral pleura on chest radiograph or other kind of imaging suitable for diagnosis pneumothorax.
Incidence of VAP	Clinical Pulmonary Infection Score (CPIS, table 2) > 5 with an infiltration on CXR.
Need for rescue therapies	ECMO, continues neuromuscular blockage, Pressure release ventilation, Dialysis, Inhaled vasodilators
Pulmonary ARDS	ARDS caused by; Pneumonia, Aspiration of gastric contents, Pulmonary contusion, Inhalation injury, Pulmonary vasculitis, Drowning.
Non-Pulmonary ARDS	ARDS caused by; Non-pulmonary sepsis, Major trauma, Pancreatitis, Severe burns, Non-cardiogenic shock, Drug overdose, TRALI.

ARDS: acute respiratory distress syndrome; VAP: ventilator–associated pneumonia; ICU intensive care unit

[A] ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA 2012;307:2526-33.

Table 2. Clinical Pulmonary Infection Score	Points
Body temperature	
≥ 36.5 or ≤ 38.4	0
≥ 38.5 or ≤ 38.9	1
≥ 39 or ≤ 36.4	2
Leucocyte count	
≥ 4.0 or ≤ 11.0 $\cdot 10^9 \cdot L^{-1}$	0
< 4.0 or > 11.0 $\cdot 10^9 \cdot L^{-1}$	1
Rod form ≥ % 50	Add 1 point
Tracheal secretion	
Absence of tracheal secretion	0
Presence of tracheal secretion	1
Abundant purulent secretion	2
Oxygenation	
$Pa0_2/Fi0_2$, mmHg > 240 or ARDS present	0
Pa02/Fi02, mmHg ≤ 240 or no ARDS	2
Pulmonary infiltration in chest X-ray	
No infiltration	0
Diffuse infiltration	1
Localized infiltration	2
Progression in pulmonary infiltration	
Radiographic progression (-)	0
Radiographic progression* (+) *After exclusion of Heart failure and ARDS	2
Pathogenic bacteria in tracheal aspirate culture	
No or few pathogenic bacteria	0
Moderate or high levels of pathogenic bacteria	1
Pathogenic bacteria to be seen in Gram staining	Add 1 point

Statistical analyses

Interim analysis (n=269)

An interim analysis will be conducted 90 days after half of the intended number of patients (n = 269) have been enrolled. The primary endpoint will be analyzed as described below on an intention-to-treat basis using a logistic mixed-effects regression model to estimate the treatment effect on 90-day mortality,with a prespecified *p*-value threshold of 0.003 based on the Lan-DeMets O'Brien-Fleming alpha spending function and with the assumption that the trial stops if a bound is crossed. The interim analysis will be adjusted for additional co-variates.

A logistic mixed-effects regression model will be used to estimate the odds ratio for 90-day mortality, with mortality as the dependent variable and treatment allocation as the main independent variable. Study center, used as a stratification factor, will be incorporated as a random effect. A two-sided significance test will be applied. The results of this analysis will lead to two possible actions:

1) If a mortality effect with $p \leq 0.003$ is observed in favor of either of the treatment arms, the study will be stopped immediately according to the predefined stopping rules outlined in the initial protocol.
2) If $p > 0.003$, the study will proceed without adjustments.

Analysis after completion (n=538)

The database will be closed when the 90-day follow up of the last participant is completed. The main paper will include all follow up data. All statistical analyses will be conducted on an intention–to–treat basis, with patients analysed according to their assigned treatment arms, unless informed consent was withdrawn. The primary endpoint will be analysed as described below, with a significance level of 0.047 and a reported 95% confidence interval. All other analyses will be performed using a two–sided superiority hypothesis test with a significance level of 0.05, and presented with two–sided 95% confidence intervals. In addition to the unadjusted *p* values for secondary outcomes, a Holm–Bonferroni procedure will be applied to control for multiple testing. Analyses will be performed using the software R (R Core Team, 2016, Vienna, Austria).

