This supplement contains the following items:

1. Study Protocol
    - Original protocol: Version 1.0, dated May 9th, 2018.
    - Final protocol: Version 2.0, dated January 3rd, 2019.
    - Summary of all changes made between the first and final protocol (i.e. Final protocol – Appendix 6: Amendment history).

2. Statistical Analysis Plan
    - Original Statistical Analysis Plan: Version 1.0, dated January 17th, 2022.
    - Final Statistical Analysis Plan: Version 2.0, dated March 17th, 2022
    - Summary of all changes made between the first and final Statistical Analysis Plan (i.e. Final statistical analysis plan – section 1: Document history).

3. Summary of Changes
    - Between original (V1.0) and final (V2.0) protocol.
    - Between original (V1.0) and final (V2.0) Statistical Analysis Plan.

Original Protocol

Version - 1.0

9th of May 2018

FULL/LONG TITLE OF THE TRIAL

A multi-center, randomized, double-blind, phase IV clinical trial on the diuretic effects of Acetazolamide (Diamox ®) in patients with Decompensated heart failure and Volume OveRload

SHORT STUDY TITLE / ACRONYM

Acetazolamide (Diamox ®) in Decompensated heart failure with Volume OveRload (ADVOR)

PROTOCOL VERSION NUMBER AND DATE

Version number: version 1.0
Version date: 09 May, 2018

RESEARCH REFERENCE NUMBERS

EudraCT Number:	2018-001345-14
Clinical trials.gov Number:	NCT03505788
SPONSOR Number:	ZOLCAR17001
KCE Trial Number:	KCE-17001

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■ SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the requirements for the conduct of clinical trials in the EU as provided for in " Directive 2001/20/EC", and any subsequent amendments, GCP guidelines, the Belgian law of May 7th 2004 regarding experiments on the human person, the Sponsor's SOPs, and other regulatory requirements as amended.

The undersigned agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

The undersigned also confirm that they will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:	
Signature:	Date:
[redacted] General Director **Ziekenhuis Oost-Limburg AV**	14/05/2018
Signature:	Date:
[redacted] Chairman **Ziekenhuis Oost-AV**	14/05/2018
Signature:	Date:
[redacted] Medical Director **Ziekenhuis Oost-Limburg AV**	11/5/18

[redacted]

Ziekenhuis Oost-Limburg

Chief Investigator:
Signature:

[redacted]

Date
May 9, 2018.

Statistician:
Signature

[redacted]

Head of CenStat - Hasselt University

Date
May 9, 2018

Acknowledged by **Funder (KCE)**
Signature:

[redacted]

Date:
14/5/2018

Print name:

[redacted]

KEY TRIAL CONTACTS

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Sponsor	Ziekenhuis Oost Limburg Autonome Verzorgingsinstelling Schiepse bos 6, 3600 Genk, Belgium Main contact person: Evi Theunissen Phone: [redacted] E-mail: [redacted] Legal contact persons: Joke Vanlangenaeker and Martine Verplancke Phone: [redacted] / E-mail: [redacted] Although KCE provides funding for the trial, KCE shall under no circumstances be considered as sponsor of the Study or assume any responsibilities or liabilities in connection therewith, and sponsor or co-sponsor shall make no representations whatsoever in this respect.
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Committees	Trial Steering Committee Trial Management Group Endpoint Adjudication Committee *See below for more information regarding these committees*

TRIAL SUMMARY

Trail Title	A multi-center, randomized, double-blind, phase IV clinical trial on the diuretic effects of Acetazolamide (Diamox ®) in patients with Decompensated heart failure and Volume OveRload
Short title	Acetazolamide (Diamox ®) in Decompensated heart failure with Volume OveRload
Clinical Phase	IV
Study type	Interventional
Planned sample size	Approximately 519 patient
Trial duration	Approximately 27 months (3 months follow-up / patient)
Planned Trial Period	2018-2021
Purpose and rationale	To investigate if combination therapy with acetazolamide improves loop diuretic efficacy to increase diuresis in decompensated heart failure (HF) patients, allowing for a better/faster decongestion and potentially resulting in improved clinical outcome and increased quality of life.
Primary endpoint	Treatment success (decongestion achieved) on the morning of day 4 without the need for escalating diuretic strategy (doubling loop diuretic dose, addition of chlorthalidone, or ultrafiltration) on the morning of day 3
Secondary endpoints	1. Combined end-point of all-cause mortality and heart failure readmission during 3 months of follow-up 2. Length of index hospital admission 3. Longitudinal changes in EuroQoL five dimensions questionnaire (EQ-5D) (baseline, the morning of day 4, any readmission, and 3 months).
Trial Design	The ADVOR study is a phase IV randomized, multi-center, double-blind study, comparing monotherapy with high-dose loop diuretics (standard of care - SOC) versus combination therapy with acetazolamide and high-dose loop diuretics in patients with decompensated heart failure and clinical signs of volume overload. The study consists of 3 phases: • screening phase: starting from identifying a study subject prior to / during hospitalization until the first dose of study medication will be given • treatment phase: starting from the first dose of study medication administration until the morning of day 4 or earlier in case of successful decongestion sooner. • follow-up phase: starting when the treatment phase ends until 3 months after the study start dose.
Trial Participants	The study population will consist of patients hospitalized with decompensated HF and demonstrating at least one clinical sign of volume overload.
Main Inclusion Criteria	• An elective or emergency hospital admission with clinical diagnosis of ADHF and at least one clinical sign of volume overload (e.g. oedema, ascites or pleural effusion) • Maintenance therapy with oral loop diuretics at a dose of at least 1 mg bumetanide or 40 mg furosemide or 20 mg torsemide for at least 1 month before hospital admission • Plasma NT-proBNP levels >1000 ng/mL or BNP levels >250 ng/mL at screening

