B3SHORT STUDY

SHORTER RECOVERY TIME AFTER CRITICAL ILLNESS

Protocol Identification Number: 2017/1334C

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PROTOCOL VERSION NO. 1.46 – 12.06.2023

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Protocol Amendment Summary of Changes Table

DOCUMENT HISTORY	
Document	**Date**
1.34: First complete REK approved protocol.	*Completed April 12th 2019. Approved by REK May 27th 2019.*
1.4:	*Completed November 26th 2019. Approved by REK December 20th 2019*
1.43:	*Completed September 9th 2020. Approved November 19th 2020. The current version.*
1.46	*To be submitted now*

Amendments prior to November 20th 2020

Not recorded systemically

Amendment 1 (November 20th 2020)

Added text to segment regarding liver function under section 4.4 "Exclusion Criteria": "Patients whose liver enzymes are elevated three fold or more above the cut off limit are normally excluded from the study. In incidences where the increased transaminases are not caused by hepatocyte damage, but by other means such as for example decreased clearance, patients may however be evaluated fin inclusion.

Amendments June 2023: (all highlighted in red color or with track changes in the appropriate sections).

1. Amendment 1 of November 20th included in protocol for submission to REK.
2. The time taken to complete all study tests is too large for some of the study participants. Study tests have therefore been listed according to priority. Please see text underneath table 3 in section 6.
3. Echo cardiograph has been removed from the list of study test (table 3, section 6) as it is not possible to carry out with current available study staff.
4. Changes in section 8 (safety) has been made as it was not clear how adverse events were to be reported in previous protocols. Reporting of adverse events is now according to hospital standard. The section has also been made tidier. See red text under section 8 and 9.2
5. The healthy control group: The healthy control group has been removed from the study due to financial and logistical challenges centered on sensitive data from the HUNT study. The placebo group is still very much part of the study. The potential statistical issues are discussed and some secondary endpoints related to epigenetic clocks will be affected by this. See sections 4.1, 4,2 and 10,1.
6. The supplementary group planned to be treated with NR dose 1000 mg x 2 has been cancelled due to time constraints (project slowed by the corona pandemic, start-up inclusion rates were slow, heavier workload per patient than anticipated (section 4.2, figure 7). This group was however not included in the power calculations and its cancellation will not affect the strength of the study.

7. Determination of sample size (10.1): Participants who died prior to reaching the primary endpoint (discharge from hospital) will not be replaced, but included in the calculations (as “infinite” hospitalization).

Signature page

Title Shorter recovery time after critical illness

Protocol ID no: 90652904

I hereby declare that I will conduct the study in compliance with the Protocol, ICH GCP and the applicable regulatory requirements:

Name	Title	Role	Signature	Date
Arne Søraas	MD, PhD	Principal Investigator		
John Arne Dahl	PhD	Research group leader		
Olaug Marie Reiakvam	MD	Investigator		
Anders B Nygaard	Phd	Research data scientist		NY June 2023
Mette Istre	MSc	Research Engineer		

PROTOCOL SYNOPSIS

Protocol title	Shorter recovery time after critical illness
Responsible institution:	Professor og avdelingsleder Fredrik Muller Address: Avd. For mikrobiologi. Rikshospitalet. PB 4950 Nydalen, 0424 Oslo
Study type	Double-blinded, randomized, placebo-controlled phase II trial
Intervention:	Dietary Supplement NR and placebo
Centre:	Oslo University Hospital
Study Period:	First pilot patient enrolled in pilot: 8th of September 2018 Anticipated recruitment period: 2 years Last patient included: October 2021
Treatment Duration:	3 months
Follow-up:	1000 days after end of treatment and until 2030 regarding death and diseases
Objectives	Administration of NR can prevent or shorten fatigue secondary to acute illness. (ICD-10 R53.83) Reduction of fatigue by NR administration will reduce complications, morbidity and mortality, and improve quality of life. Epigenetic biomarkers can predict long- and short-term patient outcome

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List of Abbreviations and Definitions of Terms

Abbreviation or special term	Explanation
AE	Adverse Event
CRF	Case Report Form (electronic/paper)
CSA	Clinical Study Agreement
CTCAE	Common Terminology Criteria for Adverse Event (for cancer trials only)
DAE	Discontinuation due to Adverse Event
REK	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Council for Harmonization
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
NR	Nicotinamide riboside
WBC	White blood cell

1 Introduction

1.1 Background – fatigue after critical illness

Patients experiencing acute illness will often have a prolonged recovery time. The cause of this is unknown, but certain factors, like age, duration and graveness of the illness, is associated with prolonged recovery. ICU-acquired weakness negatively impacts prognosis and in one study, 122 patients with ICU-acquired weakness was matched to non-weak controls and the one-year mortality was found to be almost twice as high in the weakness group[3].

1.2 Background - Therapeutic Information

During an episode of acute illness, the initial phase is dominated by tissue damage and subsequent generalized inflammation caused by the initial insult. The acute phase is followed by a more prolonged recovery phase often requiring continued hospitalization. The inflammation caused by tissue injury in acute illness activates Poly (ADP-Ribose) Polymerase (PARP) which consumes nicotinamide adenine dinucleotide (NAD+) and lowers cellular NAD+ levels throughout the body[6].

We have discovered that in the recovery phase of acute illness, mitochondrial energy production is impaired because inflammation causes a depletion of NAD+ which is a substrate for the epigenetic modifier SIRT1[1,2,7]. SIRT1 activity is necessary for mitochondrial homeostasis[2] and it has been shown that patients with protracted critical illness, as well as those with intensive care unit-acquired weakness, have disturbances in the mitochondria of striated muscles. In one study, where healthy human volunteers were compared to patients in an ICU department, the authors found a 54% reduced capacity for ATP production in the ICU group [2]. They hypothesized that the cause was changes in the stoichiometry of the mitochondrial oxidative phosphorylation (OXPHOS) complexes. These complexes act as a chain in the mitochondrial inner membrane to generate ATP and the authors found some parts of the chain were depleted; specifically, this was the

OXPHOS complexes dominantly encoded by mitochondrial genes. The same pattern has also been identified in old mice where it has been shown that administration of NAD+ precursors of the vitamin B3 family can specifically increase production of the mitochondrial encoded OXPHOS complexes [3]. This has been associated with increased ATP production, improved health and extended lifespan in mice [3, 4].

We propose that recovery after acute illness with tissue damage is impeded by a relative NAD+ deficiency causing a reduced tissue ATP supply. We also propose that it is possible to increase ATP supply by administering the oral NAD+ precursor nicotinamide riboside (NR). An orally available NAD+ precursor (NR) that restores NAD+ levels in humans have recently become available. Thus, NR may be seen as a "recovery-improvement pill". This is a completely novel approach to the recovery phase which frequently constitutes the longest part of patients` hospital stays.

1.3 Pre-Clinical & Clinical Experience with the Investigational Medicinal Product (NR)

NR is a natural constituent of cow milk (https://www.ncbi.nlm.nih.gov/pubmed/27052539) which contains approximately 1 mg/l. It is considered generally safe by the FDA (https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/PharmacyCompoundingAdvisoryCommittee/UCM553368.pdf)). It is sold as a nutraceutical. Phase 1 trials have shown that it increases NAD+ in humans (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5062546/pdf/ncomms12948.pdf and https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5876407/). It has been shown to prolong the life when administered to old mice (Zhang, Ryu et al. 2016).

1.4 Rationale for the Study and its Purpose

Recovery from acute illness invariably takes time and is associated with increased risk of new acute illness, physical dependency and mortality (figure 1)[4,5]. Delayed or incomplete recovery is common and increases with the age of the patient and the duration and seriousness of the acute disease[13,14]. Recovery time can vary between a few days in otherwise healthy subjects with influenza to months, years or never in elderly patients after major illness. During a typical episode of acute serious illness with hospitalization the patient will be treated as an inpatient during the initial recovery phase which will be followed by a prolonged period in a long-term care facility, rehabilitation institution or at home with home nursing. Younger patients may recover at home without additional care, but will often be unable to work, adding to the costs for society.

The cause of the need for extended time to recover has not been explored extensively in the scientific literature and is by some viewed as "self-explanatory" while others call for more research[13]. Prompt and adequate treatment of the acute illness, supportive treatment with fluids and nutrition and early mobilization are key focus areas in every hospital that are known to improve patient outcome and shorten recovery time. To our knowledge, no "recovery-improvement drug" exists. We aim to include patients in ICU who are in this late stabile phase and administer NR or placebo from that point and onwards for 90 days. This is a completely novel approach to the recovery phase where NR may be seen as a "recovery-improvement pill".

1.4.1 ATP supply in critically ill patients and in recovery

Patients admitted to intensive care units are critically ill and often experience a prolonged recovery phase both as inpatients and in rehabilitation centers[4,5,13,14]. In these patients, ICU-acquired weakness is associated with a worse outcome and increased mortality[15]. In a one study[3] two matched groups of weak and not-weak ICU patients the weak patients had a hazard ratio (HR) of approximately 0.7 for live weaning from mechanical ventilation and live discharge from hospital. They also had 30% increased costs for the hospital stay (+5.400 Euro) and importantly a nearly doubled 1-year mortality from 17-30%.