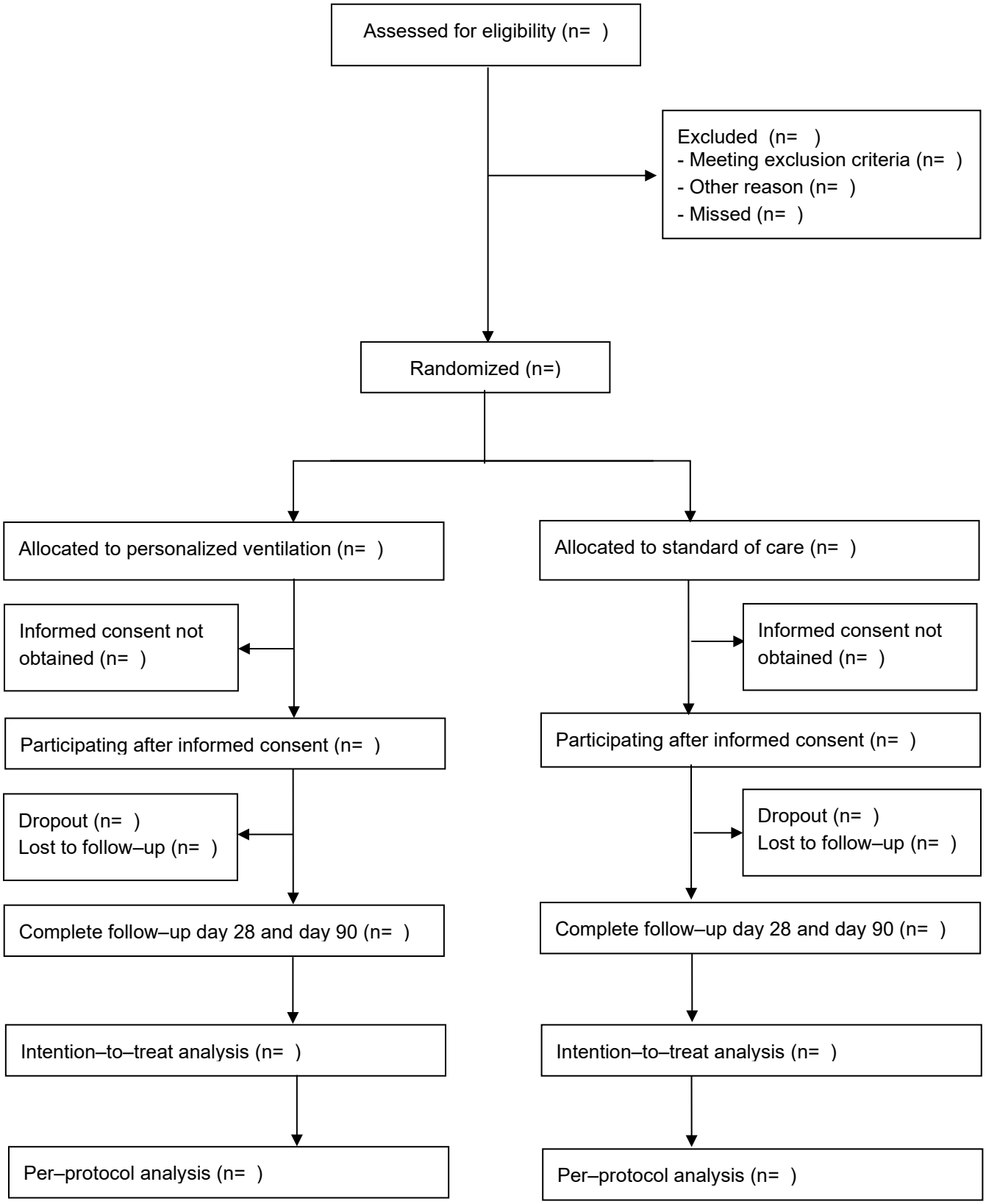
Trial profile

The timeline for a study participant is shown in **Figure 1.** Patient flow will be represented in a CONSORT flowchart (**Figure 2).**

FIGURE 1	STUDY PERIOD				
	Enrolment	Randomization	Post-allocation		Close-out
TIMEPOINT	$-t_1$	0	*72 hours*	*Extubation / death*	*Day 90*
ENROLMENT:					
Eligibility screen	X				
Lung ultrasound exam [a]	X				
Informed/ deferred consent [b]		←	→		
Randomization		X			
INTERVENTIONS:					
Personalized ventilation		←	—	→	
Standard care		←	—	→	
ASSESSMENTS:					
Demographic and baseline characteristics		X			
Ventilation data [c]		X			
SOFA score [d]		X			
Complications and rescue therapies [e]		X	X	X	X
Life status, location patient and pulmonary support [f]		X	X	X	X
Life status, duration of ventilation, ICU and hospital admission					X

Figure 1. Timeline for a study participant from enrollment until last day of follow-up. [a] If a patient has "focal" ARDS and is randomized to personalized ventilation, a LUS will be repeated every 72 hours. [b] Informed consent is obtained before randomization or deferred consent is obtained within 72 hours after randomization depending on the local regulations. [c] Until day 7 or until extubation. [d] Until day 7 or ICU discharge. [e] Until day 90 or ICU discharge. [f] Until day 90 or hospital discharge. SOFA; Sequential Organ Failure Assessment, ICU; intensive care unit.

PROPOSED FIGURE 2



Baseline characteristics

A description of the baseline characteristics of the trial participants will be presented by treatment group (**Table 3**). Discrete variables will be summarized as numbers (%). Percentages will be calculated according to the number of trial participants for whom data are available. Where values are missing, the denominator will be stated in the table and no assumptions or imputations will be made. Continuous variables will be summarized by either means and standard deviations (SD) or medians and interquartile ranges (IQR), according to the observed distribution of the variable.