Main Exclusion Criteria	• Concurrent diagnosis of an acute coronary syndrome defined as typical chest pain in addition to a troponin rise above the 99th percentile and/or electrocardiographic changes suggestive of cardiac ischemia • A previous or current diagnosis of hypertrophic, restrictive, or constrictive cardiomyopathy as documented in the medical record • History of congenital heart disease requiring surgical correction • History of cardiac transplantation and/or ventricular assist device • Systolic blood pressure <90 mmHg or mean arterial pressure <65 mmHg at the moment of admission • Expected use of intravenous inotropes, vasopressors or nitroprusside during the study. Use of nitrates is allowed only if the patient's systolic blood pressure is >140 mmHg • Estimated glomerular filtration rate (eGFR) <20 mL/min/1.73m² at screening • Use of renal replacement therapy or ultrafiltration at any time before study inclusion • Treatment with intravenous loop diuretics > 2 mg bumetanide or an equivalence of another loop diuretic during the index hospitalization before randomization • Treatment with acetazolamide during the index hospitalization before randomization • Exposure to nephrotoxic agents (i.e. contrast dye) anticipated within the next 3 days • Use of any non-protocol defined diuretic agent with the exception of mineralocorticoid receptor antagonists. Thiazides, metolazone, indapamide and amiloride should be stopped upon study inclusion. If patient is taking a combination drug including a thiazide-type diuretic, the thiazide-type diuretic should be stopped upon study inclusion. • Current use of sodium-glucose transporter-2 inhibitors • Subjects who are pregnant or breastfeeding
Exploratory tertiary end-points	• Body weight change after day 1, 2, 3, 4 and discharge compared to admission • All-cause mortality during first 3 months after study start dose • Heart failure readmissions during first 3 months after study start dose • All cause rehospitalisation during first 3 months. after study start dose • Total urinary volume and natriuresis starting from first intravenous (IV) diuretic administration at randomization until the morning of day 3 (urinary output, see figure 1 and appendix 4) • Relative plasma BNP or NT-proBNP change from baseline until the assessment of the secondary endpoint on day 4 and at 3 months • Total dose of IV loop diuretics used during first 4 days • Changes in doses of neurohumoral blockers from baseline to discharge and after 3 months. • Need for renal replacement therapy or ultrafiltration during first 3 months after study start dose • Incidence of hyponatremia during treatment phase • Hypokalaemia during treatment phase • Incidence of metabolic acidosis requiring NaHCO3 supplements during first 4 days • WRF defined as a >0.3 mg/dL increase in serum Cr, or a >20% decrease in eGFR by the CKD-EPI formula during treatment phase • Liver dysfunction on admission • plasma volume changes during treatment phase (assessed by

	albumin and hematocrit) • Occurrence of iron deficiency on admission Optional laboratory sub-study in participating centers: • Change from baseline in selected biomarkers from baseline through 3 months after study start dose in a subset of randomized patients
Safety assessments	• Adverse events • Laboratory values (including monitoring hypokalaemia, metabolic acidosis, substantial increase in creatinine (Cr), substantial decrease in eGFR)
Data analysis	• The treatment effect for the primary end-point is evaluated by means of a generalized linear mixed model. The statistical model will include a fixed treatment effect and random center effect. • For the first secondary end-point [occurrence of the combined endpoint of all-cause mortality and heart failure re-admission during 3 months of follow-up], a generalized linear mixed model for a binary outcome will be used. The model will incorporate a fixed treatment effect and random center effect. If the treatment effect on the composite endpoint of 'all-cause mortality and HF readmission' turns out to be statistically significant, both components will be evaluated separately in a hierarchical fashion with HF readmissions first and all-cause mortality second. • Length of index hospitalization and change in quality of life scores are compared among treatment arms with a linear mixed model (fixed treatment effect and random center effect). Transformation will be employed when the model assumptions (such normality) are violated. • All hypothesis are 2-sided and tested with a significance level of $\alpha=0.05$

ROLE OF STUDY SPONSOR AND FUNDER

Ziekenhuis Oost Limburg autonome verzorginsinstelling (ZOL AV), as mentioned in KEY TRIAL CONTACT shall act as sponsor of the Study, as defined in the Law of 2004, and in accordance with the Law of 2004, the sponsor shall assume, even without fault, the responsibility of any damage incurred by a study patient or, in the case of death, his rightful claimants sustained that arises either in direct or indirect connection with the experiments and shall provide compensation therefore. The Sponsor shall enter into an insurance contract in accordance with article 29 of the Law of 2004. ZOL AV shall ensure that it shall be mentioned in the Protocol, the Informed Consent Forms and in other relevant communication with the Study Subjects or the Regulatory Authorities as sponsor of the Study.

ZOL AV has designed the trial together with the aforementioned Study steering committee. ZOL AV and the steering committee will be responsible for the data analysis (with assistance of the “center of statistics”, University Hasselt), interpretation, manuscript writing, and dissemination of results. ZOL AV will have the final decision regarding any of these aspects of the trial. However, publication of the main study results will be the responsibility of the steering committee. Even in case of a negative study result, the data will be published.

ZOL AV acknowledges and agrees for the avoidance of doubt that KCE shall under no circumstances be considered as sponsor of the Study or assume any responsibilities or liabilities in connection therewith, and ZOL AV shall make no representations whatsoever in this respect.

ROLES AND RESPONSIBILITIES OF TRIAL MANAGEMENT COMMITEES

Trial Steering Committee (TSC)

The role of the Trial Steering Committee (TSC) is to provide the overall supervision of the trial. The TSC includes members who are independent of the investigators, their employing organisations or institutions, funders and sponsors. The TSC monitors trial progress, conduct and advise on scientific credibility. The TSC will consider and act, as appropriate, and ultimately carries the responsibility for deciding whether a trial needs to be stopped on grounds of safety or efficacy. The TSC shall oversee the performance of the study and discuss important topics in relation thereto.

The TSC will meet on average 3 times per year or as necessary when adapted to the stage of the trial (set-up, conduct, analysis). The TSC is composed of the CI, trial statistician, the trial PM, 7 independent experts, minimum 2 representatives of other participating centers with at least one representative of the French speaking sites and one representative of the Dutch speaking sites, up to 2 representatives of patients or the general public, 1 representative of the sponsor and 1 representative of the funder. The TSC will send reports to the sponsor and the funder. KCE shall have the right (but not the obligation) to be present at each TSC meeting.

Details of the final members of the TSC, their responsibilities, number of meetings and reporting procedures can be found in the TSC charter.

Trial Management Group (TMG)

The TMG includes those individuals responsible for the day-to-day management of the trial, such as the Chief Investigator, statistician, trial manager, and data manager. The role of the group is to monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself.

Chief Investigator: Wilfried Mullens (ZOL AV, Genk, Belgium)

Statistician: Liesbeth Bruckers (University Hasselt, Belgium)

Trial Manager: Katrien Tartaglia (ZOL AV, Genk, Belgium)

Data Manager: Liesbet Van Brussel (ZOL AV, Genk, Belgium)

Data Safety Monitoring Committee (DSMC)

The DSMC is not needed as this is a low-risk pragmatic interventional trial with a short inclusion period studying an old drug with a well-known safety profile within an accepted clinical indication.

