Statistical Analysis Plan for: The RE-ENERGIZE Trial

1 Administrative Information

1.1 SAP summary table

TRIAL FULL TITLE	A RandomizEd trial of ENtERal Glutamine to minimIZE thermal injury
TRIAL REGISTRATION	https://clinicaltrials.gov/ct2/show/NCT00985205
PROTOCOL PUBLICATION	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5965329/
CURRENT PROTOCOL DATE	April 23, 2019 (final protocol modifications)
TRIAL PRINCIPAL INVESTIGATOR	Daren K. Heyland
TRIAL SENIOR STATISTICIAN	Andrew G. Day
TRIAL COORDINATOR	Maureen Dansereau
STATISTICIAN(S) PERFORMING ANALYSIS	Xuran Jiang and Andrew G. Day
SAP AUTHOR(s)	Andrew G. Day, Xuran Jiang, Maureen Dansereau and Daren Heyland
SAP DATE	December 9, 2021
SAP STATUS	Finalized V1
SAP REVISION HISTORY	None yet
STATUS OF TRIAL AT TIME OF SAP FINALIZATION OF V1	Enrollment completed. Blinded data cleaning completed. No by arm outcome results generated yet.

1.2 Signatures

I have read and approve the enclosed SAP dated 2021-12-09 for the RE-ENERGIZE trial.

Senior Statistician & SAP Author

Name: Andrew G. Day

Signature: Andrew G. Day

Date: December 9, 2021

Statistician Performing Analysis (other than senior statistician):

Name: Xuran Jiang

Signature: Xuran Jiang

Date: 09 Dec 2021

Trial Co-ordinator

Name: Maureen Dansereau

Signature:

Date: 09 Dec, 2021

Principal Investigator

Name: Daren K. Heyland

Signature:

Date: Dec 9/2021

1.3 Purpose, usage, and target audience of this document

This document provides a detail description of the analysis plan for the RE-ENERGIZE trial. This document is meant to be used in conjunction with the study protocol. This document does not subsume the protocol, but several elements of the protocol, such as the sample size justification are reproduced herein for completeness. This document has the following purposes:

1. Provides a written agreement between the principal investigator, study co-ordinator, lead study statistician and data analysts regarding exactly what analysis will be performed.
2. Provides a record of the analysis plan specified prior to examining any outcomes by arm.
3. Provides clear specifications for the analyst(s) performing the data filtering/transformation, variable derivations, statistical analyses and report generation.

This document follows the guidance published in JAMA by Gamble et al (2017) and referenced at https://www.equator-network.org/reporting-guidelines/guidelines-for-the-content-of-statistical-analysis-plans-in-clinical-trials/ (1) The SAP checklist is completed in Appendix A.

1.4 SAP contributors and signatories

Andrew Day drafted the SAP, Xuran Jiang contributed details regarding the definition of several outcomes, Maureen Dansereau added details regarding the trial operation and data management, and Daren Heyland helped interpret the protocol and prioritize outcomes, analyses, and validation. All authors as well as Shawna Froese provided critical review and editing to all parts of the SAP. The finalized version of the SAP was approved and signed off by all authors.

Table of contents

2 Introduction to Study

2.1 Background and rationale

Copied from https://clinicaltrials.gov/ct2/show/NCT00985205

Burn injuries represent a public health problem worldwide, ranked fourth in all injuries and are among the leading cause of disability adjusted life years in low and middle-income countries. More than in any other injury, the inflammation and catabolism associated with severe burns can exacerbate nutrient deficiencies, thereby predisposing patients to impaired immune function and increased risk of developing infectious complications, organ dysfunction, and death. Consequently, over the last few decades numerous trials have evaluated the impact of different nutrition/nutrient strategies in severe burns patients. Glutamine is of particular interest in this regard as it appears vital for a number of key stress-response pathways in serious illness. The existing randomized trials of glutamine supplementation in burns patients have suggested a significant reduction in mortality, infection, and hospital length of stay. However, in other critically ill patient populations, there is a signal of increased mortality associated with glutamine administration. Given this conflicting evidence, burn practitioners are either harming or saving lives with glutamine use. We hypothesize that the inexpensive therapeutic strategy tested in this multicenter randomized controlled clinical trial of supplemental enteral glutamine in 1200 severe burn injury patients will lead to lower morbidity and mortality and reduced health care costs in an otherwise very devastating and disabling injury worldwide.