The ventilation parameters

Daily ventilation variables and clinical parameters (RASS score, SOFA score, fluid balance) will be reported for the first 7 days. Differences in between the ventialtion strategies from day 0 until day 7 will be assessed using trends, medians, and linear mixed models.

Protocol adherence

For each patient, protocol adherence is evaluated from the day of randomisation until 7 days after randomisation based on the criteria listed in **Table 4**. For the ventilation parameters PEEP, tidal volume, prone positioning, and recruitment maneuvers, adherence is assessed according to the ventilator lung morphology and randomization arm. Patients who are fully adherent to the protocol are assigned a score of 0, while non-adherence is assigned a score of 2 per ventilation parameter. Under specific circumstances outlined in the safety protocol, deviations are permitted and are still considered protocol-adherent but are assigned with a score of 1. Overall protocol adherence per patient is calculated as the average of all available observations for that patient. Perfect protocol adherence corresponds to a score of 0, while complete non-adherence corresponds to a score of 8. Scores of 2 or lower indicate good protocol adherence, scores between 2 and 4 indicate acceptable protocol adherence, and scores above 4 indicate no protocol adherence. In cases of ECMO treatment or when extubation is expected, the corresponding day of ventilation is not included in the assessment because different targets apply to these patients. If data are missing, the patient will be classified as having a protocol deviation. Protocol adherence will be presented using heatmaps and boxplots.

Table 3 – Baseline characteristics of the patients

	Personalized ventilation (*n* =)	Standard of Care (*n* =)
Age, years		
Female sex		
BMI, kg/m^2		
Prognostic score		
APACHE IV		
SAPS II score		
Charlson comorbidity index		
SOFA score		
Clinical Frailty Scale		
Reason of ICU admission		
Sepsis		
Hemorrhagic shock		
Coma		
Acute respiratory failure		
Acute metabolic disorders		
Elective surgery		
Urgent surgery		
Reason of ARDS		
Pneumonia		
Non-pulmonary sepsis		
Aspiration of gastric consent		
Major trauma		
Pulmonary contusion		
Pancreatitis		
Inhalation injury		
Severe burns		
Non-cardiogenic shock		
Drug overdose		
TRALI		
Pulmonary vasculitis		
Drowning		
Hours of ARDS before randomization		
Ventilatory variables at lung ultrasound		
Controlled ventilation		
Tidal volume, mL/kg PBW		
Plateau pressure, cmH_2O		
Maximum airway pressure, cmH_2O		
Respiratory rate, bpm		
PEEP, cmH_2O		
Driving pressure, cmH_2O		
etCO_2, mmHg		
FiO_2, %		
SpO_2, %		
Arterial blood gas at lung ultrasound		
Arterial pH		
$PaCO_2$, mmHg		
PaO_2, mmHg		
Lactate		
Bicarbonate		
Arterial saturation		
PaO_2 / FiO_2, mmHg		

BMI: body mass index; SAPS: Simplified Acute Physiology Score; SOFA: Sequential Organ Failure Assessment; ICU: Intensive Care Unit; ARDS: Acute Respiratory Distress Syndrome; PBW: predicted body weight; bpm: breaths per minute; PEEP: positive end-expiratory pressure