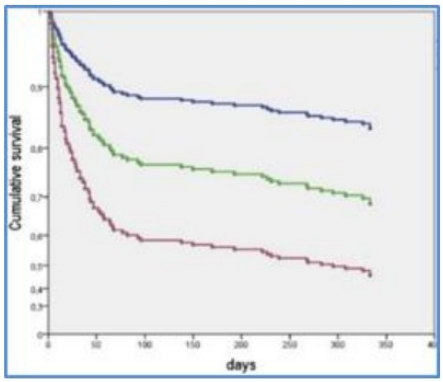


Figure 1. Weakness score at the end of ICU stay predicts first year mortality. *Cox regression estimates for survival in the first year after intensive care unit (ICU) admission in the total population of weak patients according to persistence and severity of weakness at final examination in the ICU. Weakness was diagnosed with the Medical Research Council (MCR) sum score (lower score=more weakness)[3].*

Blue: MCR>=48, Green: MRC 36-47, Red: MCR<36

1.4.2 Reduced capacity of the electron transport chain (ETC) in acute illness

The ETC is, as its name suggests, a *chain* where five different large protein complexes (I-V) in the mitochondrial inner membrane oxidize metabolites to produce an electrochemical gradient (complex I-IV) which is used by complex V to produce ATP, the universal biological energy carrier. Each component of the chain is dependent on all the other components and any stoichiometric mismatch between complexes or of protein contents within complexes will result in a reduced capacity of the whole mitochondrion.

It is known that acute illness interferes with mitochondria and energy production, but the cause of this is not fully understood. In sepsis, a common cause of critical illness the occurrence of mitochondrial dysfunction is well documented even in tissues with adequate oxygen supply and it has been suggested that this may be an adaptive response which turns maladaptive in the recovery phase[16]. In a recent development to understand the relationship between acute illness, mitochondria and ATP production biopsies from muscle were obtained from patients with protracted ICU-stays and compared to healthy controls[7]. The mitochondria of the ICU patients had a **halved** capacity for ATP production. They also had a selective depletion of the ETC complexes containing **mitochondrial** encoded proteins, and not of complex II which only contains nuclear encoded proteins. This suggests that a selective reduction in the production of mitochondrial proteins (mitochondrial biogenesis) inhibits ATP production in ICU patients.

Figure 2. The five complexes of the electron transport chain (ETC)

Mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) denotes the number of proteins in each protein complex that are encoded in the mitochondria and the cell's nucleus, respectively. For example, complex II is solely encoded by nuclear genes which are translated to proteins in the cytoplasm and transported into mitochondria while complex III contains one (key) mitochondrial encoded protein.

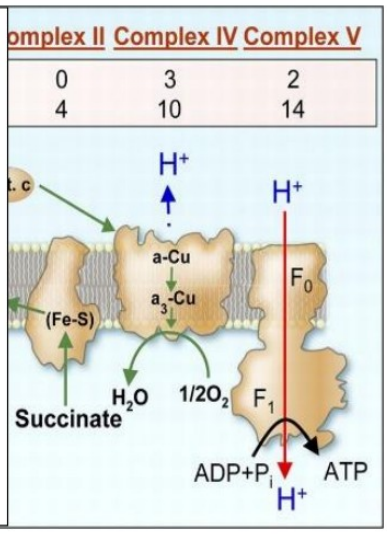
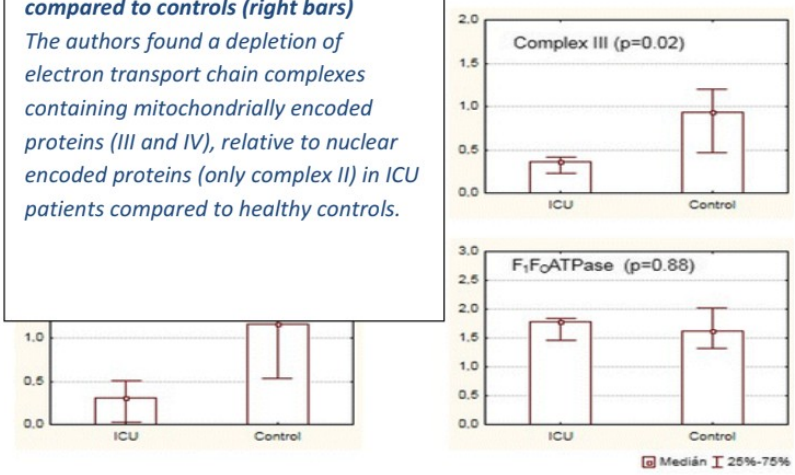


Figure 3. Selective disruption of mitochondrial electron transport chain stoichiometry in ICU patients (left bars) compared to controls (right bars)
The authors found a depletion of electron transport chain complexes containing mitochondrially encoded proteins (III and IV), relative to nuclear encoded proteins (only complex II) in ICU patients compared to healthy controls.



We have discovered that the mitochondrial signature in acute illness is indistinguishable to that of old age in mice which also showed a selective depletion of the mitochondrial encoded proteins of the ETC[2]. Intriguingly, restoration of NAD+ levels by supplementation of a NAD+ precursor restored the production of mitochondrial encoded ETC proteins and nearly doubled ATP concentration and health in these aged mice in the same paper. This may be a viable way of improving recovery after acute illness in humans.

1.4.3 Restoration of ATP levels in mice and cells

In the above-mentioned paper, the authors found a selective reduction of the mitochondrial encoded proteins of the ETC in old mice that were similar to that in muscle biopsies from ICU patients.
Reduced activity of the epigenetic modifier SIRT1 was identified as an upstream cause of reduced mitochondrial biogenesis in old mice. SIRT1 is a NAD+ dependent histone deacetylase which breaks down NAD+ and NAD+ levels are known to fall with age in mouse (and humans). In the paper, diminished SIRT1 activity inhibited mitochondrial biogenesis and replenishment of NAD+ through administration of a NAD+ precursor ("NMN") restored mitochondrial biogenesis and AT)2.

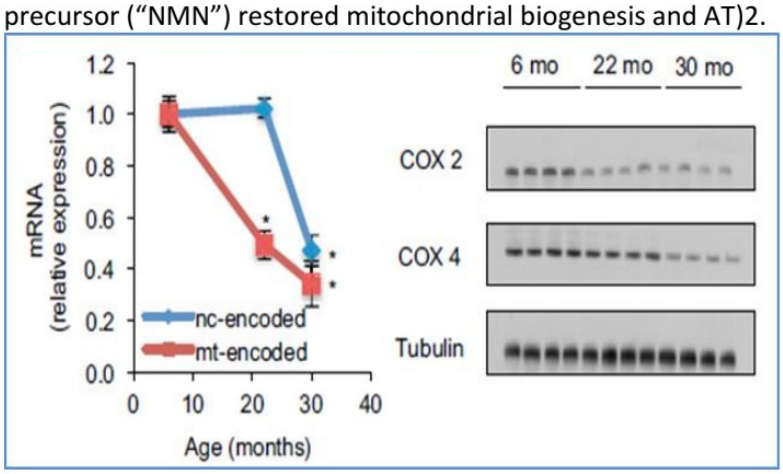


Figure 4. Selective reduction of mitochondrial encoded proteins in the electron transport chain (ETC) in old mice.

Left pane: *Messenger RNA expression of mitochondrial- and nuclear encoded electron transport chain complexes in young, old and very old mice. A changed stoichiometric relationship is seen in old mice.* ***Right pane:*** *the proteins COX2 and COX4 are both part of ETC complex IV. While the mitochondrial encoded COX2 shows a reduction in old mice COX4 is only*

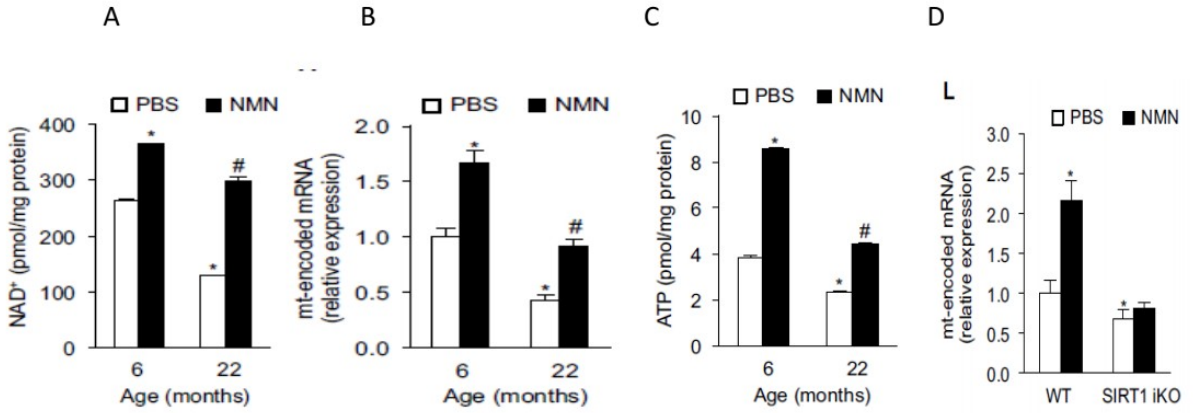


Figure 5.

a) *Supplementation of with a NAD+ precursor, nicotinamide mononucleotide, ("NMN"), but not placebo (PBS) restored cellular NAD+ levels in old mice.* ***b)*** *The restoration of NAD+ levels were accompanied by a partial restoration of mRNA for mitochondrial encoded proteins of the electron transport chain.* ***c)*** *The restoration of NAD+ was accompanied by almost a doubling of ATP content in tissue.* ***d)*** *The restoration was dependent on activity of the nuclear protein SIRT1 and knockdown protein negated the restoration by the NAD+ precursor. SIRT1 is a histone deacetylase which breaks down NAD+.*

Thus, it has been convincingly shown in mice that low NAD+ levels inhibit mitochondrial biogenesis and ATP production. It has been known for a long time that PARP together with the sirtuins (including SIRT1) are major nuclear NAD+ consumers that compete for NAD+ and that PARP is activated by the inflammatory response in range of acute illnesses because of tissue injury[6,17,18]. This depletes NAD+ levels throughout the body and through the SIRT1-mitochondrial homeostasis axis impairs ATP production and tissue energy supply[6]. We suggest that PARP induction in inflammation and critical illness leads to depletion of NAD+, loss of mitochondrial homeostasis and reduced ATP production which we propose are deleterious for recovery worsens outcome.