Endpoint Adjudication Committee (EAC)

The EAC will adjudicate the HF-related endpoints. The EAC will meet at regular intervals throughout the course of the study to assess events, and determine whether these events should contribute to the secondary endpoints of the ADVOR trial. HF readmissions are defined as either a hospital admission because of decompensated HF or an unscheduled contact at the emergency department for worsening

HF if the patient is treated with intravenous loop diuretics. The EAC will have at least two members, specializing in heart failure management. None of the EAC members will be participating investigators in the ADVOR trial.

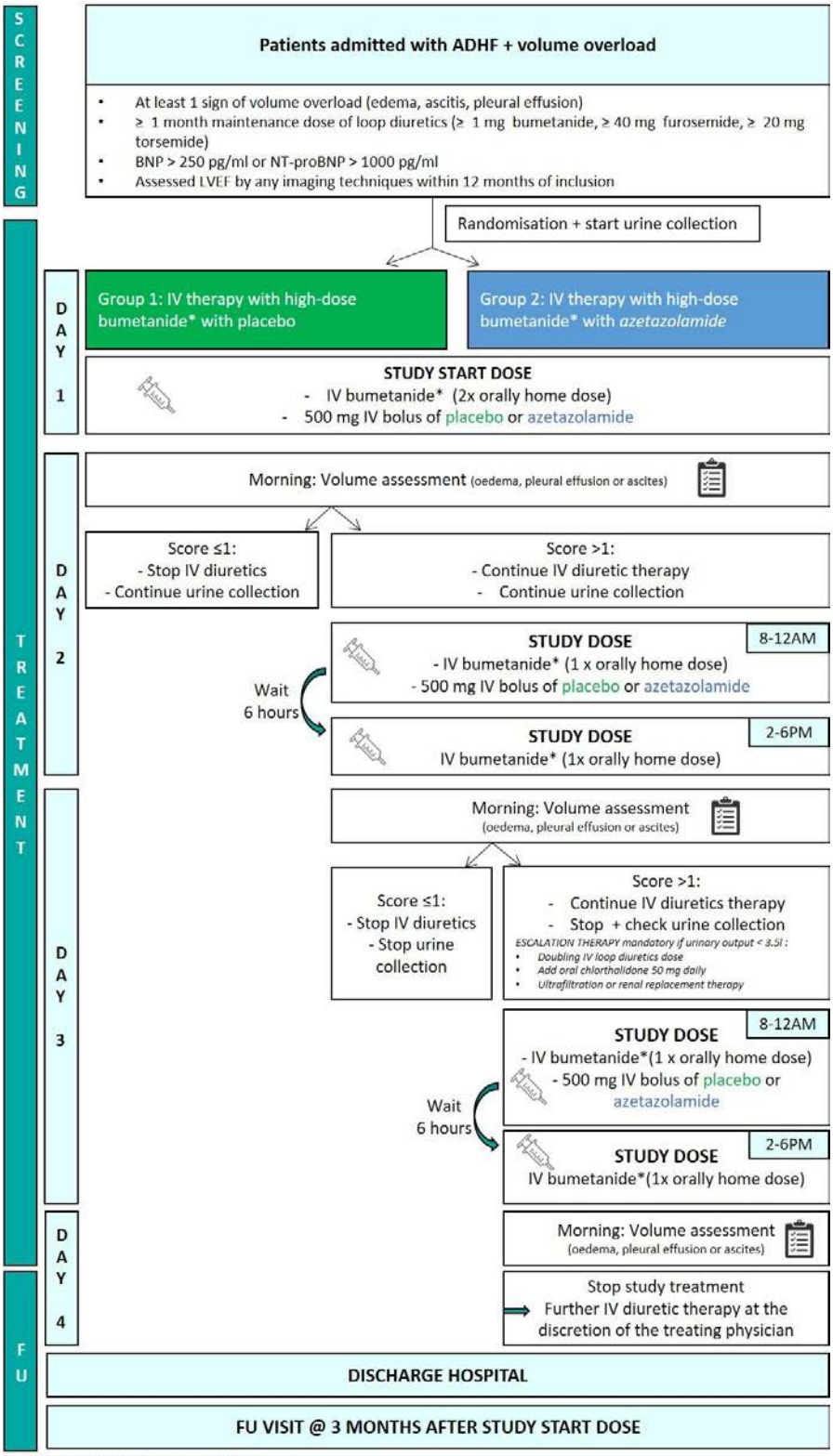
LIST OF ABBREVIATIONS

ADHF	Acute Decompensated Heart Failure
AE	Adverse Event
BID	“Bis in die” (twice a day)
BWGHF	Belgian Working Group of Heart Failure
BNP	B-type natriuretic peptide
CI	Chief Investigator
Cr	Creatinine
EAC	Endpoint Adjudication Committee
eCRF	Electronic Case Report Form
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
EQ-5D	EuroQol five dimension (questionnaire)
ESC	European society of Cardiology
GCP	Good Clinical Practice
GMP	Good Manufacture Practice
HFA	Heart Failure Association
HF	Heart Failure
HFpEF	Heart Failure with preserved Ejection Fraction
HFrEF	Heart Failure with reduced Ejection Fraction
HTA	Health technology assessment
IEC	Independent ethics committee
IMP	Investigational medicinal product
IRT	Interactive response technology
ISF	Investigator Site File
IV	Intravenous
NT-proBNP	N-terminal of pro-B-type natriuretic peptide
MG	milligram
PI	Principal Investigator
PM	Project Manager
QoL	Question of Life
RCT	Randomised Clinical Trial
RRT	Renal replacement therapy
SD	Source document
SI	Sub-investigator
SOC	Standard of care
TMG	Trial Management Group

TSC	Trial Steering Committee
VAS	Visual analogue scale
WRF	Worsening renal function
ZOL AV	Ziekenhuis Oost Limburg Autonome Verzorgingsinstelling

TRIAL FLOW CHART

Figure 1. Trial flow Chart.



*bumetanide is preferred loop diuretic agent
Conversion factor is 1 mg bumetanide = 20mg torsemide = 40 mg furosemide (IV and oral)
Bolus of bumetanide is limited to 5 mg bumetanide

1 BACKGROUND

Aging of the population and prolongation of the lifespan of cardiac patients by modern therapeutic innovations have led to an increased incidence of HF (1). During the last two decades, important progress has been made in the treatment of ambulatory HFrEF patients. Renin-angiotensin system blockers, β-blockers, mineralocorticoid receptor antagonists, ivabradine, neprilysin inhibition, and cardiac resynchronization therapy have all been demonstrated to reduce morbidity and/or mortality in ambulatory HFrEF patients (2-18).