Table 4. Protocol adherence definitions

	PEEP	Tidal Volume*	Proning	Recruitment
Standard of Care				
Full adherence	The protocolized PEEP/FiO_2 table is followed during increment of ventilation parameters, a 10% FiO_2 deviation is allowed.	4-8 ml/kg PBW.	Proning is applied; No proning is applied with PaO_2/FiO_2 ≥150 mmHg.	No recruitment manoeuvres.
Safety protocol	Any PEEP if the pH is below 7.3 and tidal volume (<6.5 ml/kg PBW) and respiratory rate (RR ≥30) are optimized, with Pmax >28 cm H_2O.	>8 ml/kg PBW when pH <7.25 and RR >30; <4 ml/kg PBW when pH >7.45 and RR <15.	X	Recruitment manoeuvre performed.
Protocol deviation	>10% FiO_2 deviation from the protocolized PEEP/FiO_2 table within lung protective ventilation range (Pmax <29 cm H_2O).	>8 ml/kg PBW without permissive hypercapnia; <4 ml/kg PBW without hyperventilation.	No proning is applied with PaO_2/FiO_2 <150 mmHg.	X
Focal ARDS, personalized ventilation				
Full adherence	PEEP ≤9 cm H_2O.	6-8 ml/kg PBW.	Proning is applied; No proning is applied with PaO_2/FiO_2 ≥200 mmHg and PEEP ≤5 cm H_2O.	No recruitment manoeuvres.
Safety protocol	PEEP >9 cm H_2O with FiO_2 ≥80% or PaO_2/FiO_2 <100 mmHg.	>8 ml/kg PBW when pH <7.25 and RR >30; <6 ml/kg PBW when pH >7.45 and RR <15.	X	Recruitment manoeuvre performed.
Protocol deviation	PEEP >9 cm H_2O with FiO_2 <80% or PaO_2/FiO_2 ≥100 mmHg.	>8 ml/kg PBW without permissive hypercapnia; <6 ml/kg PBW without hyperventilation.	No proning is applied with PaO_2/FiO_2 <200 mmHg or PEEP >5 cm H_2O.	X
Non-focal ARDS, personalized ventilation				
Full adherence	Mandatory ventilation and PEEP ≥15 cm H_2O; Spontaneous ventilation and PEEP ≥10 cm H_2O; Any PEEP if PaO_2/FiO_2 ≥200 mmHg and FiO_2 ≤40%.	4-6 ml/kg PBW.	No proning is applied; Proning is applied with PaO_2/FiO_2 ≤150 mmHg.	Mandatory ventilation and a recruitment manoeuvre is performed; Spontaneous ventilation and no recruitment manoeuvre is performed.
Safety protocol	Any PEEP if the pH is below 7.3 and tidal volume (<4.5 ml/kg PBW) and respiratory rate (RR ≥30) are optimized, with Pmax >28 cm H_2O.	>6 ml/kg PBW when pH <7.25 and RR >30; <4 ml/kg PBW when pH >7.45 and RR <15.	Proning is applied with PaO_2/FiO_2 >150 mmHg.	Spontaneous ventilation and a recruitment manoeuvre is performed.
Protocol deviation	PEEP <15 cm H_2O within lung protective ventilation range (Pmax <29 cm H_2O).	>6 ml/kg PBW without permissive hypercapnia; <4 ml/kg PBW without hyperventilation.	X	Mandatory ventilation and no recruitment manoeuvre is performed.

**If patient is spontaneous ventilated, there is always full protocol adherence.*
PBW: predicted body weight; RR: Respiratory Rate expressed in breaths per minute; PEEP: positive end-expiratory pressure.

Primary outcome

We will fit a logistic mixed-effects regression model to estimate the treatment effect on 90-day mortality, with the outcome as dependent variable and treatment allocation as the main independent variable. Age, clinical frailty score, and PaO_2/FiO_2 at inclusion enter as fixed covariates (linear terms, pre-specified). Study center will be included as a random (intercept) effect because it is used as a stratification variable (6).

The primary contrast is the adjusted odds ratio (OR) for treatment, with 95% confidence interval and Wald p-value. This estimand corresponds to the within-center, covariate-adjusted effect on death at 90 days, under a treatment-policy strategy (i.e. intention-to-treat paradigm) for intercurrent events. It reflects how treatment changes the odds of death for patients of the same age, frailty, and baseline PaO_2/FiO_2 within a typical center.

To aid interpretation and comparability with other trials, we will also report marginal treatment effects obtained by standardization of model predictions over the empirical distribution of covariates and centers. These will include the risk difference (absolute difference in 90-day mortality) and the risk ratio (relative risk of death) between intervention groups, each with 95% confidence intervals. These estimands correspond to the population-average treatment effects on the absolute and relative scales. Specifically, they represent the expected change in 90-day mortality if the entire trial population were assigned to the treatment versus the control group, while maintaining the observed distribution of baseline risk factors (age, frailty, and oxygenation).

<u>Sensitivity analyses</u>

As mortality is inherently a time-to-event endpoint, we will conduct supportive analyses using survival methods. Kaplan–Meier curves will be used to visualize cumulative mortality up to day 90 by treatment group. Treatment effects will be quantified with a Cox proportional hazards model including a shared frailty term for center, thus accounting for clustering by site and adjusted for the same co-variates listed above. Hazard ratios with 95% confidence intervals will be reported, where a hazard ratio < 1 indicates lower hazard of death in the treatment arm. The proportional hazards assumption will be assessed by Schoenfeld residuals; if violated, time-varying effects will be explored.

Secondary outcomes

The distribution of VFD28 is expected to be characterized by two point masses (–1 and 0) and a continuous, bounded component on (0,28). To account for this structure, we will use a two-point-inflated, transformed Beta regression joint model. This involves a two-step procedure: first we model outcomes using a multinomial logit model to estimate the probabilities of: Death (–1), Alive, ventilated 28 days (0), Alive, ≥1 ventilator-free day (>0). In the second step, for patients with >0 VFD, outcomes will be transformed to the (0,1) interval by $y*$=VFD/28. These will be modelled using a Beta regression with a logit link for the mean parameter μ and log link for the precision parameter ϕ. Treatment assignment and covariates will be included.

This model will provide the following estimands.