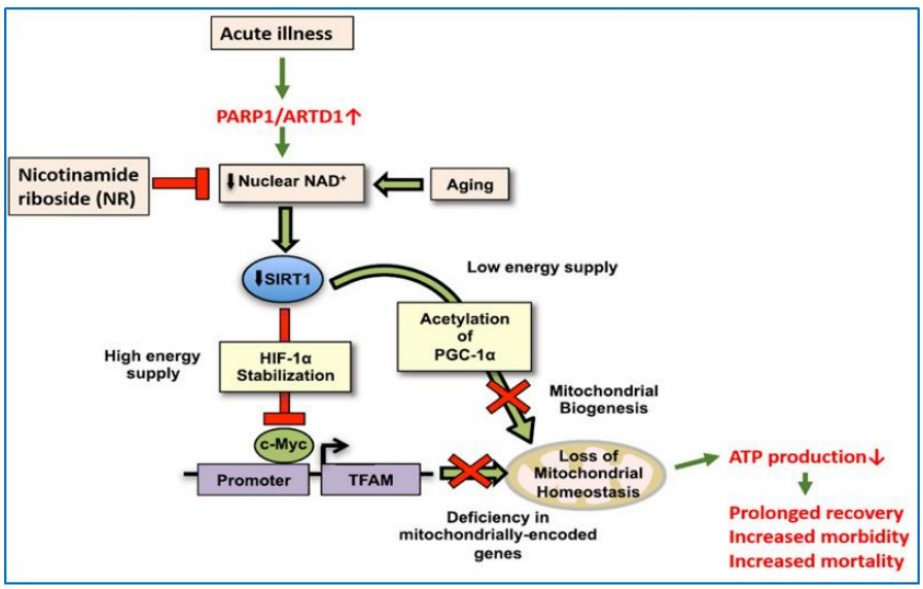


Figure 6. Model for reduced ATP production in critical illness with tissue injury and inflammation extended from[2]. *Acute illness leads to PARP activation which consumes nuclear NAD+ and thus the NAD+ dependent histone deacetylase SIRT1 is inhibited leading to loss of mitochondrial homeostasis and reduced ATP production. ATP is essential in for almost every biological process.*

Project design, method selection and analyses

To ensure the best possible validity, we have decided to go for a randomized placebo-controlled study design with double blinding. In addition to a pilot phase with four-six patients which has started the study will have four phases with 16-26 randomized patients each, firstly into a low dosage group (250mg x1) and then the same number of patients into progressively higher dosage groups (250mg x2, 250mg x4 and 250 mg x8) in the next phases (Figure 8). Patient will be treated with the study drug for three months to ensure coverage over the whole recovery period for most patients.

Some unevenness in the randomization is likely given the relatively small number of patients and because patients in ICUs are a very heterogeneous group. The relatively strict exclusion criteria will on the other hand make the participants more homogenous.

1.4.4 Measuring and targeting aging

Epigenetic alterations are one of the root causes of aging proposed by Lopez-Otin in the seminal paper "Hallmarks of Aging"(Lopez-Otin, Blasco et al. 2013). Epigenetics refers to chemical modifications of DNA and proteins related to DNA that affect gene expression and cellular function. Ground-breaking work by our collaborator Steve Horvath published in 2013 used information on aging-associated site-specific DNA methylation changes to develop a predictive model of chronological age and demonstrated the existence of an 'epigenetic clock'. He thus established a tool to directly measure one of the proposed root causes of aging (Hannum, Guinney et al. 2013, Horvath 2013). It was later established that the epigenetic clock not only carried information about chronologic age, but also about the rate of aging which could be measured as a higher (or lower) predicted than actual age. These differences are termed "age acceleration" and have repeatedly been shown to predict all-cause mortality (Marioni, Shah et al. 2015). It was soon realized that with access to old stored blood samples from cohorts including deceased participants one would be able to use Horvath's method to develop much improved predictors for mortality and biological age and recently two such predictors have been published(Zhang, Wilson et al. 2017, Levine, Lu et al. 2018). The predictor by Zhang et. al. (Zhang predictor) has been shown to outperform the 34-item Frailty Index (FI) with a wide margin in predicting mortality up to 15 years into the future(Zhang, Saum et al. 2018). The predictor constructed by Steve Horvath outputs "biological age" ("PhenoAge") as a number which is the same as the chronological age in persons with a normal rate of aging and seems to perform as well as or better than the Zhang predictor(Levine, Lu et al. 2018, Zhang, Saum et al. 2018). The rate of aging determined by PhenoAge is relatively in longitudinal samples from the same person. These predictors probably contain information about biological age**,** which will have profound impact on life sciences, medicine and geroscience. However, their long-term predictive value is not established and this is important because biological age measures by the most common definition should be able to predict future mortality relatively early in life(Butler, Sprott et al. 2004). As both predictors have been published during the past year their attenuation or acceleration by drugs like NR or disease and whether this might influence later outcome has not been explored yet. We will validate explore their usefulness in evaluating outcome of a pharmacological intervention.

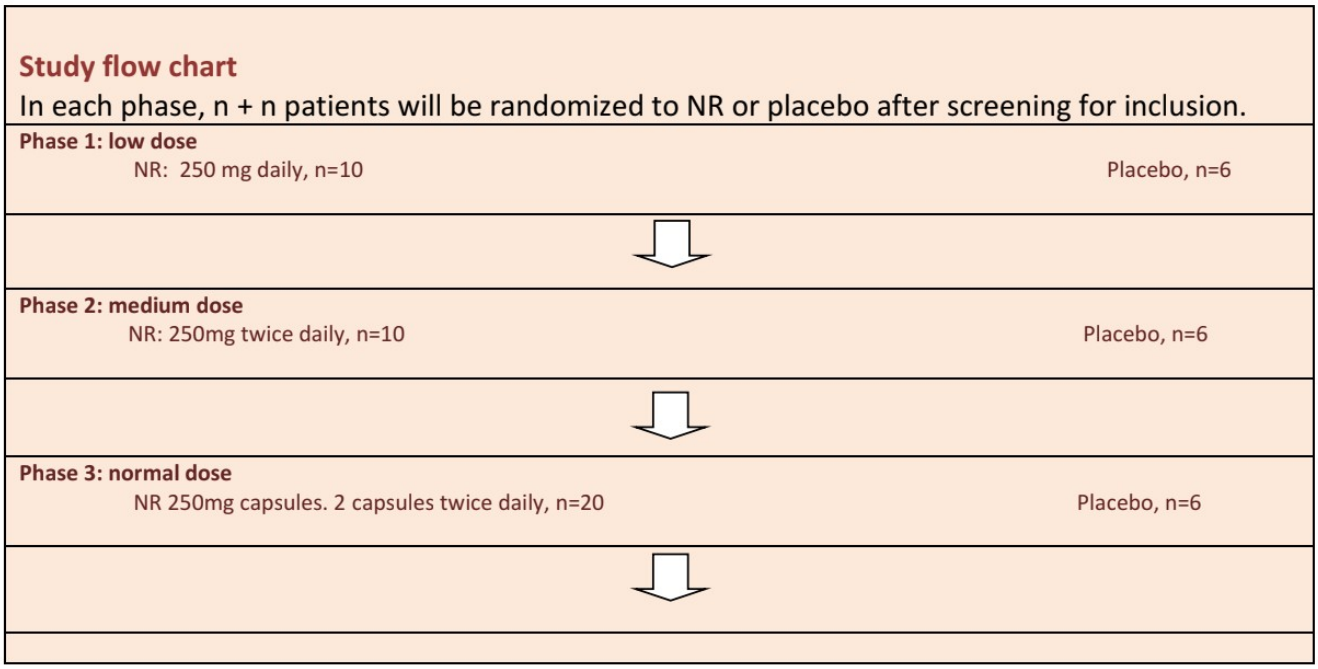


***Figure 7. Study flow chart**. In each phase 16-26 patients from a medical or* surgical ward will be randomized to NR or placebo. *In addition to the randomized patients, 30 healthy controls will be included for the purpose of representing a baseline for the validation of the use of epigenetic clocks in pharmaceutical studies.*
A safety committee will be monitoring the study. One of their tasks will be to evaluate if it is safe to proceed to a higher dose in the next phase.

2 STUDY OBJECTIVES AND RELATED ENDPOINTS

2.1.1 Primary aim

We aim to shorten recovery time after acute illness by supplementing patients with NR during recovery after such illness.
We aim to investigate the use of epigenetic biomarkers in clinical studies.

2.1.2 Hypotheses

• We hypothesize that recovery time after illness with tissue injury and inflammation will be reduced in patients supplemented with the NAD+ precursor NR because such disease depletes NAD+ and thus reduces tissue energy supply through the inhibition of the SIRT1-mitochondrial homeostasis axis.
• We hypothesize that impaired tissue energy supply prolongs recovery after acute illness.

2.1.3 Aims

• To measure recovery time after acute illness in patients supplemented by NR or placebo
• To identify characteristics of the patients that benefits most from NR supplementation after acute illness
• To identify the optimal NR dose yielding the largest reduction in recovery time in patients after acute illness
• To measure NAD+ levels during and after acute illness in patients using NR and placebo
• To investigate epigenetic mechanisms of how NR may shorten recovery time after acute illness
• To validate the usefulness of epigenetic clocks in evaluating outcome of a pharmacological intervention.

- Investigate whether NR influences epigenetic age measured by the available epigenetic age measurements at the time of analysis.
- Lay the grounds for developing NR as a patented drug for use during acute illness.

See 10.5 for endpoints

3 OVERALL STUDY DESIGN

The study is a double blinded randomized three stage superiority study where four different doses of NR will be compared to placebo for its efficacy of shortening hospital stays after acute illness. The study will be carried out in multiple departments of Oslo University Hospital and nearby hospital(s).