Despite these important advances, many patients are still hospitalized frequently with decompensated HF demonstrating most often signs and symptoms of systemic congestion and volume overload, which is associated with worse outcome (19). Treatment in these cases mainly focuses on symptomatic relief through administration of diuretics, although clear evidence on the optimal agent, dosing schedule, and administration route is lacking. Coexisting renal dysfunction often complicates decongestive treatment and worsening renal function (WRF), often defined as a 0.3 mg/dL rise in serum Cr, is a common finding in this context (20). However, the prognostic impact of WRF defined as Cr change is unsure as it might be associated with worse, neutral or even better outcome (21-23). In contrast, persistent congestion and volume overload, as a reflection of the renal inability to preserve sodium homeostasis, has been more consistently associated with higher mortality and more frequent readmissions in HF (24). This suggests that achieving a net negative fluid balance might be an attractive treatment target in decompensated HF.

Loop diuretics are by far the most commonly used agents to achieve decongestion in decompensated HF. Especially in diuretic-naïve patients, they are often very effective to relief dyspnoea and congestive symptoms. However, in the Diuretic Optimization Strategies Evaluation (DOSE) trial, which is the only randomized clinical trial on diuretic therapy for decompensated HF patients, no differences in patients' global assessment of symptoms or change in renal function were observed when loop diuretics were administered by bolus as compared with continuous infusion or at high versus low dose during a hospitalization for decompensated HF (25). Also, only a minority of patients (15%) were adequately decongested after 72 h in the DOSE trial, thereby indicating the urgent need for more effective decongestive therapies. Furthermore, guidelines from international cardiac societies lack high-quality data on the optimal dosing, timing and method of delivery of diuretic agents. Importantly, there are several reasons why loop diuretics might be less effective or even harmful in HF. First, loop diuretics directly stimulate renin production by inhibiting the $Na^+/K^+/2Cl^-$-cotransporter on the luminal side of the macula densa, which depletes intracellular chloride levels in the macula densa. The consequence is an increased cyclo-oxygenase-2 and nitric oxide synthase I activity in macula densa cells, leading to paracrine prostaglandin E_2 and nitric oxide secretion (26). Both prostaglandin E_2 and nitric oxide work in concert to stimulate renin release by granulosa cells of the afferent arteriole and further detrimental activation of the renin-angiotensin-aldosterone axis. Second, impaired secretion of loop diuretics in the proximal tubules of HF patients, especially when there is concomitant renal dysfunction, results in lower concentrations at the place where these agents act – the luminal side of the thick ascending limb of Henle's loop. Third, increased sodium reabsorption in the proximal tubules might result in less sodium offered to the thick

ascending limb of Henle's loop, especially if glomerular filtration is concomitantly impaired, hampering the efficacy of loop diuretics.

Recent advances in heart failure (HF) biomarker studies suggest promise from markers enhancing traditional method of assessing affected patients. B-type natriuretic peptide (BNP) and its biologically inert, amino-terminal pro-peptide counterpart (NT-proBNP)[32,33] have quickly become an essential component in the diagnosis and determining prognosis in HF. With a large number of biomarkers now or soon to be available, an understanding of the role that biomarkers may play in HF care is necessary.

2 RATIONALE

From a pathophysiological point of view, targeting sodium reabsorption in the proximal tubules has several potential benefits in HF. First, it is the place where most sodium is reabsorbed, especially in decompensated HF. Second, greater delivery of chloride to macula densa cells decreases renin production, ceasing neurohumoral activation. Third, endogenous natriuretic peptides (acting in the distal nephron) will possibly regain their effects (27). The carbonic anhydrase inhibitor acetazolamide (Diamox®), which is approved for the treatment of mountain sickness, inhibits sodium reabsorption in the proximal tubules. Despite the pathophysiological rationale for inhibition of proximal sodium reabsorption in decompensated HF, acetazolamide is now a largely forgotten diuretic. One observational study in patients with decompensated HF and marked volume overload found that the addition of acetazolamide improved loop diuretic efficacy with ~100 mmol Na+ excreted per 40 mg of furosemide equivalent dose (28). Thus, although the diuretic and natriuretic capacity of acetazolamide is poor on its own, it might well be a very efficient booster of diuretic efficacy in combinational diuretic therapy with loop diuretics. This concept is further supported by one small randomized trial including 24 patients with volume overload refractory to loop diuretic therapy (29). All these patients demonstrated a greatly reduced fractional sodium excretion, which was easily overcome by the addition of acetazolamide. We've conducted a small two-center trial to see if improved diuretic efficacy with acetazolamide in a patient population with heart failure and cardio-renal syndrome at high risk for diuretic resistance translates into better natriuresis. (Clinical Trial NCT01973335). The study has just been finished with analysis of the results ongoing. Importantly, the promising concept of blocking proximal nephron sodium absorption with acetazolamide has been published over the last couple of years (30).

The ADVOR study has an innovative primary end-point. As abundant evidence has consistently linked persistent volume overload after decongestive therapy in decompensated HF with poor outcomes, decongestion itself is a valid surrogate end-point (24). It has been demonstrated from reanalysis of the DOSE trial and CARRESS that the persistent oedema has excellent prognostic ability to predict death, readmissions or unscheduled medical contacts (31). In ADVOR, a more exhaustive congestion score with the emphasis on relief of volume overload will be used. Secondary end-points in ADVOR will be very clinically relevant. A more thorough decongestion should also translate into less readmissions for recurrent decompensation, better renal preservation, and eventually lower mortality. This will be assessed as a key secondary end-point after hospital stay. Additionally, time to decongestion is a major determinant of hospital stay and combinational therapy with acetazolamide might significantly shorten

this. Finally, improved quality of life for patients is expected with better decongestion and will be the last secondary end-point. Importantly, acetazolamide is an easy and cheap drug to use (add on, bolus infusion, no special monitoring required), with a potentially favourable cost-efficiency profile. Important health economic data, specifically for the Belgian situation, will be obtained through the ADVOR study.

During the ADVOR study centers can decide to participate in the optional laboratory sub-study. The urine and blood samples collected through this laboratory sub-study will be stored in the University Biobank of Limburg and it will become the biggest databank of its kind within diuretic studies for patients with decompensated heart failure.