In our pilot study (Critical Care Medicine, 2003, 31:2444) we found a protective effect of glutamine against blood infection in severely burned adult patients. In addition, a significant decrease in mortality was observed with glutamine. These results should be tested with a multi-center trial because our study was small and did not have mortality as an end point.

2.2 Overall aim

The overall aim of the study is to determine the overall efficacy and safety of glutamine in burn patients. The cost-effectiveness of glutamine administration may also be measured if the results show a decrease in length of care or a reduced incidence of acquired bacteremia due to Gram negative organisms with glutamine.

2.3 Study hypotheses

Among adult patients with severe thermal burn injuries, compared to placebo, enteral glutamine administration will:

1. reduce time to live hospital discharge,
2. increase 6-month survival,
3. improve health related quality of life and physical functioning among survivors at 6 months,
4. reduce incidence of acquired bacteremia due to Gram-negative organisms,
5. reduce hospital mortality,
6. reduce time to live ICU discharge,
7. reduce hospital stay among 6-month survivors.

3 Study Methods

3.1 Trial design

A randomized, parallel, two-arm, placebo-controlled, double-blind, multicentre definitive trial of ~1200 randomized patients across 54 sites in United States, Canada, Europe, UK, Paraguay, Brazil, Singapore, Thailand and the Dominican Republic. The study was funded by a grant from the Canadian Institutes of Health Research.

3.2 Modification to trial design from initial protocol

Due to slower than expected accrual, in November 2018 we modified the protocol to swap the primary and secondary outcomes to time to discharge alive as primary and 6-month mortality as secondary and we changed the target sample size from 2,700 patients to 1,200 patients. Because of this change, we also dropped the planned second interim analysis after 1, 350 patents. However, by this point we had already performed our first interim analysis after 600 patients were followed for 6 months.

3.3 Randomization

After informed consent was obtained (within 72 hours of admission to ICU) and baseline data were collected, the local study co-ordinator logged on to the CERU central web-based randomization system to confirm patient eligibility and to obtain a blinded participant number. At that time the unblinded local pharmacist was sent a notification of the new blinded participant number and their treatment assignment so they could prepare and deliver the appropriate blinded study product to the ICU. Randomization was stratified by centre using permuted blocks of random size (randomly selected from block sizes of 2, 4 and 6 but this was not disclosed until after enrollment was complete).

3.4 Sample size considerations

The original sample size justification in the published protocol (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5965329/) called for a total of 2700 patients to provide 80% power at a two-sided alpha=0.05 to detect a 25% relative risk reduction from 15% to 11.25%.

The modified total sample size of 1, 200 patients was justified in our updated protocol as follows:

> We plan to enroll 600 patients per arm. We assessed the power of our primary outcome, time to live hospital discharge within 90 days, using 10, 000 simulations with the same pooled mortality

rate, and daily live discharge rates as our current pooled (unblinded) observed data (n=544), but applying various effect sizes for reduction in mortality and increased daily rate of discharge alive among survivors. Currently 3% of the randomized patients are missing the time to discharge alive (mostly due to consent withdrawals); our simulations assume 5% missing data. Our current 90-day mortality rate is 14%, so we will assume that the 14% is the midpoint of the two arms. Using this approach, we found that if the either arm had a 20% relative reduction in 90-day mortality (from 15.56% to 12.44%) and 20% relative increase in discharge among 90 day survivors, then this study would achieve 82% power at a two-sided alpha=0.05 by the Wilcoxon rank-sum test ranking patients according to their discharge time with decedents ranked highest and patients still in the hospital after 90 days as the next highest. The planned interim analysis will have a trivial (<1%) effect on the power of the study for the primary outcome.

Our secondary outcome is 6-month mortality. Our pooled 6-month mortality rate among the 498 patients currently followed for 6 months is 16%. With 570 patients per arm, allowing for 5% lost to follow-up, this sample size will achieve 84% power to detect a 33.3% relative risk reduction in mortality from 19.2% to 12.8% using a Chi-Squared test (or two independent proportion z-test) at a two-sided alpha=0.05. The power allowing for the interim analysis plan is 82%

With 600 participates per arm the time-to-event competing risk approach by Fine and Gray will achieve 90% power to detect a 25% increase in the rate of live discharge under the assumptions that by day 40 in the control arm half the patients have been discharged alive, 10% have died and 5% have been lost to follow-up.