Study Period	Estimated date of first patient enrolled: April, 2019 Anticipated recruitment period: 2 years Estimated date of last patient completed: November 2021.
Treatment Duration:	3 months
Follow-up:	Patients will be followed up for 1000 days as described below (table 5.1). In addition, epigenetic clocks will be further evaluated by yearly checking the National Death Registry until all patients are diseased.

4 STUDY POPULATION

4.1 Selection of Study Population

Patients will be included from Medical Intensive Care units and regular wards at Oslo University Hospital, Norway, but may be extended to other wards at OUS or nearby hospitals to ensure an adequate number of both categories of participants.

The patients will be identified by screening of hospital records by the study staff and invited to participate and informed about the study by the study staff after consultation with the treating physician. If deemed necessary to include an adequate number of patients with existing samples in existing bio banks, prospective participants may also be screened by querying the bio bank (Janus) for information about existing samples from the prospective participant.

4.2 Number of Patients

58 patients

4.3 Inclusion Criteria

1. Adults > 18 years old, admitted to hospital with tissue damage, can be included when they are considered medically stable though still expected to remain hospitalized for at least 7 more days (from inclusion).

2. Preferably: Previously included in the Janus Cohort or any other cohort or study with stored biological samples.

4.4 Exclusion criteria

Exclusion Criteria

1. Allergy to NR or ingredients in capsules or placebo.
2. Patients expected to pass away within 90 days.
3. Patients unable to give their consent
4. Unstable patients:

i. Uncontrolled infection (clinical septicaemia, inadequate response to treatment, inadequate control of source of infection or at treating physician's discretion).
ii. MAP <70 mm Hg and symptoms of hypotension.
iii. Patients requiring dialysis at the time of inclusion or GFR <25
iv. Liver failure with Child-Pugh class B or C or any class associated with hepatic encephalopathy (any grade), ALT or AST >3 times upper limit. If liver transaminases are elevated for other reasons than hepatocyte damage, patients may be included regardless of ALT/AST values *
v. Severe peripheral oedema and/or pulmonary oedema, any unstable cardiac rhythm, ongoing myocardial ischemia, troponin T >500.
vi. Signs of elevated intracranial pressure (headache, vomiting and depressed global consciousness in conjunction with focal neurological signs, papilledema, spontaneous periorbital bruising and a triad of bradycardia, respiratory depression and hypertension).
vii. Arterial pH <7.30 or >7.50
viii. Serum potassium under 3, 2 or over 5 mmol/L.

5. Pregnancy or breastfeeding **
6. Any cancer not in full remission for >10 years (for non-melanoma skin cancers, full remission for >1 year)
7. Use of St John's Wort based supplements during the past 30 days
8. Patient has undergone solid organ transplantation
9. Participation in any clinical trial with unknown medications
10. Major gastrointestinal or other major internal bleeding past 2 days.
11. Logistical challenges after discharge. Patient must be able to attend follow up.
12. Intracerebral haemorrhage or major stroke past week.
13. The treating physician considers the patient unfit or unable to participate.

*Please also see Amendment number 1.

** All fertile women must have an HCG test.

5 TREATMENT

Patients will be receiving either NR or placebo.

5.1 Identity, Supply and Storage

NR, in the shape of Tru Niagen (ChromaDex, Inc.) will be used in this study. Both Niagen and placebo capsules can be stored for up to 1 year at room temperature. A 3-month supply of the appropriate dose of either placebo or NR capsules will be packaged into kits by persons that are otherwise not involved in the study (presumably medical students recruited by the study staff). In addition to the required information, the boxes will be marked with dose, administration guidelines and an identification number. The link between the ID number and the content of each box (placebo or NR) will only be known by persons involved in this step. Please see 5.9 "Drug labelling" for more information.

5.2 Dosage and Administration

The capsules are taken orally and contain 250 mg of NR or placebo. Placebo or NR capsules will be administered after randomization as described below. (Please also see flow chart under point 5).

- First 16 patients enrolled: 1 capsule 250 mg every morning.
- Following 16 patients enrolled: 1 capsule of 250 mg morning and evening for three months.

- Following 26 patients enrolled: 2 capsules of 250 mg morning and evening for three months.
- High dose of 1000 mg morning and evening has now been cancelled (12.06.2023)

During hospitalizations the capsules will be administered by the hospital personnel while the capsules will be self-administered after discharge. Three months of NR or placebo will be released to the patient at randomization.

The capsules should ideally be swallowed whole, but if necessary, they may be opened before administration and the content poured into a small amount of water for administration in PEG/nasogastric tube. The content should not be chewed or crushed. The absorption of Tru~~e~~ Niagen remains the same if administered in this fashion, but unusual administration methods will be registered. The capsules may be opened by the hospital staff, but not by the study staff.

Due to strict inclusion criteria, and the expected frailty level of such patients, it is unlikely that two included patients will meet. It is thus unlikely that opening the capsules will lead to patient un-blinding.

A missed dose should be taken as soon as possible, but not any later than 6 hours after the usual time. If more than 6 hours have passed, the patient should wait until the next dose is scheduled.

5.3 Premedication and Monitoring

No pre-medication will be used. After administration of the first capsule, clinical status shall be monitored every half hour for the first three hours. In case of suspected adverse events, or at the discretion of the treating physician, administration of the study drug can be postponed and this will be recorded in the CRF. In this case the patient will continue to use the medication until all three months' worth of capsules is consumed.

5.4 Concomitant Medication

The following medication is not allowed while the patient is included in the study:

- St Johns Worth

The use of antibiotics, the specific use of aminoglycosides, and use of vitamin B3 containing drugs will be recorded in the patient's file and CRF.

5.5 Subject Compliance

Compliance will be ensured by administration of the study intervention as part of the normal hospital routine while the patients are hospitalized. After hospitalization compliance at home will be ensured by regular contact with the study staff including the end of study interview and a phone call one-month post inclusion to remind patients to take tablets. Patients will also be encouraged to buy a pill organizer and unused tablets and packaging should be brought to the end of study visit for control. The study staff will also ensure administration of the study drug while the patients are at long term care facilities, rehabilitation institutions or under the formal care of other caregivers. Every effort will be made to ensure that the study drug is mentioned in the discharge letter under the section "new medications". This list of medications is routinely circulated to patients, caretakers and the patient's general practitioner.

Finally, compliance will be ensured by measuring mononuclear white blood cell NAD+ levels which are known to be affected by the study drug. These data will be sent from the laboratory to the safety committee and not included in the database before after un-blinding.

5.6 Accountability

One batch containing three months of NR will be distributed to each participant. This batch will be kept and used by the hospital ward during hospitalization and then released to the participant at discharge. Any remaining medication will be collected and counted at the end of study or if a participant withdraws prior to this. Left over medication is to be disposed of in the risk waste boxes for incineration in accordance to the hospital policy.

5.7 Labelling

NR 'a 250 mg and placebo will have a label permanently affixed to the outside of the container. Labelling will be in accordance with ICH, GCP and national regulations, and will clearly state that the material is for clinical trial / investigational use only and that it should be kept out of reach of children.

Label:
Information on the labels is printed in Norwegian. It`s English translation follows:

Investigating product or placebo
Contains: 250 mg nicotinamide riboside capsules or placebo.
Batch number.
Expiration date.
Dosage: Corresponds to the phase of the study, please see 4.2

For clinical investigation only.
To be kept refrigerated or in room temperature.
Keep away from children.
The capsules can be swallowed or opened and the content poured into water.

Patient inclusion number.
Patient`s initials.
Protocol code
Date dispensed
Name of prescribing doctor

The sponsor of this study is: Oslo Universitetssykehus, Avdeling for molekylær mikrobiologi, Sognsvannsveien 20, Oslo
Contact persons: Medical doctor Olaug Marie Reiakvam (mobil: 91302935) or Principal investigator Arne Søraas (mobil: 90652904).

Studiepreparat for B3Short studien eller placebo.
Innehold: 1 kapsel inneholder 250 mg nikotinamide riboside eller placebo **Batch nummer:** **Utløpsdato:**
Dosering Til klinisk utprøvning. Oppbevares i romtemperatur eller kjøleskap. Oppbevares utilgjengelig for barn. Kapslene svelges hele el åpnes og innholdet løses opp i vann og drikkes.
Pasientens inklusjonsnummer: **Pasientinitialer:** **Protokollnummer:** 90652904 **Startdato:** **Utskrivende lege:**
Studiesponsor: OUS, Avd. for Molekulær Mikrobiologi, Sognsvannsveien 20, Oslo. **Kontaktpersoner:** Olaug Marie Reiakvam (tlf 91302935), Arne Søraas (tlf 90652904).

Empty lines to be filled in during packaging (Batch number, expiration date and dose, patient`s inclusion number) or by the investigator at inclusion (patient`s initials, starting date and prescribing doctor).

5.8 Subject Numbering

Each subject is identified in the study by a unique subject number that is assigned when subject signs the Informed Consent Form and takes the first capsule. Once assigned the subject number cannot be reused for any other subject. The first patient included in the study will be included in phase 1 and thus receive the ID number 101, the second patient 102 and so on. The first patients enrolled in the following phases will hence be subject number 201, 301 and so forth.