Multinomial logit model:

- Odds ratio for death vs alive with VFD >0.
- Odds ratio for prolonged ventilation (0 vs >0 VFD) for the odds of higher VFD versus lower VFD under treatment. This quantifies the treatment effect on inability to wean within 28 days, while accounting for death as a competing category in the multinomial model.

Beta regression

- Effect on μ quantifies the shift in ventilator-free days among patients with partial liberation.
- Secondary derived quantities (probability alive and ventilator-free by day 28, median VFD among survivors) will be estimated from the joint model with uncertainty expressed via confidence or credible intervals.

Time to extubation (competing risk analysis):

To evaluate treatment effects on liberation from invasive ventilation, we will fit a competing risks proportional hazards model, treating extubation as the event of interest and death as a competing event. Cumulative incidence functions will be plotted by treatment group to visualize the probability of extubation over time while properly accounting for the competing risk of death. Hazard ratios with 95% confidence intervals will be reported, where a hazard ratio > 1 indicates a higher rate of extubation under treatment.

Ordinal analysis of ventilator-free days:

In parallel, we will model the ordinal distribution of ventilator-free days (VFDs) using a mixed-effects cumulative logistic regression, including a random intercept for center to account for within-center clustering. This approach respects the ordered nature of the VFD outcome (–1 =

death, 0 = ventilated all 28 days, 1–28 = ventilator-free days). A positive treatment coefficient indicates benefit, i.e. a shift toward more ventilator-free days. The associated score test statistic approximates the Wilcoxon rank-sum test, a widely accepted nonparametric method for this outcome.

Presentation of results:

For transparency and interpretability, we will also present summary statistics (means ± standard deviations of VFDs) and visualize the full outcome distribution with a cumulative incidence plot showing the proportion of patients in each outcome category, with non-survivors explicitly coded as –1.

Estimand interpretation: Both models target the treatment effect on time to liberation from ventilation within 28 days, while acknowledging death as a competing event. The competing risks model yields a cause-specific hazard ratio for extubation, and the cumulative logistic model yields a shift in the ordinal distribution of VFDs, each under a treatment-policy strategy for intercurrent events.

Duration of ventilation in survivors / ICU LOS / hospital LOS:

Duration of ventilation in survivors is analyzed separately to estimate treatment effects on ventilation duration, complementing the composite VFD outcome. Continuous outcomes such as duration of ventilation in survivors, ICU length of stay, and hospital length of stay will be analyzed using mixed-effect median regression models, with centers included as a clustering effect to estimate median differences and the same prespecified covariates as the primary analysis (age, Clinical Frailty Score, baseline PaO_2/FiO_2). The primary estimand is the covariate-adjusted median difference between treatment groups (conditional on center). 95% confidence intervals will be obtained by bootstrap clustered at center if analytical standard errors are unavailable. As sensitivity analyses, we will log-transform the outcomes and fit linear mixed models to obtain geometric mean ratios, and repeat analyses in survivors censored at a prespecified administrative cutoff to assess influence of extreme values.

Incidence of pneumothorax, VAP, need for rescue therapies:

These binary events will be analysed with with mixed-effects logistic regression including a random intercept for center and adjustment for the prespecified covariates. We will report adjusted odds ratios with 95% CIs and Wald p-values, and complement these with model-standardized absolute risks and risk differences (with 95% CIs) for clinical interpretability. For

very rare events we will report exact or penalised (Firth) logistic regression results as sensitivity analyses and provide cluster-robust standard errors if model convergence is problematic.

ICU mortality / Hospital mortality / All-cause 28-day mortality:

These binary mortality endpoints will be analysed analogously to the primary outcome using mixed-effects logistic regression (random intercept for center, same covariates) to produce within-center adjusted ORs with 95% CIs and Wald p-values. Marginal risk differences and risk ratios will be obtained by standardization over the empirical covariate/center distribution. Sensitivity analyses will include time-to-event methods (Kaplan–Meier and Cox models with shared frailty for center) to account for censoring and to check consistency with the logistic estimates. Proportional hazards will be assessed and time-varying effects explored if needed.

Change in clinical frailty score between pre-admission and day 90 in survivors:

The change in frailty is ordinal and will be analysed using a mixed-effects proportional odds (cumulative logit) model with a random intercept for center and adjustment for baseline frailty, age and PaO_2/FiO_2. The primary estimand is the common odds ratio (shift toward lower frailty) with 95% CI. We will check the proportional odds assumption (graphically and with formal tests) and, if violated, report a partial proportional odds model or present category-specific ORs. For clinical clarity we will also report the marginal probabilities of clinically relevant change categories (improved/same/worse) standardized to the trial population.

Per–protocol analysis

The per-protocol population will include all patients who demonstrated acceptable or good protocol adherence. Two per-protocol populations will be defined: one group with strict adherence with average protocol adherence ≤ 2, and one with the broader population with average adherence ≤ 4. All primary and secondary outcomes will be analyzed in these two per-protocol populations using the same models as in the intention-to-treat analysis (mixed-effects logistic regression for mortality, two-part Beta regression for VFD28, mixed-effects median regression for continuous outcomes, and mixed-effects logistic regression for binary outcomes).