This laboratory sub-study will investigate more in detail the mechanistic and potential favourable effect of acetazolamide and loop diuretics. Furthermore, this laboratory sub-study will provide new insights into the pathophysiology of decompensated HF and potentially will allow for identifying a high risk patient population. This could ultimately lead to improved and patient tailored treatment strategies. Biomarker sub-studies have become a valuable source of data fur such analysis, and offer unique insights in to mechanistic and pathophysiologic pathways in a well selected and phenotyped patient population.

2.1 Assessment and management of risk

The study will examine if the addition of acetazolamide will lead to a better decongestion in decompensated HF patients with volume overload. It's expected the better decongestion will lead to less HF readmissions, reduced all-cause mortality, improved quality of life, reduced hospital stay duration and significant reduction in HF related health care expenditure.

The ADVOR trial will investigate if adding acetazolamide, which can be very easily administered, in every hospital, without additional extra testing or invasive monitoring, through a bolus infusion in acute decompensated heart failure (ADHF) patients might lead to faster, safer and easier decongestion. If proven beneficial, this approach can easily be adopted by every hospital in a quick manner with considerable cost-savings with regards to health care expenditure and improvements of quality of life for patients.

Importantly, ADVOR will examine if an improved application of existing decongestive therapies (not novel drugs), based on strong scientific reasoning, will result in a better outcome for patients and society. Therefore, data from the study will provide information regarding the safety and efficacy of acetazolamide treatment in above mentioned patient population.

As such, the ADVOR study was specifically designed to have maximum benefit without additional risk for this frail patient population. The study will:

1) Be conducted with limited additional testing
2) Have minimal or no additional expected risk for the patient (comparison of standard diuretic regimen with standard diuretic regimen + addition of acetazolamide)

3) Have very clinically meaningful endpoints. Achieving a faster decongestion with reduced risk for escalation of therapy, which often increases complication rates as well as length of hospital stay. This will be beneficial for the patients in the short-term. Additionally, a more thorough decongestion should also translate into less readmissions for recurrent decompensation and improved quality of care

Therefore, ADVOR can be considered a 'Low-intervention clinical trial' as:

1) Acetazolamide, the investigational medicinal products, which already has been authorised, has a very low risk profile and is well-known to the general cardiologist
2) According to the protocol of the clinical trial, Acetazolamide the investigational medicinal product, will be used in accordance with the terms of the marketing authorisation
3) The additional diagnostic or monitoring procedures do not pose more than minimal additional risk or burden to the safety of the subjects compared to normal clinical practice

3 OBJECTIVES AND ENDPOINTS / OUTCOME MEASURES

ADVOR will investigate if combination therapy with acetazolamide improves loop diuretic efficacy to increase diuresis in decompensated HF patients with volume overload, allowing for a better/faster decongestion and a lower total dose of loop diuretics. A better / faster decongestion should lead to less HF readmission, reduced all-cause mortality, shorter length of stay and improved quality of life.

3.1 Primary objective

The ADVOR study is a phase IV randomized, multi-center, double-blind study, comparing monotherapy with high-dose loop diuretics (standard of care - SOC) versus combination therapy with acetazolamide and high-dose loop diuretics in patients with decompensated HF and clinical signs of volume overload. While the SOC results in 15% effective decongestion, it's estimated - based on strong scientific reasons - that the combination therapy should have a success rate of 25%, which represents a clear meaningful benefit of 10% more patients with appropriate decongestion after 72 h.

- Population; patients hospitalized with decompensated HF and demonstrating signs of volume overload.
- Intervention: combination therapy with high-dose loop diuretics + acetazolamide
- Comparison: monotherapy with high-dose loop diuretics + placebo
- Outcome: % decongestion, need for escalating diuretic therapy, HF readmission, all-cause mortality, length of stay, QoL
- Time: 72 h for primary endpoint, 3 months for secondary endpoint

3.2 Secondary objectives

1. Combination therapy with acetazolamide improves loop diuretic efficacy to increase diuresis in decompensated HF patients, allowing for a better/faster decongestion and a lower total dose of loop diuretics.
2. Combination therapy with acetazolamide leads to lower occurrence of diuretic resistance and escalating diuretic therapy in decompensated HF
3. Combination therapy with acetazolamide will potentially lead to improved clinical outcome in decompensated HF (less heart failure readmissions, lower all-cause mortality)
4. Combination therapy with acetazolamide will shorten the length of stay in patients with decompensated HF, which is expected to reduce health care expenditure
5. Combination therapy with acetazolamide will potentially lead to improved quality of life

3.3 Endpoints

The ADVOR study has an innovative primary end-point. As abundant evidence has consistently linked persistent volume overload after decongestive therapy in decompensated HF with poor outcomes, decongestion itself is a valid surrogate end-point. It has been demonstrated from reanalysis of the DOSE trial and CARRESS that the persistent oedema has excellent prognostic ability to predict death,

readmissions or unscheduled medical contacts. In ADVOR, a more exhaustive yet easy to use – 'volume assessment' score - will be used. Importantly, ADVOR will be a double blind randomized trial thereby excluding any potential for bias in the clinical judgement of the treating physician for any of the endpoints including the primary endpoint of decongestion.

Secondary endpoints in ADVOR will be very clinically relevant. A more thorough decongestion should also translate into less readmissions for recurrent decompensation, and eventually lower mortality. This will be assessed as a key secondary end-point after hospital stay. Additionally, time to decongestion is a major determinant of hospital stay and combinational therapy with acetazolamide might significantly shorten this. Finally, improved quality of life for patients is expected with better decongestion and will be the last secondary endpoint. Importantly, acetazolamide is an easy drug and very cheap drug to use (add on, bolus infusion, no special monitoring required), which will therefore be easily adopted by the health care. Endpoints have also been discussed with "Mon Coeur Entre Parenthèses" which is a HF patient association (VZW) representing HF patients and their peers and deemed to be important by them.

In addition, the primary and secondary outcome measures are in line with the COMET (Core Outcome Measures in Effectiveness Trials) initiative. Indeed 'decongestion' (being dry is for the patient a very important improvement in symptomatology), heart failure readmission, all-cause mortality, length of stay, quality of life….can all be considered standardised relevant core outcomes sets. Therefore, the core outcomes relevant for any acute heart failure study will be collected and reported, making it easier for the results of ADVOR to be compared, contrasted and combined as appropriate with other trials (Zannad F et al, European Journal of Heart Failure, 2013;15:1082-1094). Additionally, we will continue to explore other tertiary outcomes, which are often mechanistically very interesting, as well.