6 STUDY PROCEDURES

6.1 Timing of investigations

Table 1: Timing of laboratory tests and biobanking

	Inpatients				Outpatients		
	Day 1*	Day 3 +/-1	Day 7 +/-1	Weekly	Day 90 +/-10	Withdrawl visit	Week 65 and 150
Na	X	X	X	X	X	X	X
K	X	X	X	X	X	X	X
Calcium	X	X	X	X	X	X	X
Mg	X	X	X	X	X	X	X
Phosphate	X	X	X	X	X	X	X
Urea	X	X	X	X	X	X	X
Creatinin	X	X	X	X	X	X	X
GFR	X	X	X	X	X	X	X
Albumin	X	X	X	X	X	X	X
Bilirubin	X	X	X	X	X	X	X
CRP	X	X	X	X	X	X	X
ALP	X	X	X	X	X	X	X
ASAT	X	X	X	X	X	X	X
ALAT	X	X	X	X	X	X	X
LT	X	X	X	X	X	X	X
GT	X	X	X	X	X	X	X
Pancreas amylase	X	X	X	X	X	X	X
Uric acid	x		x		x	x	x
Homocystein	X	X	X	X	X	X	X
Ferritin	X	X	X	X	X	X	X
Hb	X	X	X	X	X	X	X
Leucocytes	X	X	X	X	X	X	X
Trombocytes	X	X	X	X	X	X	X
INR	X	X	X	X	X	X	X
v-PH	X	X	X	X	X	X	X
v-HCO3	X	X	X	X	X	X	X
Pro-BNP	X		X	X	X	X	X
Troponin-t	X	X	X	X	X	X	X
CK	X	X	X	X	X	X	X
HbA1c	X				X	X	X
TSH	X		X	X	X	X	X
Ft4	X		X	X	X	X	X
Folate	X		X	X	X	X	X
LDL	X		X	X	X	X	X

HDL	X	X	X	X	X	X
Total Cholesterol	X	X	X	X	X	X
BIOBANKING:						
4x EDTA	X	X		X	X	X
1x Serum	X	X		X	X	X
1x Plasma	X	X		X	X	X
2x CPT	X	X		X	X	X
Urine, faeces, saliva,buccal swabs **	x	x		x	x	x

* Prior to administration of intervention drug

**Selected number of patients only

Table 2: Timing of inclusion and procedures.

	Screening period	**Treatment Period** At inclusion (1), day 3, 7[i] And on discharge (DC)	**Mid-treatment follow-up phone call**	**Last week of treatment visit at 90 days +/- 10**	**End of study visit ca 65 wks.**	**Withdrawal visit**	**Follow up period**
Screen patients [ii]	x						
Informed consent	x						
Inclusion + exclusion criteria	X						
Medical history, prior treatment.	X		X				
Treatment dispensed/returned		1		x		x	
Concomitant medication[iii]	X	1-7, DC	X	X	X	X	
Physical examination [iv]	X	1-7		X	x	X	
Vital signs [v]	X	1-7		X	X	X	
Tests/questionnaires[vi]		1-7,		X	X	X	
Adverse events		1-7, DC	X	X	X	X	X
Survival assessment		1-7, DC	x	x	X	X	x

(DC: Discharge)

[i] And every 7th day onwards if still hospitalized.

[ii] All ICU patients and patients on the following medical wards are screened: Infectious diseases, internal medicine, pulmonary medicine and acute medicine. See also own section on screening.

[iii] Antibiotics and vitamin B3 containing drugs only.

[iv] Full physical examination of all major organ systems.

[v] Blood pressure, pulse, temperature, respiration rate and 02 saturation.

[vi] See table 3 below for details.

Table 3: Overview of scoring forms and other assessment methods**

CLINICAL TEST/scoring systems	TIMING
EQ5D (filled in by investigator in the presence of the patient).	2 weeks prior to admission. On day 1 and on day 90.
MOCA	Day 7 and 90 and week 65.
Trail making A	Day 7 and 90 and week 65.
Trail making B	Day 7 and 90 and week 65.
Forward and Backward Recall.	Day 7 and 90 and week 65.
NEWS (completed by nursing staff as part of standard treatment).	- Baseline morning and afternoon, day 3 and day 7.
4 meter walking test	At baseline, day 7, day 90 and week 65.
Clinical Frailty score (filled in by investigator)	Pre-baseline, baseline, day 7, day 90 and week 65.
Grip strength	At baseline, day 7, at discharge, day 90 and week 65. (and every occation at hospital).
RASS (completed by nursing staff as part of standard treatment of sedated patients).	Day 0, 1, 3, 7 and every 7th day thereafter for the duration of stay in ICU
Charlson comorbidity index (Filled in by investigator)	Baseline
Hearing Assessment **	At baseline, day 7, day 90 and week 65.
SAPS2 (Filled in by investigator).	On admission to ICU or first day at hospital for patients never at ICU.

(*) Portable audiometry, tympanometry and otoscopy.

(**) Patient's level of fitness will vary considerably and individual evaluations will be made for all patients regarding their ability to complete all or some of these assessments. For patients who are capable of competing all individual tests, but for whom the sum of the assessments is too large, the assessments should for the most part be prioritized as follows:1: Grip strength.

2: EQ5D.

3: Forward-backward recall.

4: Trailmaking A.

5: 4 meter walking test.

6: Trailmaking B.

7: MOCA

8: Hearing assessment.

6.2 By Visit

Patients will be identified by the study staff screening patient records in collaborating wards. After conversation with the treating physician, a preliminary evaluation will be made regarding possible inclusion. After the patient has been thoroughly informed about the study and inclusion is deemed possible, all points mentioned in the above table will be carried out (flow chart 5.1). Informed consent must have been given voluntarily by each subject before any study specific procedures are initiated. Signed consent forms will be kept in a locked drawer inside the investigator's office.
Blood will be drawn early the following morning PRIOR to administration of NR/placebo. In addition to parameters mentioned in 5.1, NAD+ metabolites will also be measured. NR/placebo will be given shortly after this.

Staff at collaborating wards may contact the study staff out of hours.

6.2.1 Before Treatment Starts

The following will be carried out prior to starting treatment:

- To evaluating patient eligibility, patients must meet all inclusion criteria and none of the exclusion criteria. The patient must give his or her informed consent and sign the appropriate paperwork. Please see table 1 for additional information.
- Baseline values of parameters used as end-points will be assessed (see 9.4)

6.2.2 During Treatment

Patients will be monitored and assessed routinely as long as they remain in hospital. Possible adverse reactions are likely to occur during this time, and only patients thought to remain in hospital for at least 7 days are included. This allows the study staff to keep a close eye on all included patients. See 5.1 for detailed description of visits during and after hospitalization.

6.2.3 End of Treatment Visit

An end-of-treatment visit will be carried out during the last week of treatment. For logistical reasons, and because potential effects of NR are assumed to be established prior to 90 days, we will allow for a window of 90 days +/- 7 days. Please see 5.1 for details.

6.2.4 Withdrawal Visit

Patients who withdraw from the study will be followed up as described in the flow chart above (5.1).

6.2.5 After End of Treatment (Follow-up)

Please see table 1 flow chart "End of treatment visit", "End of study visit" and "Follow-up period". Survival information and information regarding re-admissions will be collected using hospital charts and the causes of death registry until all participants are deceased.

6.2.6 Criteria for Patient Discontinuation

Patients may be discontinued from study treatment and assessments at any time. Specific reasons for discontinuing a patient for this study are:

- Voluntary discontinuation by the patient who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment.
- Safety reason as judged by the Principal Investigator
- Incorrect enrolment i.e. the patient does not meet the required inclusion/exclusion criteria for the study
- Patient lost to follow-up
- A female patient becoming pregnant (treatment will be stopped, but follow up continues as described otherwise).
- Deterioration in the patient's condition which in the opinion of the Principal Investigator or treating physician warrants study medication discontinuation.
- Compliance issues.

6.3 Procedures for Discontinuation

6.3.1 Patient Discontinuation

Patients who withdraw or are withdrawn from the study, will stop further treatment. Patients who withdraw will be followed up at the time of discontinuation and at the end of the study (see also flow chart 5.1). The reason for discontinuation will be recorded and significant adverse events will be followed up. All patients randomized will be included in the study population.
Patients who withdraw or are withdrawn from the study after randomization cannot be replaced.

6.3.2 Trial Discontinuation

The whole trial may be discontinued at the discretion of the PI or the sponsor in the event of any of the following:

- Occurrence of AEs unknown to date in respect of their nature, severity and duration
- Medical or ethical reasons affecting the continued performance of the trial
- Difficulties in the recruitment of patients
- The project leader will immediately inform Helsetilsynet in the case of serious unexpected adverse events that are likely to be caused by the research.
- The project leader or other personnel are obliged to inform Helsetilsynet about research practices that might pose a danger to the safety of participants. In the case of deaths that can have been caused by the research the police should be informed.

6.4 Laboratory Tests

Routine blood samples will be taken at all study visits. During hospitalization these samples will be taken as part of normal hospital routines and usually in the morning. The tests to be taken are listed in table 1, but minor changes may be amended at a later point.
It is beneficial for data analysis to obtain blood tests from as early on during the current illness as possible. Collection of such samples will be done in a pragmatic way by contacting the relevant biobanks or laboratories that might store samples from the participant. This may also include asking the laboratory to pre-emptively freeze patient samples instead of destructing them at the standard time.
Biobanking for other analyses will be performed immediately before the first capsule is administered. If not mentioned otherwise samples will be bio banked according to the groups own SOP (Standard Operating Procedure.

1) A separate venous or arterial sample will be collected immediately before the first capsule is administered to analyze NAD+ and metabolites. This sample will be drawn by either the ward staff or the study staff depending upon the location of the patient and presence of existing

arterial/venous access. Sampling will mainly be venous at the 90 day follow up when most participants will no longer be in-patients. The study staff will always be present to facilitate swift handling of the sample. This sample should be taken not more than 6 hours prior to the first capsule and at the same time of day on day 7 and 90 if at all possible. NAD+ is unstable and the sample must therefore be transported swiftly to the lab for immediate handling.