3.5 Framework

This is a confirmatory (i.e. hypothesis testing) superiority RCT comparing the efficacy and safety of enteral glutamine to placebo in severe burn patients.

3.6 Interim analyses

Although glutamine is recommended by current guidelines and used in about half of all burn cases, some safety concerns had emerged before commencing the definitive trial. Therefore, we planned and executed an interim analysis that tested for excess mortality (secondary outcome) in the glutamine arm after 600 patients were followed for 6 months. This one-sided interim analysis was tested at a nominal one-sided p-value of 0.01. However, the final assessment after all patients completed the 6-month follow-up will be 2-sided. In order to maintain an overall type I error rate of 0.025 in each direction for this secondary outcome, the final analysis will test for higher mortality in the glutamine arm at a nominal p-value of 0.019 while lower mortality in the glutamine arm will be tested at the traditional 0.025. This approach will maintain an overall type I error rate of 0.05 without affecting the power to detect a glutamine benefit. However, the power to detect increased mortality with glutamine will be decreased slightly. We feel this was justified in order to allow the possibility of stopping the study early if there had been a strong signal of increased mortality. Details of and justification for this approach are provided in Appendix 2 of the study protocol. This interim analysis did not alter our type I error rate of the current primary outcome (time to discharge alive).

3.7 Timing of final analysis

All outcomes will be analyzed once all data is collected and cleaned and after finalization of the statistical analysis plan.

3.8 Timing of outcome assessments

Survival and health related quality of life were assessed 6 months after randomization. All other outcomes were assessed while in hospital up to 3 months post randomization except daily nutrition that was assessed for the first 12 days in the ACU.

4 Statistical Principals

4.1 Confidence intervals and P-values

95% confidence will be presented for selected key outcomes. P-values will be two-sided without adjustment for multiplicity. We will use the traditional two-sided $p \leq 0.05$ to indicate statistical significance for the primary outcome. For the secondary outcome, in order to maintain an overall two-sided type I error rate of 0.05 while accounting for they interim analysis, we will test for higher mortality in the glutamine arm at a nominal p-value of 0.019 while lower mortality in the glutamine arm will be tested at the traditional 0.025. We will consider the multiplicity of tests when interpreting the significance of the additional outcomes.

4.2 Adherence and protocol deviations

The following are the key metrics that we reported on throughout the study and will be reported overall (not by site) in the final statistical report:

1. **Intervention**
    - timing from ACU admission to randomization
    - timing from ACU admit to start of intervention
    - time from randomization to start of intervention
    - duration of study intervention
    - proportion of patients that exit the study with < 7 days of study intervention
    - compliance with study intervention (of all doses the patients was to receive, how many did they receive)
    - #protocol violations (<80% dose over 3 day average)
2. **Nutritional adequacy indices**
    - timing of start of EN from ACU admit (days)
    - use of and duration of EN, PN or both
    - In tube feed patients, total nutritional adequacy (calories and protein from all sources including propofol and changes to prescription over time)
    - motility agents in <80% adequacy
    - frequency of use of restricted EN formulas
3. **Compliance with study protocol**
    - missing data for baseline demographics

- missing ACU LOS
- missing hospital LOS
- % missing 6 month mortality data
- % with useable SF36 at 6 months (*Not collected in Pilot*)
- ACU mortality (% patients with this data)
- hospital mortality (% patients with this data)

4.3 Analysis populations

The primary analysis will be a modified intention-to-treat including all patients to the arm they were randomized regardless of study compliance except we will exclude randomized patients who deemed ineligible and did not receive any study treatment. An exploratory per-protocol analysis will be performed on the primary and secondary outcomes by only including patients who had at least 7 days and had a minimum of 90% compliance overall with study IP.

5 Trial population

5.1 Eligibility criteria:

Published at https://clinicaltrials.gov/ct2/show/NCT00985205 .