We will also collect the dosages of neurohumoral blocker therapy throughout the study period, thereby hopefully facilitating a better implementation of guideline recommended therapy. As guidelines recommend a specific dosage for each of these drugs in HFrEF, it's easy to standardize the intake of such medications to be used in an accurate analysis. This will be done as an exploratory tertiary end-point.

3.4 Primary endpoint

Treatment success (decongestion achieved) on the morning of day 4 without the need for escalating diuretic strategy (doubling loop diuretic dose, addition of chlorthalidone, or ultrafiltration) on the morning of day 3.

3.5 Secondary endpoints

1. Combined end-point of all-cause mortality and heart failure readmission during 3 months of follow-up
2. Length of index hospital admission
3. Longitudinal changes in EuroQoL five dimensions questionnaire (EQ-5D) (baseline, the morning of day 4, at any readmission, 3 months)

3.6 Exploratory tertiary endpoints

1. Body weight change after day 1, 2, 3, 4 discharge compared to admission
2. All-cause mortality during first 3 months after study start dose
3. Heart failure readmissions during first 3 months after study start dose
4. All cause rehospitalisation during first 3 months after study start dose
5. Total urinary volume and natriuresis starting from first intravenous (IV) diuretic administration at randomization until the morning of day 3
6. Relative plasma BNP or NT-proBNP change from baseline until day 4 and at 3 months follow up visit
7. Total dose of IV loop diuretics used during first 4 days
8. Changes in doses of neurohumoral blockers from baseline to discharge and after 3 months.
9. Need for renal replacement therapy or ultrafiltration during first 3 months after study start dose
10. Hyponatremia during treatment phase
11. Hypokalaemia during treatment phase
12. Incidence of metabolic acidosis requiring NaHCO3 supplements during first 4 days
13. WRF defined as a >0.3 mg/dL increase in serum Cr, or a >20% decrease in eGFR by the CKD-EPI formula during treatment phase
14. Liver dysfunction on admission
15. plasma volume changes during treatment phase (assessed by albumin and hematocrit)
16. Occurrence of iron deficiency on admission
17. Optional laboratory sub-study in participating centers: Change from baseline in selected biomarkers from baseline through 3 months after study start dose in a subset of randomized patients

4 TRIAL DESIGN

The ADVOR study is a phase IV randomized, multi-center, double-blind study, comparing monotherapy of high-dose loop diuretics (SOC) versus combination therapy of acetazolamide with high-dose loop diuretics in patients with decompensated HF and volume overload. Data from the study will provide information regarding the safety and efficacy of acetazolamide treatment in the above-mentioned patient population. Randomized clinical trial with 2 treatment arms; therapy with high-dose loop diuretics and placebo vs therapy with high-dose loop diuretics and acetazolamide. An automated web-based system is used to randomly assign patients in a 1:1 ratio with variable blocks sizes, stratified for LVEF according to study center. To ensure an equal proportion of HFpEF versus HFrEF patients in both study arms, the population will be stratified at inclusion according to LVEF ≤40% versus >40%. Permuted block randomisation, at center and LVEF stratum level will be used to achieve this. Objective is to demonstrate superiority of the combination therapy with regards to achieving decongestion at 72 hours.

The study consists of 3 phases (cfr Figure 1 Flow Chart):

- screening phase: starting from identifying a study subject prior / during hospitalization until the first dose of study medication will be given
- treatment phase: starting from the first dose of study medication administration until the morning of day 4 or earlier in case of successful decongestion sooner
- follow up phase: starting when the treatment phase ends until 3 months after the study start dose

5 STUDY SETTING

The study population will consist of patients hospitalized for decompensated HF with clinical signs of volume overload. The goal is to randomize approximately 519 patients in approximately 24 centers in Belgium. Importantly, participating centers are located in Flanders, Wallonia and Brussels, which also ensures that the study population is representative of the real-life patient population in which the study drug will be used in case the study is positive.

The study will be supported by the members of the Belgian Working Group of Heart Failure (BWGHF). This is a national scientific non-profit group which was established in 2004 as one of the official working groups of the Belgian Society of Cardiology. Therefore, a positive result will almost immediately be adopted by the Belgian cardiology community as all members of the BWGHF are considered leaders in the field.

Finally, only centers who have fulfilled all the duties with regards to study selection and training will be allowed to randomize patients.

6 ELIGIBILITY CRITERIA

Decompensated HF patients with volume overload independent of ejection fraction might be included. This is important as 50% of decompensated HF patients are HFpEF patients (HF with preserved ejection fraction) and 50% of the decompensated HF patients are HFrEF patients (HF with reduced ejection fraction). In addition, patients do not need to have an echocardiogram at study inclusion to establish left ventricular ejection fraction which further simplifies the inclusion procedure. Finally, the DOSE trial (only other RCT studying the effects of diuretic therapy) also included patients without pre-specification of EF. To ensure an equal proportion of HFpEF versus HFrEF patients in both study arms, the population will be stratified at inclusion according to LVEF ≤40% versus >40% (assessed within 12 months before inclusion). Permuted block randomisation according to center and LVEF stratum will be used to achieve this.

Importantly, though the diagnosis of HF sometimes is difficult to establish in HFpEF, the main inclusion criteria are; 1) clinical diagnosis of decompensated HF and at least one clinical sign of volume overload (e.g. oedema, ascites or pleural effusion), 2) increased BNP / NT-ProBNP to ensure the volume overload is secondary to congestion/heart failure and 3) maintenance therapy with oral loop diuretics at a dose of ≥ 1 mg bumetanide or equivalent dose for ≥ 1 month before hospital admission will be remained. The combination of these three criteria will guarantee that only decompensated HF patients with volume overload will be included.