2) Cytokines (serum/plasma)
3) EDTA full-blood rapidly frozen for:
   a) DNA extraction for DNA methylation arrays
   b) Sequencing
   c) SNP arrays
   d) ChIP-seq and other molecular procedures
4) EDTA plasma for biochemical analyses and other analyses
5) WBCs for RNA seq
6) *Urine
7) *Buccal swab
8) *Saliva
9) Frozen WBC for ChIP-seq/ATAC seq

*Denotes samples that will only be collected from selected patients which probably will be the second and second last randomization batch (of 5+3 or 10+3 patients) and a similar number of healthy controls (matched by age and sex).

7 Assessments

7.1 Assessment of Efficacy / Pharmacokinetic / Pharmacodynamic / Response

See 5.4 – laboratory tests for NAD+ metabolites. As these may cause unblinding, these results will be kept away from study staff until unblinding.

7.2 Safety and Tolerability Assessments

Safety will be monitored by the assessments described below as well as the collection of AEs at every visit. Significant findings that are present prior to the signing of informed consent must be included in the relevant medical history/ current medical condition page of the CRF. For details on AE collection and reporting, please see flow chart 5.1 and point 7 below.

7.3 Other Assessments

We hypothesize that recovery time after acute illness with tissue injury and inflammation will be reduced in patients supplemented with the NAD+ precursor NR. Hence, it is natural to collect data with regards to the following:

- Measure NAD+ levels before (before the first capsule is administered) during and after acute illness in patients using NR and placebo. See also point 5.4. We will connect the clinical outcome measures to the effect by measuring NAD+ level in peripheral blood mononuclear cells using liquid chromatography-mass spectrometry and histone acetylation using our own world leading ChIP-seq method (25).
- To investigate epigenetic mechanisms of how NR shortens recovery time after acute illness.
- See “Primary Endpoints“ and “Secondary Endpoints” under section on Protocol Synopsis and section 9 below.

8. Safety monitoring and reporting

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE). Each patient, ward nurses and the treating physicians will be instructed to contact the investigator immediately if they manifest any signs or symptoms they perceive as serious.
The methods for collection and reporting safety data are described below. Despite NR`s good safety profile and few expected side effects, all possible adverse events will be included in our study.

We will however refrain from reporting any other AE/SAE besides SUSARS in the hospitals own reporting system, Akilles, as this is putting an unnecessary strain on the reporting system.

8.1 Definitions

8.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient administered the study intervention and which does not necessarily have a causal relationship with this treatment.
An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the intervention. The term AE is used to include both serious and non-serious AEs.

8.1.2 Serious Adverse Event (SAE)

If an abnormal laboratory value/vital sign are associated with clinical signs and symptoms, Serious Adverse Event (SAE) is defined as any untoward medical occurrence, that at any dose:

1. Results in death
2. Is immediately life-threatening
3. Requires in-patient hospitalization or prolongation of existing hospitalization
4. Results in persistent or significant disability or incapacity
5. Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

Medical and scientific judgment is to be exercised in deciding on the seriousness of a case. We will be using the CTCAE (Common Terminology Criteria for Adverse Events, US department of Health and Human Services, V5, 2017) for guidance. Important medical events may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent one of the listed outcomes in the definitions above. In such situations, or in doubtful cases, the case should be considered as serious.

Hospitalization for administrative reason (for observation or social reasons) is allowed at the investigator's discretion and will not qualify as a serious adverse event unless there is an associated adverse event warranting hospitalization. Also, pre-planned hospitalization admission (i.e., elective or scheduled surgery arranged prior to the start of treatment) for pre-existing condition is not considered to be a serious adverse event.

8.1.3 Suspected Unexpected Serious Adverse Reaction (SUSAR)

Adverse Reaction: all untoward and unintended responses to an investigational medicinal product related to any dose administered.

Unexpected Adverse Reaction: an adverse reaction, the nature or severity of which is not consistent with the applicable product information.

Suspected Unexpected Serious Adverse Reaction: Unexpected Adverse Reaction that is serious, i.e.:

1. Results in death
2. Is immediately life-threatening
3. Requires in-patient hospitalization or prolongation of existing hospitalization
4. Results in persistent or significant disability or incapacity.
5. Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

8.2 Expected Adverse Events

Previous studies have showed that NR in general is well tolerated. In a 2019 study of healthy, obese individuals it was found that type, incidence and severity of AE were similar across the different intervention groups and the placebo group. (*Conze, D., Brenner, C. & Kruger, C.L. Safety and Metabolism of Long-term Administration of NIAGEN (Nicotinamide Riboside Chloride) in a Randomized, Double-Blind, Placebo-controlled Clinical Trial of Healthy Overweight Adults. Sci Rep **9,** 9772 (2019). https://doi.org/10.1038/s41598-019-46120-z)*
There were no statistically significant differences in the NIAGEN and placebo-groups with respect to any clinical chemistry parameter either, and no serious AE or reports of flushing were registered. Importantly, all AEs were resolved by the end-of-study.

No other animal- or human studies published have demonstrated any serious side effects of NR. In a study (reference 17 in the protocol) from 2017 there were no significant differences in the incidence of AEs in the intervention group compared to the placebo group. In the intervention group, six adverse events (mild nausea, moderate fatigue, mild headache, moderate dyspepsia, moderate abdominal discomfort and moderate diarrhoea) were possibly related to NR. All participants reporting AEs recovered and there were no SAEs reported in this clinical study either.

8.3 Disease Progression

Events which are definitely due to disease progression (worsening of symptoms related to known acute or chronic illness that is known to fluctuate/expected to fluctuate in similar scenarios) will be recorded as AE or SAE in the study`s own reporting system, but not in Akilles.
Death due to progressive disease during the treatment phase is to be recorded as a SAE or SUSAR as appropriate.

8.4 Time Period for Reporting AE and SAE

For each patient the standard time period for collecting and recording AE and SAEs will begin at day 1 when the first capsule is administered and will continue until 30 days after the end of study visits at day 90. Patients will be asked about side effects, and specifically flushing, at all points of contact with the investigator.
During the course of the study all AEs and SAEs will be proactively followed up for each patient; events should be followed up to resolution, unless the event is considered by the investigator to be unlikely to resolve due to the underlying disease. Every effort should be made to obtain a resolution for all events, even if the events continue after discontinuation/study completion.

8.5 Recording of Adverse Events

SE and SAE will be recorded on SA/SAE forms and evaluated according to CTCAE criteria.
Any unexpected laboratory value or vital sign will also be recorded as an AE. The CTCAE system will be used to identify levels of abnormality that require reporting. Related clinical signs and symptoms must be noted. Abnormal laboratory results/vital signs will be considered additional information if related to a symptom, sign or diagnosis that warrant reporting on it`s own.

The following information will be noted in the AE/SAE forms:

- Dose, way of administration and duration of treatment.
- The nature of the event(s) will be described by the investigator in precise standard medical terminology (i.e. not necessarily the exact words used by the patient).
- The duration of the event will be described in terms of event onset date and event ended data.
- The result of the AE/SAE (Death, life threatening, not yet resolved/still treated for SAE/AE, treated and resolved, improved with sequelae, resolved without sequelae).
- The grade (1-5) according to the CTCAE system.
- The Causal relationship of the event to the study medication will be assessed as one of the following (the definitions below are according to WHO):

Unrelated:
There is not a temporal relationship to investigational product administration (too early, or late, or investigational product not taken), or there is a reasonable causal relationship between non-investigational product, concurrent disease, or circumstance and the AE.

Unlikely:
There is a temporal relationship to investigational product administration, but there is not a reasonable causal relationship between the investigational product and the AE.

Possible:
There is reasonable causal relationship between the investigational product and the AE. De-challenge information is lacking or unclear.

Probable:
There is a reasonable causal relationship between the investigational product and the AE. The event responds to de-challenge. Re-challenge is not required.

Definite:
There is a reasonable causal relationship between the investigational product and the AE.

- **Action taken:**

The outcome of the adverse event:

- Has the event resolved or is it still on-going?
- Make note of treatments and investigations carried out.
- Record if the study drug has been continued or not.

8.7 Reporting Procedure

8.7.1 AEs and SAEs

SE are to be recorded in the study`s own forms and the SMC (Safety Monitoring Committee) will be updated every forth night. AE/SAE will be discussed at the study`s weekly meetings. The SMC should be informed of any SAE at the earliest convenience and no later than 4 days. If the SAE is considered to be either probably or definitely related to the study drug, the SMC must be informed within 1 day.

The leader of the SMC, Vidar Ormaasen, will receive the report in the form of a password protected excel sheet that is available on K:sensitive, see example below:

AE and SAE in the B3Short study:

Pasient ID og hoveddiagnoser	Alder/kjønn	Spurt om AE?	Inklusjonsdato	Andre relevante medisiner	Dato for AE/SAE	SAE	AE

Gradering	Konsekvens	Ekstra ved SAE: Dødsårak, obduksjon, SUSAR?	PI/SMC info	Sammenheng med studiemedisin?	Studiemedisinener:

The head of the Department of Microbiology (where PI and PhD candidate are employed), Doctor Fredrik Muller will be the recipient of reports in the Achilles system.
The study safety committee keeps detailed records of all SAEs reported by the investigators and performs an evaluation with respect to causality and expectedness. Based on, among other, SAE reports the sponsor will evaluate whether the risk/benefit ratio associated with the study has changed.

8.7.2 SUSARs

SUSARS will also be recorded in the hospital`s own reporting system (Achilles). If the seriousness of the SUSAR is exceeding the expected risk in similar scenarios, the OUS Department of Quality and patient safety will be contacted for a discussion no later than 24 hours after the event (phone number 23 02 70 32. If deemed necessary, the allocation code can be broken and the Norwegian Board of Health Supervision notified as appropriate.