5.2 Screening, recruitment, patient flow/follow-up

A CONSORT style flow diagram will present the numbers of patients screened and all reasons excluded prior to randomization (2). This figure will also present the number randomized to each arm and then will work down to the numbers included in the assessment of the primary and secondary outcomes with counts of all post-randomization exclusion reasons. In addition, a table will provide the study ID error code (if applicable) and description of the reason for each randomized patient being excluded from the evaluation of the primary or secondary outcome. Finally, variables collected on screened patients will be compared descriptively between screened patients randomized vs. not randomized.

5.3 Baseline characteristics

Baseline characteristics will be described by arm and overall using descriptive statistics only. Categorical variables will be described as counts (%). Continuous variables will be described as mean±SD (min to max) and/or median [Q1 to Q3].

The following baseline patient characteristics will be described:

Age, sex, BMI, ethnicity, APACHE II score, modified SOFA Score, Charlson comorbidity index, tobacco use, burn size (expressed as percent total body surface area %TBSA), type of burn, hours from hospital admission to randomization, hours from ICU admission to randomization, hours from burn injury to randomization, patient co-enrolled in another academic ACU study, high dose vitamin C as part of resuscitation protocol, mechanically ventilated at baseline (i.e. on MV at time of randomization) and geographic region (Canada, USA, South America, Europe and Asia).

6 Analysis

6.1 Outcome definitions

See section 3.2 which describes the modification of the sample size and switching of the primary and secondary outcomes due to slower than expected accrual.

6.1.1 Primary outcome:

Time to discharge alive from hospital [time frame 3 months]

6.1.2 Secondary outcomes:

6-month mortality

6.1.3 Other registered outcomes:

1. Health-Related Quality of Life - in particular the physical function domain of the SF-36, ADL, and IADL questionnaires. [Time frame: 6 months]
2. Incidence of acquired bacteremia due to Gram negative organisms [Time frame: 3 months] Note: all infections were captured during the pilot trial and will be reported on the pilot study patients only.
3. Hospital Mortality [Time frame: 3 months]
4. Duration of Mechanical Ventilation [Time frame: 3 months]
5. ICU Stay [Time frame: 3 months]
6. Hospital Stay [Time frame: 3 months]

6.1.4 Additional unregistered outcomes:

7. Modified Persistent Organ Dysfunction score (PODs-free days and binary outcome at 30 days and 90 days) [Time frame: 3 Months]
8. Heart Rate (daily highest and lowest) Time frame: 3 Months]
9. Renal Replacement Therapy (ever used and duration among users) Time frame: 3 Months]
10. Burn-related Operative procedures (frequency count) Time frame: 3 Months]
11. Nutrition variables (only those mechanically ventilated, report timing, adequacy, composition, see section 4.2) [Time frame: 12 days]
12. Concomitant Medication Use ('ever' use of Beta-Blockers, Oxandrolone, nandrolone, and Testosterone) [Time frame: 3 Months]
13. Key Laboratory Parameters (daily report of Creatinine **µmol/L**, Urea **mmol/L**, T. bilirubin **µmol/L** and glucose **mmol/L**) [Time frame: 3 Months]

6.1.4 Serious adverse events:

All reported by arm and difference in patient level counts tested by Fisher's exact test.

- Number of SAEs reported and patients with an SAE
- Number of possibly, probably or definitely related SAEs reported and patients with these

- Number of SAEs reported and patients with SAE by category

6.1.5 Economic evaluation:

A cost-effectiveness analysis may be conducted if the trial demonstrates improved health outcomes in the glutamine. Dr. Ana Johnson, a health economist at Queen's University, was recruited at study inception to lead this analysis. Details of the pre-planned cost-effectiveness analysis are described in appendix 3 of the trial protocol. If a cost-effectiveness analysis is perused, the full methodology will be detailed in a separate document.