6.1 Inclusion criteria

- Signed written informed consent must be obtained before any study assessment is performed
- Male or female patients 18 years of age or older
- An elective or emergency hospital admission with clinical diagnosis of decompensated HF with at least one clinical sign of volume overload (e.g. oedema (score 2 or more), ascites confirmed by echography or pleural effusion confirmed by chest X-ray or echography)
- Maintenance therapy with oral loop diuretics at a dose of at least 1 mg bumetanide or an equivalent dose for at least 1 month before hospital admission (Conversion: 1 mg bumetanide = 40 mg furosemide = 20 mg torsemide)
- Plasma NT-proBNP levels >1000 ng/mL or BNP levels >250 ng/mL at the time of screening.
- Assessed LVEF by any imaging technique; i.e. echocardiography, catheterization, nuclear scan or magnetic resonance imaging within 12 months of inclusion

6.2 Exclusion criteria

- Concurrent diagnosis of an acute coronary syndrome defined as typical chest pain in addition to a troponin rise above the 99th percentile and/or electrocardiographic changes suggestive of cardiac ischemia
- History of congenital heart disease requiring surgical correction
- History of a cardiac transplantation and/or ventricular assist device

- Systolic blood pressure <90 mmHg or mean arterial pressure <65 mmHg at the moment of admission
- Expected use of intravenous inotropes, vasopressors or nitroprusside during the study. Use of nitrates is allowed only if the patient's systolic blood pressure is >140 mmHg
- Estimated glomerular filtration rate <20 mL/min/1.73m² at screening
- Use of renal replacement therapy or ultrafiltration at any time before study inclusion
- Treatment with intravenous loop diuretics > 2 mg bumetanide or an equivalence of another loop diuretic during the index hospitalization and prior to randomization
- Treatment with acetazolamide during the index hospitalization and prior to randomization
- Exposure to nephrotoxic agents (i.e. contrast dye) anticipated within the next 3 days
- Use of any non-protocol defined diuretic agent with the exception of mineralocorticoid receptor antagonists. Thiazides, metolazone, indapamide and amiloride should be stopped upon study inclusion. If patient is taking a combination drug including a thiazide-type diuretic, the thiazide-type diuretic should be stopped
- Current use of sodium-glucose transporter-2 inhibitors
- Subjects who are pregnant or breastfeeding

7 TRIAL PROCEDURES

7.1 Recruitment

Patients might be enrolled during an admission of decompensated HF with volume overload, on the condition of fulfilling inclusion and not fulfilling exclusion criteria. In addition, patients might already be informed that they could participate in study during an outpatient visit (if they need to be hospitalized for decompensated HF). Patients will be recruited by the sites within a period of approximately 24 months.

7.1.1 Patient identification

It is recommended to inform the medical team (cardiology and the health care providers) who might have first contact with potential study subjects (e.g. emergency room physicians) about the trial. They should inform the subject regarding the trial. Only a member of the patient's existing clinical care team should have access to patients' records without explicit consent in order to identify potential participants and check whether they meet the inclusion criteria or make the initial approach to patients. In case the treating physician is not a member of the ADVOR trial (a principal investigator (PI) or sub-investigator (SI)), he/she could refer the patient to the ADVOR investigator. The screening process can start only if a written informed consent is obtained. The investigator of the ADVOR trial should confirm eligibility of the subject.

Potential participants might also be recruited through publicity (posters, leaflets) which can be made publically available in cardiology outpatient departments only if approval has been obtained by the ethics committee.

7.1.2 Screening

Following screening technical requirements are necessary to meet any noted inclusion or exclusion criteria:

- laboratory tests (BNP or NT-proBNP part of inclusion criteria and eGFR part of exclusion criteria)
- chest X ray or chest ultrasound in case pleural effusion is used as inclusion criteria. Importantly, a chest X-ray is considered standard of care in case of decompensated HF (guidelines of the European Society of Cardiology (ESC))
- abdominal ultrasound in case ascites is used as inclusion criteria

No other technical procedure needs to performed as part of routine care. However, the LVEF assessed by any technical exam (echocardiogram, nuclear scan, MRI, catheterization) within the last 12 months will need to be recorded. As only patients already treated with a loop diuretic are allowed in the study, we expect that every patient has a LVEF recorded within 12 months preceding study entry.

We don't expect screen failures i.e. patients who do not meet eligibility criteria at time of screening to be able to enter the study at a later stage as we expect them to have received already more than 2 mg loop diuretics intravenously (which will count as an exclusion criterium).

Patients will not receive any special incentives or compensation through participation in the study. No specific study visits are needed. The 3 month FU visit is standard of care as recommended by the guidelines of the ESC. Study related exams (BNP or NT-proBNP) will be reimbursed by the study.

7.2 Consent

The Principal Investigator (PI) retains overall responsibility for the informed consent of participants at their site and must ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained and competent to participate according to the ethically approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki. If delegation of consent is acceptable then details should be provided.

Informed consent must be obtained prior to the participant undergoing procedures that are specifically for the purposes of the trial and are outside of standard routine care at the participating site (including the collection of identifiable participant). In case the patient agrees to participate in the optional laboratory sub-study, an additional signed and dated informed consent must be obtained before the laboratory sub-study blood and urine samples will be collected.

The right of a participant to refuse participation without giving reasons must be respected.

The participant must remain free to withdraw at any time from the trial without giving reasons and without prejudicing his/her further treatment and will be provided with a contact point where he/she may obtain further information about the trial. Where a participant is required to re-consent or new information is required to be provided to a participant it is the responsibility of the PI to ensure this is done in a timely manner.

The PI takes responsibility for ensuring that all vulnerable patients are protected and participate voluntarily in an environment free from coercion or undue influence.

Eligible patients may only be included in the study after providing written (witnessed, where required by law or regulation), Independent Ethics Committee (IEC) approved informed consent or if patient is incapable of doing so, after such consent has been provided by a legally acceptable representative(s) of the patient. In cases the patient's representative gives consent, the patient should be informed about the study to the extent possible given his/her understanding. If the patient is capable of doing so, he/she should indicate assent by personally signing and dating the written informed consent document.

The process of obtaining informed consent should be documented in the patient source documents.

Once the informed consent has been signed by the patient, a new patient file should be created in the electronic Case Report Form (eCRF) by the PI or the qualified person to whom this task has been delegated. Once the patient file is created, the patient is enrolled in the study and the eCRF system will automatically generate a trial number for this patient. Once a number is assigned to a patient, the patient number can't be re-used.

7.3 The randomisation scheme

Randomized clinical trial with 2 treatment arms; therapy with high-dose loop diuretics and placebo vs therapy with high-dose loop diuretics and acetazolamide. An automated web-based system is used to randomly assign patients in a 1:1 ratio with variable blocks sizes, stratified for LVEF according to study center. To ensure an equal proportion of HFpEF versus HFrEF patients in both study arms, the population will be stratified at inclusion according to LVEF ≤40% versus >40%. Permuted block randomisation according to center and LVEF stratum will be used to achieve this.