8.8 Procedures in case of emergency

Medical emergencies will be taken care of by the treating physician present at the hospital at all times. An unblinding procedure follows below. This procedure will be available at the ward (see also chapter 9.2). The investigators will also be available by phone at all times and concerning any study-related question.

The investigators **(Olaug Marie Reiakvam ph. no 91 30 29 35** (investigator) and **Arne Søraas ph. no 90 65 29 04** (primary investigator)**)** are responsible for assuring that there are procedures and expertise available to cope with emergencies during the study.

Every effort should be made to contact the investigators before unblinding. Unblinding is permissible when the treatment of the patient is dependent on whether the patient was given NR or not.
For information regarding code breaking, please see section 9.2.3 Envelopes are kept at the medical ICU at OUS (phone: 22118320) and each has a kit number written on the outside. The corresponding kit number is also recorded in DIPS to allow hospital staff to identify the correct envelope.

Details of un-blinding procedure:
In case of need for emergency un-blinding the study staff will be contacted by the doctor requesting un-blinding. If un-blinding is deemed necessary, this will be done by opening of the envelope belonging to the patient who will be kept in the medical ICU at Ullevål hospital which always has staff on duty. The date and time of un-blinding must be documented in the eCRF and in the patient's hospital records.

In the event of an SAE, the Investigator may only break the treatment code if the appropriate future management of the patient necessitates knowledge of the current treatment. Although it is advantageous to retain the blind for all patients prior to final trial analysis, when an SAE may be a serious adverse reaction unexpected or otherwise judged reportable on an expedited basis, it is recommended that the blind should be broken only for that specific patient, by an independent person in the Clinical Trials Unit or its designee, even if the Investigator has not broken the blinding.

8.9 Data Monitoring Committee (Called Safety Monitoring Committee in this study).
An un-blinded safety committee will monitor clinical outcome in the included patients before each increasing dose.

9. Data Management and monitoring

9.1 Case Report Forms
The Clinical Data Management System (CDMS) used for the eCRF in this study is "Web Form" ("Nettskjema") at University of Oslo. The setup of the study specific eCRF in the CDMS will be performed by the investigators.

The designated investigator staff will enter the data required by the protocol into the eCase report forms (eCRF). The Investigator is responsible for assuring that data entered into the eCRF is complete, accurate, and that entry is performed in a timely manner. The signature of the investigator will attest the accuracy of the data on each eCRF. If any assessments are omitted, the reason for such omissions will be noted on the eCRFs. Corrections, with the reason for the corrections will also be recorded.

After database lock, the investigator will receive a digital copy of the subject data for archiving at the investigational site.

9.2 Source Data
Nettskjema will be the primary source of source data for the study and information from physical examinations will be recorded here. Additionally, relevant data from the hospital patient records will be recorded in the paper version of the study file or via Nettskjema. A single excel sheet is the source data for adverse events. The audiometry results will be transferred from the audiometer to K: sensitive. When the source data is in paper form, a digital copy will be taken using the Nettskjema secure photo app and stored in tsd. The hospital records will be updated with findings from study examinations where this is deemed relevant by the study staff.
To achieve this, the medical records of each patient will clearly describe at least:

- That the patient is participating in the study, e.g. by including the enrolment number and the study code or other study identification;
- Date when Informed Consent was obtained from the patient and statement that patient received a copy of the signed and dated Informed Consent;
- Results of all assessments confirming a patient's eligibility for the study;
- Diseases (past and current; both the disease studied and others, as relevant);
- Surgical history, as relevant;

- Treatments withdrawn/withheld due to participation in the study;
- Results of assessments performed during the study if relevant.
- Treatments (NR) given, changes in treatments during the study and the time points for the changes.
- Visits to the clinic / telephone contacts during the study, including those for study purposes only;
- Non-Serious Adverse Events and Serious Adverse Events (if any) including causality assessments if relevant.
- Date of, and reason for, discontinuation from study treatment;
- Date of, and reason for, withdrawal from study;
- Date of death and cause of death, if available;
- Additional information according to local regulations and practice.
- Kit number as well as the production codes of all flasks contained in a kit.
- Information regarding un-blinding procedures, contact numbers and reporting side effects.

The eCRF will be the source data for adverse events and self-reporting questionnaires will be filled out electronically through Nettskjema. For other data the hospital records will be the source data.

9. 3 Study Monitoring

The investigator will be visited on a regular basis by the Clinical Study Monitor, who will check the following:

- Informed consent process
- Reporting of adverse events and all other safety data
- Adherence to protocol
- Maintenance of required regulatory documents
- Study Supply accountability
- Facilities and equipment (example: laboratory, pharmacy, ECG machine, etc…) if applicable
- Data completion on the CRFs including source data verification (SDV).

The monitor will review the relevant CRFs for accuracy and completeness and will ask the site staff to adjust any discrepancies as required.
The institution's representatives (e.g. monitors, auditors) will be allowed access to source data for source data verification in which case a review of those parts of the hospital records relevant to the study will be required.
The study will be monitored according to GCP standards by OUS, Department of Research Support.

9.4 Confidentiality

The investigator shall arrange for the secure retention of the patient identification and the code list in e-biobank. Patient files shall be kept for the maximum period of time permitted by each hospital. The study documentation (CRFs, Site File etc) shall be retained and stored during the study until 31.12, 2030 (the date defined in the REK application for the study). All information concerning the study will be stored in a safe place inaccessible to unauthorized personnel.

9.5 Database management

Data management will be performed by the study team. A data management plan and data control plan have been prepared describing the data management in detail. Appropriate SOP's are also prepared and can be found in the TMF along with the previously mentioned documents. A data handling report will be prepared following database closure.

The data will be stored in a dedicated and secured area at TSD at UIO/OUS with a backup located at an external drive kept locked in the office of the PhD student of the project and not together with the code-list. Data will be stored in a de-identified manner, where each study participant is recognisable by his/her unique trial subject number. The data will be stored until 2035 or later if very long term follow up will be conducted after consultation the Ethics committee at that point in time. The code-list connecting the personal identification of each patient to the study number will be kept on paper in the TMF locked in the investigators office and a digital copy will be made by use of the Nettskjema photo app and stored in tsd. An electronic backup will be made on an IronGuard encrypted memory.

10 Statistical methods and data analysis

10.1 Determination of Sample Size

A total of 58 patients will be recruited The number of dropouts is unknown, but the power calculation assumes only 58 randomized patients.

Assuming the time of hospitalization after randomization follows a Weibull distribution with a scale parameter $\lambda = 18$ and shape parameter $\kappa=2.2$ (corresponding to a median of 15 and an IQR of 10 – 21), we need to include 58 patients (18 in the placebo group, 10 in the 250 mg and 500 mg group and 20 in the 1000 mg group) in order to reach approximately 80% power to show a significant dose-response in this study with a maximal reduction in time of hospitalization of 33%. This calculation is based on optimal designs in dose-finding studies according to the MCPMod methodology with three alternative dose-response curves (linear, e-max and exponential)(Pinheiro, Bornkamp et al. 2014).

10.2 Randomization

10.2.1 Allocation- sequence generation

Key elements to specify regarding allocation of treatment are:

- The department of research support, OUS will generate the allocation sequence
- The allocation ratio in the three phases of the study will be 10:6, 10:6, 20:6 and 20:6
- Block randomization will be used.

10.2.2.Allocation- procedure to randomize a patient

The allocation sequence will be used to pre-package tablets of NR or placebo in separate boxes for each patient for the four phases of the study and to create envelopes for emergency un-blinding.
Each patient will receive one pre-packaged box with capsules for the whole study period when the first capsule is taken.

The OUS Department of Research Support will create the randomization list. NR capsules and placebo will hold the new FDA standard; food-good manufacturing practice (GMP) quality. Both will be provided from the largest producer of NR (Chromadex Inc, USA). Since the NR capsules are stable at room temperature and have a long shelf life, we consider distribution through the hospital pharmacy unnecessary and the study staff will distribute the study medication/placebo to the patients through the treating hospital ward or institution. After discharge study medication will be supplied to the patients themselves or necessary arrangements will be made with the home nursing staff where the patient lives. In patients unable to swallow capsules, but with nasogastric access, capsules may be opened and mixed with food/nutrition by the department staff, but not study staff.

10.2.3.Blinding and emergency un-blinding

The patients, department staff, investigators, personnel assessing outcomes and statistician/study staff preparing the data will be blinded to the allocation of patients until the database is locked.
For emergency un-blinding see 7.6

10.3 Population for Analysis

The following populations will be considered for the analyses:

Intention to treat (ITT) population: All randomized participants, regardless of protocol adherence.
Per-protocol population (PP): Includes all subjects who have consumed the appropriate dose of NR for more than 30 days (primary population)
Safety population: Includes all subjects who have received at least one dose of study medication. Subjects who withdraw from the study will be included in the safety analysis. A list of withdrawn subjects, with the reasons for withdrawal, will be made.

Primay population: the per-protocol population. **Total number of patients admitted to ICU or the following medical wards: infectious diseases, respiratory medicine, internal medicine or acute medicine during the inclusion period, n**
↓
1. Does not meet inclusion criteria, n → **Stop.** 2. Meet inclusion criteria, n
↓
1. Exclusion criteria present, n → **Stop.** 2. No exclusion criteria present, n
↓
1. Not consented to inclusion, n → **Stop.** 2. Consented and included, n

10.4 Planned analyses

The main statistical analysis is planned when

- The planned number of patients has been included OR the required number and more are included after a period of 2 years.
- All included patients have either finalized their last assessment or has/is withdrawn according to protocol procedures
- All data have been entered, verified and validated according to the data management plan

Prior to the main statistical analysis, the data base will be locked for further entering or altering of data. The treatment allocation will be revealed after the database lock and used in the statistical analysis.

Deviation from the original statistical plan will be described and justified in the Clinical Study Report. Amendments to plan can be done until day of DB lock.