6.2 Analysis Methods

6.2.1 Primary outcome

The primary outcome of this study is time to live discharge from hospital up to 90 days. Death will be considered a competing risk precluding live discharge. Patients remaining in the hospital at 90 days will be censored at that time. We expect minimal loss to follow-up (LTFU) before hospital discharge, but if LTFU does occur prior to 90 days due to hospital transfer, consent withdrawal or other reasons, patients will be censored at the last time known to be in the hospital. Patients who withdrew consent for prospective data collection while in the hospital will be censored at date of consent withdrawal. The cumulative incidence function (CIF) curves will be displayed by arm and the difference in the CIF between arm will be tested by the Gray test as implemented in the SAS LIFETEST procedure (3). Gray's test is essentially a log-rank test where decedents are censored after the end of follow-up (i.e. after 90 days). The median and quartiles of time to live discharge from the CIF will be reported by arm. The between arm difference will be summarized by the unadjusted subdistribution hazard ratio as estimated by the Cox proportional hazards model (4). A sensitivity analysis will use a shared frailty model to incorporate ICU as a random effect (5). This shared frailty analysis will censored decedents at 91 days (after end of follow-up) which will mimic the Fine and Gray approach and provide a subdistribution hazard ratio while controlling for ICU. The subdistribution hazard ratios will be reported with 95% confidence intervals as estimated by PROC PHREG in SAS. We will also use a rank based approach employing the Wilcoxon rank sum-test with survivors ranked in the order of discharge and hospital decedents given the highest (worst) rank. For both the time-to-event and rank based approach we will consider patients who die within 72 hours of hospital discharge as decedents who were not discharged alive.

6.2.2 Secondary outcome

The secondary outcome of this study is 6-month mortality which will be compared between arms using the z-test for two independent proportions. This is equivalent to the Chi-Squared test for symmetric two-sided tests, but allowed us to implement one-sided interim analyses to test for increased mortality in the glutamine arm (see section 4.6). Patients remaining alive after 90 days but subsequently lost to follow-up will be assumed to be 6-month survivors for the binary survival

outcome. Patients lost to follow-up prior to 90 days will be considered missing for the primary outcome. The effect size will be reported as an unadjusted relative risk with 95% confidence intervals. A secondary analysis will employ the generalized mixed effects model with a random ICU effect. This will provide a within site interpretation of effect, will allow us to explore between site heterogeneity, and will meet regulatory guidance suggesting that site be incorporated in a sensitivity analysis if it is not used for the primary analysis. (6-8)

We analyzed 6-month survival as binary rather than a time-to-event because censoring should be trivial and we are interested in longer term survival which would indicate recovery form burn injury, but we are not interested the ordering of early deaths among decedents. However, we will provide Kaplan-Meier curves depicting the survival rate over the first 6 months in each arm and the Kaplan-Meier curves will be used to estimate the survival rate in each arm at 6 months, but again the primary test will be based on the binary survival rate at 6-months rather than the log-rank test which considers the order of deaths.

6.2.3 Other registered outcomes

Duration of mechanical ventilation and ICU Stay will be analyzed using the same approach as time-to-discharge alive where death is considered a competing risk precluding discharge. We will also compare hospital length of stay among people discharged alive from hospital.

Remaining binary outcomes including incidence of acquired bacteremia due to Gram negative organisms and hospital mortality will be described as proportions by arm with effect sizes described as relative risks with 95% CIs and correspond p-values estimated by the Chi-Squared test.

Health-Related Quality of Life outcomes include the 8 domain scores and 2 summary scales of the SF-36, the ADL summary score, and the IADL summary score. Each of these outcomes is defined only for survivors. Thus, we will limit our inference of HRQoL to survivors, so any comparison of the outcomes is conditional on 6-month survival. We will report the mean and standard dilation of all 12 scores within arm. We will report the mean difference between arms with 95% confidence intervals and p-values estimated by the linear mixed effect model with ICU included as a random effect and treatment arm as a fixed dummy variable.

6.2.4 Additional unregistered outcomes

The mean (SD) of modified PODS free days by day 30 and by day 90 will be reported by arm. Also, the count and proportion of patients pods free at day 30 and day 90 will be reported by arm. The PODS free days by day 30 and 90 will be compared between arms by the Wilcoxon Rank-sum test.

We will start counting PODS free days on study day 1. Patients who die by day 30 or 90 will be considered to have 0 PODS free days for the 30 and 90-day timeframe respectively.

The modified PODS will not consider vasopressor therapy because it was not collected beyond baseline. A patient will be considered pods free on a given day if they are not mechanically ventilated or on renal replacement therapy according to the following definitions:

(1) **Mechanical ventilation**: if any part of the calendar day is on or between the start and stop date of any invasive mechanical ventilation period, or the patient restarted invasive mechanical ventilation within 48 hours of the current day, then then the day is not a free day.
(2) **Renal replacement therapy**: if any part of the calendar of the calendar day is on or between the start and stop date of any renal replacement therapy period then the day is not a free day.