The per-protocol analysis will provide a sensitivity check to assess the robustness of the treatment effect under conditions of adequate adherence to the assigned intervention, helping to distinguish between the effect of the treatment itself and deviations from the protocol. Results will be reported as effect estimates (ORs, median differences, hazard ratios) with 95% CIs and compared with the intention-to-treat results to identify potential differences due to adherence.

Phenotype-informed analyses

Phenotype stratification

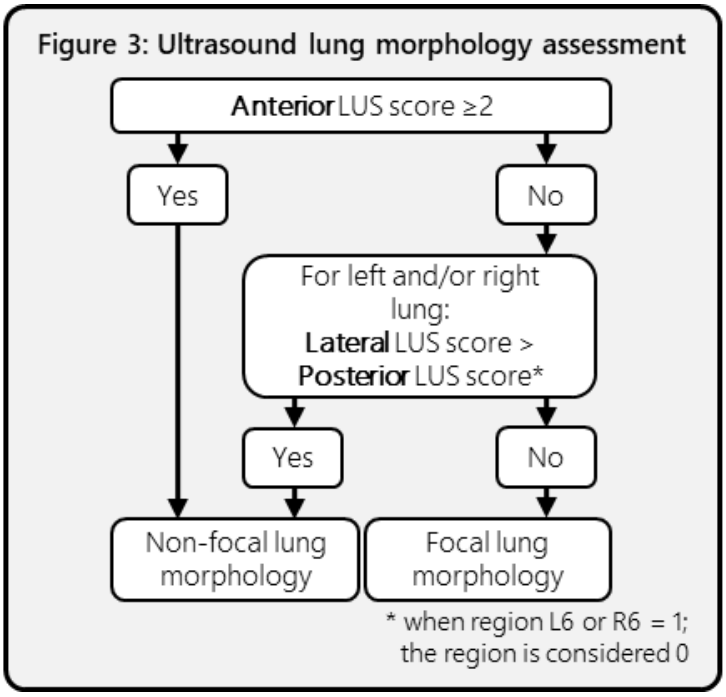
The PEGASUS study evaluates two distinct interventions, depending on the patient's lung morphology (**Figure 3**). To assess whether the intervention is effective within either phenotype, we will evaluate the primary estimand, as outlined above, in two pre-defined strata:

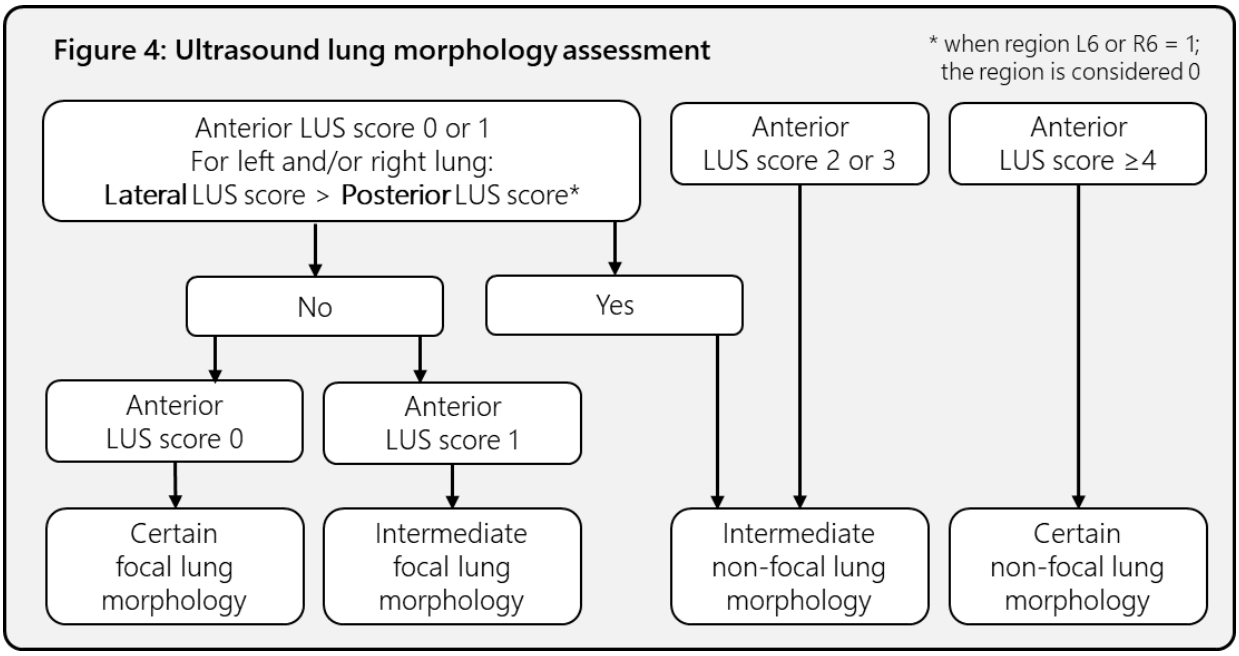
- Focal ARDS ventilated with personalized ventilation vs. Focal ARDS ventilated according to the standard of care;
- Non-focal ARDS ventilated with personalized ventilation vs. Non-focal ARDS ventilated according to the standard of care.