10.5 Statistical Analysis

Details of the statistical analyses will be described in the Statistical analysis plan (SAP). An outline of the SAP is described here.

10.5.1 Primary Endpoint

Length of stay from randomization to discharge from hospital

The number of days will be calculated as number of hours/24 from randomization to final discharge from hospital (to home, long term care facility or rehabilitation centre or other facility with a lower care level). Admission to other hospitals after transfer from the first will be included in the total length of stay.

10.5.2 Secondary Endpoint of Special Interest:

Change in the level of frailty measured as grip strength at inclusion, on day 7, and on day 90. Should a patient withdraw from the study, grip strength will be measured at the withdrawal visit.

Grip strength is a simple measure used to indicate overall muscle strength (34, 33). It has been shown to inhabit health-related prognostic value and evidence suggests that it is a powerful predictor of future disability, falls, morbidity and mortality (33, 35-37). Grip strength will be measured in both arms using a dynamometer. Each participant will be tested twice on both the right and left, and the highest score on either side will be registered.

10.5.3 Other Secondary Endpoints:

Comparing the intervention group with the placebo group with regards to the following:

1. Time to normalization of blood pressure and resolution of fever.
2. Respirator days after randomization.
3. Length of stay from randomization to medically fit for discharge.
4. 90 days, one year and very long-term mortality from randomization.
5. Measure NAD+ and metabolite levels BEFORE, during and after acute illness in patients using NR and placebo.
6. Number of new infections and re-admission during follow-up.
7. If significant differences in endpoints are found in the NR and placebo group, we wish to investigate the following:
    a. Epigenetic mechanisms of how NR shortens recovery time after acute illness.
    b. To identify characteristics of the patients that benefits most from NR supplementation after acute illness.
    c. To identify the optimal NR dose yielding the largest reduction in recovery time.

    See also table 3 with secondary endpoints (except variables only collected at baseline)

End points related to epigenetic clocks

1. Influence of intervention on epigenetic age measured by available chronological and biological epigenetic clocks.
2. Influence of disease on available epigenetic clocks.
3. Predictive power of epigenetic clocks for the outcome of the intervention and disease.
    a. Epigenetic clock measurements at baseline and
    b. Epigenetic clock measurements obtained from old bio-banked samples.

10.5.4 Secondary analyses

Details on analysis of secondary endpoints (more details will be provided in the statistical analysis plan)

Variable	Definition	Definition 2	Statistical test
Blood pressure	Changes in blood pressure after inclusion (morning day of inclusion vs day 1 + 4 hours, day 3 and day 7).		Wilcoxon signed rank
Blood pressure II	Standardized blood pressure measured at inclusion and end of study visit.		T test for change from before study (latest measurement before start of disease) and start of study to end of study in SBT and DBT in intervention vs placebo group
Respiration support	Number of hours on respiration support from first tablet is taken to patient is off all respiration support for at least 12 hours.		Wilcoxon signed rank
Body temperature	Number of days with temperature above 38 at any point from inclusion to discharge.		Wilcoxon signed rank
Body temperature	Number of days with temperature above 38 at any point from inclusion to discharge divided on number of days from inclusion to discharge		T test for average in intervention vs placebo group.
NEWS score	Change in NEWS score from -4 hrs - 0 hrs before first tablet to 1, 3 , 7 days after first tablet		T test for average in intervention vs placebo group
Quality of life	EQ 5D (paper version) 2 weeks prior to admission, on day 1 and at 90 days		T test for average in intervention vs placebo group
Duration of stay in ICU	Hours after first capsule is taken to transfer to other non-ICU department or home		Wilcoxon signed rank
Number of infections	Number of newly diagnosed infections		Wilcoxon signed rank

	from inclusion to end of trial		
Number of infections 2	Number of newly diagnosed infections with identified agent from inclusion to end of trial		Wilcoxon signed rank
Changes in hearing (clinical examination, portable audiometer, tympanometer and otoscopy	Changes in hearing thresholds from first week of inclusion compared to 90 days after inclusion.		
Antibiotic use	Days on antibiotics after inclusion to end of trial		Wilcoxon signed rank
Antibiotic use 2	Days from inclusion to first antibiotic free day		Wilcoxon signed rank
Highest CRP	Highest CRP from inclusion to end of trial		T test for average in intervention vs placebo group
Frailty	1) 4 meters walking test and grip strength measured at inclusion, day 7, and at 90 days. 2) Clinical Frailty Score 2 weeks prior to admission and at 90 days.		T test for average in intervention vs placebo group
Cognitive function	Forward and backward recall, trail making test and MOCA at day 7* and 90 and week 65.		T test for average in intervention vs placebo group
NAD+ metabolites			
Epigenetic age changes	Hannum, Horvath I, Levine Phenoage, Horvath GrimAge, Zhang, two of the most relevant and newer algorithms available at the time of analysis	Change from baseline to end of treatment. Predictive power of previous epigenetic age. Prediction of treatment outcome.	T test for average in intervention vs placebo group and vs the dedicated non-hospitalized control group
DNA methylation genome wide	Differentially methylated regions between baseline and end of study visit and between treatment groups		Genome wide significance.
Histone modifications	See 9.5.4		

*Or on discharge if before day 7, or later if the patient cannot be tested on day 7.

10.5.5 Safety analyses

See SAP.

Tabular safety analyses will be performed for all collected variables.

10.5.6 Exploratory analyses

Histone modifications and DNA methylation.

Our lab possess world leading ChIP-seq technology as evidenced by recent breakthroughs (Dahl, et al, Nature 2016) which we will use to connect the mechanism of action of NR to the clinical findings. The proposed mechanism of action of NR is restoration of intracellular NAD+ which is a rate limiting substrate for the epigenetic modifier SIRT1. SIRT1 is a lysine deacetylase which to large degree targets histones and thus acts as a genetic modifier directly affecting gene expression. We will do whole genome ChIP-seq analyses for the most important histone acetylations like H3K9ac and H4K16ac before and after treatment in all participants to identify which histones are differentially acetylated in the intervention group and to explore the genomic context of these histones.

10.5.7 Other analyses (eg health economics, patient reported outcomes etc)

Economic costs per group will be calculated and compared.

10.5.8 Descriptive statistics

The following variables will be analyzed descriptively:

Age
Gender
Ethnicity
NEWS score
Laboratory tests at inclusion
Vital measurements
Diabetes I
Diabetes II
Heart failure
Liver disease
COPD
Myocardial infarction
Peripheral vascular disease
Stroke/TIA
Dementia
Rheumatic disease/Connective tissue disease
AIDS
Type of housing before admittance
ECOG score before admission
All questionnaires used in baseline assessment.
Worst NEWS score
Highest CRP
Respiratory support at during hospitalization before inclusion
Lowest GFR
Lowest blood Ph
Lowest MAP
Lowest Glasgow coma scale
SAPS II score at admittance

11 STUDY MANAGEMENT

11.1 Steering committee

A steering committee with representatives from participating departments will meet regularly during the study period to evaluate safety, administrative issues, progression, results and advice the PI on study related questions.

11.2 Investigator Delegation Procedure

The principal investigator is responsible for making and updating a "delegation of tasks" listing all the involved co-workers and their role in the project. He will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information of relevance to the performance of this study is forwarded to the staff involved.

11.3 Protocol Adherence

Investigators ascertain they will apply due diligence to avoid protocol deviations.
All significant protocol deviations will be recorded.

11.4 Study Amendments

If it is necessary for the study protocol to be amended, the amendment and/or a new version of the study protocol (Amended Protocol) must be notified to and approved by the the Ethics Committee.

12 Ethical requirements

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice and applicable regulatory requirements. Registration of patient data will be carried out in accordance with national personal data laws.

12.1 Ethics Committee Approval

The study protocol, including the patient information and informed consent form to be used, will be approved by the regional ethics committee before enrolment of any patients into the study.
The investigator is responsible for informing the ethics committee of any serious and unexpected adverse events and/or major amendments to the protocol as per national requirements.
The project has been approved by the Regional Ethics Committee with ID number: 2017/1334C.

12.2 Trial registration and user Involvement

The trial was registered in www.clinicaltrials.gov during autumn 2018, and will be activated once inclusion of participants commences.
Users appointed by two patient's organizations have been involved in designing the study and will be involved in conducting the study.

12.3 Informed Consent Procedure

The investigator is responsible for giving the patients full and adequate verbal and written information about the nature, purpose, possible risk and benefit of the study. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.
It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever she/he wants. This will not prejudice the patient's subsequent care. Documented informed consent will be obtained for all patients included in the study before they are registered in the study.

A copy of the patient information and consent will be given to the patients. The signed and dated patient consent forms will be filed by the investigator. A professional interpreter will be used if required.

12.4 Subject Identification

The investigator is responsible for keeping a list of all patients (who have received study treatment or undergone any study specific procedure) including patient's date of birth and personal number, full names and last known addresses.
The patients will be identified in the eCRFs by patient number, initials and date of birth.

13 Trial sponsorship and financing

The study is sponsored by Norsk Forskiningsråd with project number 288579. ChromaDex (USA), the company producing the NR supplies NR and placebo for free.

14 Trial insurance

Since True Niagen is classified as a nutritional supply and not a drug, the Produktansvarslov does not apply. Participants may however apply for compensation to the the Norwegian Health Service Litigation Authority (Norsk Pasienterstatning) should they suffer injury or develop adverse reactions to NR.

15 Publication policy

Upon study completion and finalization of the study report, the results of this study will be submitted for publication in an international peer reviewed journal.
All personnel who have contributed significantly with the planning and performance of the study (Vancouver convention 1988) may be included in the list of authors.

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17 APPENDICES

A. Forms and questionnaires

B. Safety committee charter
C. Statistical analysis plan
D. Consent form
E. Amendment 1