Variables collected daily including: the highest and lowest heart rate, daily nutritional adequacy, and key laboratory parameters will be displayed by clustered boxplots depicting the daily distribution of the two arms side by side. These comparisons will be descriptive without formal hypothesis testing.

Nutrition variables will be reported only among the subgroup of patients who were mechanically ventilated for at least 48 hours in the first 12 days. Nutritional adequacy will be calculated daily for days on mechanical ventilation the entire day as the total calories and protein received by all collected sources divided by the corresponding baseline prescriptions multiplied by 100. Days on mechanical ventilation the entire day without any nutrition support received will be counted as 0 adequacy. The boxplots will be depicted by study day from day 1 to day 12 regardless of start of mechanical ventilation. The number of patients contributing to each boxplot will be annotated on the bottom of the figures.

The remaining "additional unregistered outcomes" are binary and will be reported by arm as counts and percentages with corresponding chi-squared tests.

6.3 Adjustment for covariates

For the primary and secondary outcomes, we include a sensitivity analysis that control for ICU as a random effect. Analysis of continuous outcomes will include ICU as a random effect We will also perform a sensitivity analysis of the primary and secondary outcomes adjusting for age, APACHE II score, Baseline SOFA, %TBSA, Charlson comorbidity score, and geographic region in addition to site as a random effect. For the primary time to hospital discharge alive outcome, this will use the shared frailty model describe in section 6.2.1 and for the 6-month mortality approach this will use the generalized mixed effects model with a random ICU effect described in section 6.2.2.

6.4 Assumption checking

The proportional hazards (PH) assumption of the primary outcome (time to live hospital discharge) will be assessed visually based on the roughly parallel CIF curves and log-negative log survival vs. log of time. Violations of the PH assumption do not invalidate the tests, but complicate the interpretation of the hazard ratio. If there is an important violation of the PH proportion hazard assumption then, emphasis

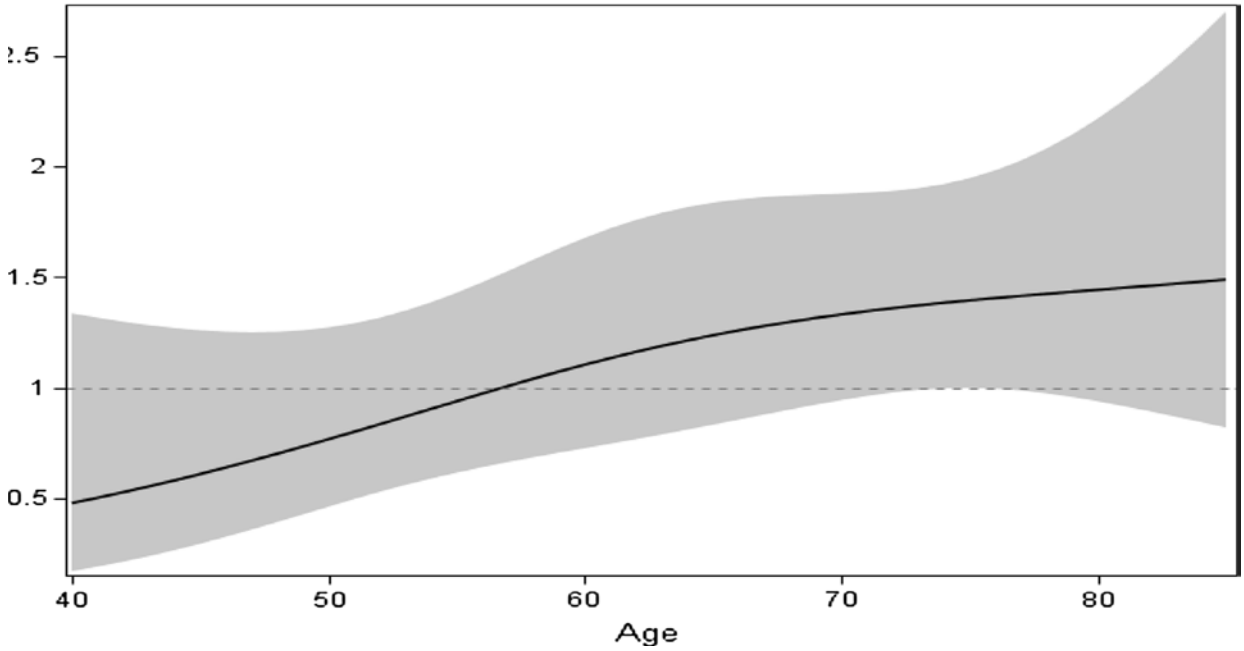
will be placed on the overall CIF curves and the median time to live discharge rather than the subdistribution hazard ratio.