Misclassification

Although multiple measures were taken to prevent misclassification, we know there is uncertainty about phenotype allocation in a subset of patients, as outlined in **Figure 4**. To assess whether the intervention is effective in patients with more certainty of phenotype allocation, we will evaluate the primary estimand, as outlined above, in two pre-defined strata:

- Certain phenotype allocation defined by ventral LUS score of 0 (focal) or equal or more than 3 (non-focal).
- Uncertain phenotype allocation defined by a ventral LUS score between 1 and 3.





Subgroup analysis

Potential heterogeneity of treatment effect for the primary outcome across subgroups will be examined via a test for treatment–by–subgroup interaction in the cumulative logistic model, irrespective of whether there is evidence of a treatment effect. Results will be summarized by subgroup and presented as common odds ratio with two–sided 95% confidence intervals. Lack of a significant interaction will imply that the results are consistent across subgroups and that the overall effect estimated are the most appropriate estimates of treatment effect within each subgroup. The results will be presented in a forest plot with a solid line of reference in the number 1 and a dashed line of reference in the overall effect. The following subgroups will be assessed:

- Pulmonary vs. non-pulmonary ARDS;
- Moderate vs. severe ARDS;
- Restrictive vs. Efficient ARDS (7)

Planned secondary analyses

After publication of the main study, **a Bayesian analysis** of the primairy endpoint will be performed. This analysis will make use of informative priors, designed to represent the position of a reasonable adversary, which is a well-informed skeptic of a large treatment effect. This is motivated by concerns raised from prior studies (e.g., LIVE trial) about potential overestimation of treatment effects, heterogeneity across ARDS trials, and the risk of early stopping inflating observed benefit. The analysis will be based on a random-effects logistic regression meta-model.

The prior for pooled treatment effect will be based on the empirical ("realistic") treatment effect distribution estimated in the ARDS domain of an ongoing study (8). If unavailable at the time of analysis, we will default to a normally distributed prior for the log-odds ratio with mean 0 (OR = 1, no effect) and SD = 0.2. This reflects equipoise, while constraining the probability of very large benefits or harms. On the OR scale, the 95% prior predictive interval is 0.68–1.48, implying <2.5% prior probability of a true OR below 0.68 or above 1.47.

The prior for between-study heterogeneity will be defined as log-normal with location -3.50 and scale 1.26, based on the predictive distribution of meta-analytical heterogeneity for mortality outcomes among trials testing nonpharmacological interventions (9). This entails an expected heterogeneity (tau) of 0.03 but allows for smaller or larger heterogeneity if supported by the data (95% predictive interval: 0.003–0.36).

This approach ensures that evidence from PEGASUS is interpreted within a conservative framework that accounts for expected heterogeneity and the limited reproducibility of large effects in ARDS trials.

Statistical analysis Plan Checklist

Section/Item	Index	Description	Reported on page #
Section 1: Administrative information			
Trial and Trial registration	1a	Descriptive title that matches the protocol, with SAP either as a forerunner or subtitle, and trial acronym (if applicable)	1
	1b	Trial registration number	1
SAP Version	2	SAP version number with dates	1
Protocol Version	3	Reference to version of protocol being used	1
SAP revisions	4a	SAP revision history	NA
	4b	Justification for each SAP revision	NA
	4c	Timing of SAP revisions in relation to interim analyses, etc.	NA
Roles and responsibility	5	Names, affiliations, and roles of SAP contributors	1
Signatures of:	6a	Person writing the SAP	2
	6b	Senior statistician responsible	2
	6c	Chief investigator/clinical lead	2
Section 2: Introduction			
Background and rationale	7	Synopsis of trial background and rationale including a brief description of research question and brief justification for undertaking the trial	3
Objectives	8	Description of specific objectives or hypotheses	3
Section 3: Study Methods			
Trial design	9	Brief description of trial design including type of trial (e.g., parallel group, multi-arm, crossover, factorial) and allocation ratio and may include brief description of interventions	4
Randomization	10	Randomization details, e.g., whether any minimization or stratification occurred (including stratifying factors used or the location of that information if it is not held within the SAP)	5
Sample size	11	Full sample size calculation or reference to sample size calculation in protocol (instead of replication in SAP)	6
Framework	12	Superiority, equivalence, or noninferiority hypothesis testing framework, including which comparisons will be presented on this basis	5
Statistical interim analysis and stopping guidance	13a	Information on interim analyses specifying what interim analyses will be carried out and listing of time points	9