The only assumption for the binary outcomes is that missing data is are missing completely at random (see section 6.6 for assessment of this) and that each observation is independent. We have a planned sensitivity analysis that will control for site as a random effect, and we will examine the primary and secondary outcome by geographical region with a test for effect modification by region by modelling a region by treatment arm interaction effect in the multivariable models.

The HRQoL scores will all be analyzed as if they are approximately normal, but these scores are bounded and thus not susceptible to extreme outliers. The linear mixed effects model is robust to departures from normality, and given the large sample size, p-values should be valid and confidence interval coverage rates nominal regardless of departures from normality. Furthermore, the equality of variance assumption is unimportant since the two treatment groups are approximately equal size and treatment arm will be balanced within each ICU due to pre-stratification. Nevertheless, we will assess influence diagnostics by site to ensure removal of any one site does not meaningfully alter the results.

6.5 Subgroup analyses

We will perform subgroup analysis to assess effect modification for the primary and secondary outcomes. Subgroups will be based on burn severity (%TBSA), age and age+%TBSA. The subgroup analysis will consider TBSA, age and age+%TBSA as continuous treatment effect modifiers. In order to model the treatment effect across the TBSA and age, separate models will be constructed which include an indicator for treatment arm, the subgroup variable as continuous and a term that is the product of the treatment indicator and the continuous subgroup variable. If strong non-linearity is present then a restricted cubic spline with 5 knots will be used to model the continuous subgroup variable. The treatment effect across the range of the subgroup variable will be depicted as shown in the figure below with an overall test of significance for the interaction between the subgroup and treatment group variables presented. For this analysis we will consider a p-value of <=0.1 as suggestive of effect modification if it coincides with a clinically important difference in treatment effect over the range of the subgroup variable. The a priori hypothesis is that older and more severely burned patients will have a greater treatment effect than younger less severely burned patients.



6.6 Missing data

The number of missing (or conversely non-missing) values will be reported by arm for every outcome. For the primary outcome (time to live hospital discharge) we expect minimal loss to follow-up since this outcome is not followed beyond the index hospital admission. The reasons of all missing primary and secondary outcomes will be reported by arm. For the primary outcome we will perform a sensitivity analysis using a graphical pattern mixture tipping point approach demonstrating the treatment effect over the possible range of missing outcomes. (9, 10).

6.7 Additional analysis

The database generated from the RE-ENERGIZE trial will be used for additional secondary analyses exploring questions other than assessing the safety and efficacy of enteral glutamine in severe burn patients. Plans for these additional secondary analyses are to be determined and are not part of the primary RE-ENERGIZE analysis.

6.8 Statistical software

The main analysis was performed using SAS 9.4 TS level 1M2 and SAS/STAT version 15.1 under Windows 7 Professional version 10.0.18362. The independent validation of selected items (see section 8.2) was performed using the same software and operating system except SAS 9.4 was level TS1M6.

7 Quality Assurance

7.1 Data quality

Data was entered into REDCap by trained local site personal. Each user with access to REDCap had a unique username and password. Access to REDCap was secure and an audit trial was maintained to keep track of the username, time, and values of all data entry and modification. A custom secure randomization module was used to implement the randomization list and maintain concealment of future allocations. A custom query module was used to implement extensive value, range, logical (including date sequence) data checks. Any violation of the pre-defined data checks triggered data queries that were tracked and required resolution (either correction or acceptance by central staff) prior to data being marked as finalized.

Key data items from 2 patients at each site were monitored via source verification once they had randomized 2 patients. After the initial 2 patients were monitored, sites were assessed for risk and follow-up monitoring only conducted when needed. The REDCap database was downloaded and converted into a multi-table analytic SAS database. Some filtering, data transformation, and variable derivation was performed in SAS. Boxplots were generated for all continuous variables and outliers were queried; all outliers were either corrected or verified as correct.