	13b	Any planned adjustment of the significance level due to interim analysis	6
	13c	Details of guidelines for stopping the trial early	9
Timing of final analysis	14	Timing of final analysis, e.g., all outcomes analysed collectively or timing stratified by planned length of follow-up	9
Timing of outcome assessments	15	Time points at which the outcomes are measured including visit “windows”	10
Section 4: Statistical Principals			
Confidence intervals and *P* values	16	Level of statistical significance	9
	17	Description and rationale for any adjustment for multiplicity and, if so, detailing how the type 1 error is to be controlled	9
	18	Confidence intervals to be reported	9
Adherence and Protocol deviations	19a	Definition of adherence to the intervention and how this is assessed including extent of exposure	12, 14
	19b	Description of how adherence to the intervention will be presented	12
	19c	Definition of protocol deviations for the trial	14
	19d	Description of which protocol deviations will be summarized	14
Analysis populations	20	Definition of analysis populations, e.g., intention to treat, per protocol, complete case, safety	9, 19
Section 5: Trial Population			
Screening data	21	Reporting of screening data (if collected) to describe representativeness of trial sample	5
Eligibility	22	Summary of eligibility criteria	5
Recruitment	23	Information to be included in the CONSORT flow diagram	11
Withdrawal/ Follow-up	24a	Level of withdrawal, e.g., from intervention and/or from follow-up	11
	24b	Timing of withdrawal/lost to follow-up data	11
	24c	Reasons and details of how withdrawal/lost to follow-up data will be presented	11
Baseline patient characteristics	25a	List of baseline characteristics to be summarized	13
	25b	Details of how baseline characteristics will be descriptively summarized	13
Section 6: Analysis			
Outcome definitions		List and describe each primary and secondary outcome including details of:	
	26a	Specification of outcomes and timings. If applicable include the order of importance of primary or key secondary end points (e.g., order in which they will be tested)	5, 6, 15-18

	26b	Specific measurement and units (e.g., glucose control, hbA1c [mmol/mol or %])	6
	26c	Any calculation or transformation used to derive the outcome (e.g., change from baseline, QoL score, Time to event, logarithm, etc.)	18
Analysis methods	27a	What analysis method will be used and how the treatment effects will be presented	15-18
	27b	Any adjustment for covariates	15, 17, 18
	27c	Methods used for assumptions to be checked for statistical methods	15-18
	27d	Details of alternative methods to be used if distributional assumptions do not hold, e.g., normality, proportional hazards, etc.	15-18
	27e	Any planned sensitivity analyses for each outcome where applicable	15, 19, 20
	27f	Any planned subgroup analyses for each outcome including how subgroups are defined	21
Missing data	28	Reporting and assumptions/statistical methods to handle missing data (e.g., multiple imputation)	6
Additional analyses	29	Details of any additional statistical analyses required, e.g., complier-average causal effect analysis	22
Harms	30	Sufficient detail on summarizing safety data, e.g., information on severity, expectedness, and causality; details of how adverse events are coded or categorized; how adverse event data will be analysed, i.e., grade 3/4 only, incidence case analysis, intervention emergent analysis	17
Statistical software	31	Details of statistical packages to be used to carry out analyses	9
References	32a	References to be provided for nonstandard statistical methods	NA
	32b	Reference to Data Management Plan	5
	32c	Reference to the Trial Master File and Statistical Master File	5
	32d	Reference to other standard operating procedures or documents to be adhered to	5
Statistical analysis Plan Checklist v 1.0 2019 (10)			

References

1. Sinnige JS, Smit MR, Ghose A, de Grooth H-J, Itenov TS, Ischaki E, et al. Personalized mechanical ventilation guided by ultrasound in patients with acute respiratory distress syndrome (PEGASUS): study protocol for an international randomized clinical trial. Trials. 2024;25(1):308.
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6. (EMA) EMA. Guideline on adjustment for baseline covariates in clinical trials. 2015.
7. Meza-Fuentes G, Delgado I, Barbé M, Sánchez-Barraza I, Retamal MA, López R. Machine learning-based identification of efficient and restrictive physiological subphenotypes in acute respiratory distress syndrome. Intensive Care Medicine Experimental. 2025;13(1):29.
8. van den Beuken M, Derde L, Harhay M, De Grooth H-J. Realistic effect sizes in critical care trials. 2025.
9. Turner RM, Jackson D, Wei Y, Thompson SG, Higgins JP. Predictive distributions for between-study heterogeneity and simple methods for their application in Bayesian meta-analysis. Statistics in medicine. 2015;34(6):984–98.
10. Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Doré C, et al. Guidelines for the content of statistical analysis plans in clinical trials. Jama. 2017;318(23):2337–43.