Quality assurance reports were run periodically throughout the trial to assess the completes, timeliness, validity and quality of trial implementation and data capture by site. Issues were flagged and resolved with participating sites in real time.

7.2 Validation of SAS database and analysis

The study PI and study co-ordinary will sense check all results to make sure they are not highly suspicions and that all counts are consistent with the patient flow diagram.

A second statistician who did not perform the primary analysis will independently verify the patient flow counts and re-analyze the primary and secondary outcomes of: 1) Time to discharge alive from hospital and 2) six-month survival.

8 References

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9 Appendix A: Statistical Analysis Plan (SAP) Checklist v 1.0 2019

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	1
	4b	Justification for each SAP revision	1
	4c	Timing of SAP revisions in relation to interim analyses, etc.	1
Roles and responsibility	5	Names, affiliations, and roles of SAP contributors	2
Signatures of:	6a	Person writing the SAP	1, 3
	6b	Senior statistician responsible	1
	6c	Chief investigator/clinical lead	1
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	6
Objectives	8	Description of specific objectives or hypotheses	6
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	7
Randomization	10	Randomization details, e.g., whether any minimization or stratification	7

		occurred (including stratifying factors used or the location of that information if it is not held within the SAP)	
Sample size	11	Full sample size calculation or reference to sample size calculation in protocol (instead of replication in SAP)	7
Framework	12	Superiority, equivalence, or noninferiority hypothesis testing framework, including which comparisons will be presented on this basis	8
Statistical interim analysis and stopping guidance	13a	Information on interim analyses specifying what interim analyses will be carried out and listing of time points	8
	13b	Any planned adjustment of the significance level due to interim analysis	8
	13c	Details of guidelines for stopping the trial early	8
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”	9
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	9-10
	19b	Description of how adherence to the intervention will be presented	9-10
	19c	Definition of protocol deviations for the trial	9-10
	19d	Description of which protocol deviations will be summarized	9-10

Analysis populations	20	Definition of analysis populations, e.g., intention to treat, per protocol, complete case, safety	10
Section 5: Trial Population			
Screening data	21	Reporting of screening data (if collected) to describe representativeness of trial sample	10
Eligibility	22	Summary of eligibility criteria	10
Recruitment	23	Information to be included in the CONSORT flow diagram	10
Withdrawal/ Follow-up	24a	Level of withdrawal, e.g., from intervention and/or from follow-up	10
	24b	Timing of withdrawal/lost to follow-up data	10
	24c	Reasons and details of how withdrawal/lost to follow-up data will be presented	10
Baseline patient characteristics	25a	List of baseline characteristics to be summarized	10
	25b	Details of how baseline characteristics will be descriptively summarized	10
Section 6: Analysis			
Outcome definitions		List and describe each primary and secondary outcome including details of:	11
	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)	11
	26b	Specific measurement and units (e.g., glucose control, hbA1c [mmol/mol or %])	11
	26c	Any calculation or transformation used to derive the outcome (e.g., change from baseline, QoL score, Time to event, logarithm, etc.)	12-14
Analysis methods	27a	What analysis method will be used and how the treatment effects will be presented	12-14
	27b	Any adjustment for covariates	14
	27c	Methods used for assumptions to be checked for statistical methods	14-15
	27d	Details of alternative methods to be used if distributional assumptions do not hold, e.g., normality, proportional hazards, etc.	12-14

	27e	Any planned sensitivity analyses for each outcome where applicable	12-14
	27f	Any planned subgroup analyses for each outcome including how subgroups are defined	15
Missing data	28	Reporting and assumptions/statistical methods to handle missing data (e.g., multiple imputation)	16
Additional analyses	29	Details of any additional statistical analyses required, e.g., complier-average causal effect10 analysis	16
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	11
Statistical software	31	Details of statistical packages to be used to carry out analyses	16
References	32a	References to be provided for nonstandard statistical methods	18
	32b	Reference to Data Management Plan	NA
	32c	Reference to the Trial Master File and Statistical Master File	NA
	32d	Reference to other standard operating procedures or documents to be adhered to	NA

Taken from the paper: 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.

Abbreviations: CONSORT, Consolidated Standards of Reporting Trials; hbA1c, haemoglobin A1c; QoL, quality of life; SAP, statistical analysis plan.