Only the PI or qualified person to whom he/she has delegated this study task can randomize the patient in the automated web-based system.

7.4 Blinding

This is a double-blind study. Therefore, after randomization, the study team and patient will not be aware of which treatment (acetazolamide or placebo) is administrated to the trial participant.

Once a patient is assigned to a study group (treatment group or control group), he/she will remain in that arm and all efforts will be made to provide the optimal therapy specified for that treatment assignment. In the unforeseen circumstance that this is clinically not feasible, the patient will remain in the assigned treatment arm for statistical analysis based on the intention-to-treat principle, as it represents a normal medical situation of success and failure of delivering the planned medical therapy.

7.5 Unblinding

Patient, site personnel, sponsor personnel and data analysts will remain blinded to the identity of the treatment from the time of randomization until database lock. Though we do not foresee serious adverse events related to the study drug, the study code should only be broken for valid medical or safety reasons e.g. in the case of a severe adverse event where it is necessary for the investigator or treating health care professional to know which treatment the patient is receiving before the participant can be treated. It is not mandatory but strongly encouraged to contact the chief investigator before unblinding any patients' treatment assignment. Patient and members of the research team should remain blinded.

Following rules apply for unblinding;

- Rapid unblinding of a patient can be performed by a physician of the study team. Detailed information concerning the unblinding procedures is provided in the Manual of Operations.
- On receipt of the treatment allocation details the PI or treating health care professional will continue to deal with the participant's medical emergency as appropriate.
- The PI/Investigation team documents the breaking of the code and the reasons for doing so on the medical notes and eCRF. It will also be documented at the end of the trial in any final study report and/or statistical report.

- the PI/Investigation team will notify the Sponsor in writing within one working day following the code break detailing the necessity of the code break.

Randomization data are to be kept strictly confidential until the time of unblinding of the trial and will not be accessible by anyone else involved in the trial with the following exceptions: (1) the PM of the company responsible for the labelling and packaging of the IMP, (2) the IRT system programmers who work on the randomization and drug management system; and (3) the data manager who prepares reports required for regulatory reporting (suspected unexpected serious adverse reactions [SUSAR] reporting). These individuals will not be involved in the day-to-day running of the study.

7.6 Screening phase assessments

Screening testing will occur after the consent process has been finalized. The screening data gathering needs to be performed prior to the administration of the study diuretic agents. The following assessments are required and data need to be collected:

- Obtain written informed consent
- Check inclusion and exclusion criteria
- Collect demographics (collect age, gender, race and ethnicity)
- Collect medical history and notification of LVEF
- Collect concomitant medication: all chronic medication but only doses of neurohumoral blockers (ace-I, ARB, beta-blockers, spironolactone, eplerenone, sacubutril/valsartan) and diuretics (loop diuretics, thiazides) need to be recorded
- Collect vital signs including body weight, arterial blood pressure and heart rate
- EQ-5D patient questionnaire to be completed by the patient (Appendix 2 and 3)
- Perform volume assessment by 1 heart failure cardiologist who has been trained in the study volume assessment. Volume assessment is based on presence of oedema, ascites, pleural effusion (Figure 3 in section 8)
- Collect blood sample for local laboratory assessment: serum hemoglobin, hematocrit, electrolytes (Na, K, Cl, HCO_3), serum osmolality, serum urea, serum Cr, total protein, serum albumin, Fe, ferritin, TSAT, LDH, troponin and plasma BNP or NT-proBNP.
    - In a subset of randomized patients participating in the laboratory sub-study blood collected during screening will be shipped to the University Biobank Limburg (see section 7.10)
- Perform pregnancy urine test (applicable for all pre- menopausal women who are not surgically sterile, as well as women of childbearing potential)
- Randomization (see section 7.3)

7.7 Treatment phase assessments

7.7.1 Day 1

- Request patient to empty their bladder before administration of the first dose of loop diuretics.
- Administer the first bolus of loop diuretics and acetazolamide or placebo to the patient.
- Start the urinary collection immediately after first bolus of loop diuretics and acetazolamide or placebo is administered. The collection will stop the latest as close to but prior to the morning bolus of study medication on day 3. Urinary catheter insertion is strongly recommended but not mandatory to achieve an optimal urine collection. However, in case of urinary incontinence, placement of a urinary catheter is mandatory. Care should be taken to ensure ALL urine is collected. For more details regarding the study urinary collection see appendix 4.

Screening phase assessments and day 1 assessments are performed on the same day. If the patient is enrolled between midnight and 6 a.m., day 1 assessments and day 2 assessment can be performed on the same day but only if there is a difference of minimum 6 hours between the start of the study medication on day 1 and study medication on day 2.

7.7.2 Day 2, Day 3 and Day 4

- Collect vital signs including body weight, arterial blood pressure, heart rate
- Perform volume assessment by 1 heart failure cardiologist who has been trained in the study volume assessment (Figure 3 in section 8)
- Collect morning blood sample for local laboratory assessment: serum haemoglobin, haematocrit, electrolytes (Na, K, Cl, HCO_3), serum urea, serum Cr, serum albumin.
- On the morning of Day 2 and Day 3, collect an urine sample from urine collection period 1 and period 2 respectively for analysis in local lab. In case patient is participating in the optional laboratory sub-study, collect an additional urine sample for each urine collection period for shipment to the University Biobank Limburg for storage and additional research (see section 7.10). Urinary collection will stop at the morning of day 3 (appendix 4).
- In case patient is still volume overloaded continue study treatment as described in section 8
- Collect all new medication started, and doses of loop diuretics need to be recorded
- Document and assess adverse events (section 9)
- In addition, only on the morning day 4:
    - EQ-5D to be completed by the patient
    - Collect blood sample for local laboratory assessment of the BNP or NT-ProBNP level. In a subset of randomized patients participating in the laboratory sub-study blood collected on day 4 will be shipped to the University Biobank Limburg (see section 7.10)
    - Collect all new medication started, and doses of neurohumoral blockers (ace-I, ARB, beta-blockers, spironolactone, eplerenone, sacubutril/valsartan) need to be recorded

7.8 Follow-up phase assessments

- At Discharge:
    - Perform volume assessment by 1 heart failure cardiologist who has been trained in the study volume assessment (Figure 3 in section 8)
    - Collect weight
    - Record only neurohumoral blockers (ace-I, ARB, beta-blockers, spironolactone, eplerenone, sacubutril/valsartan) and diuretics (loop diuretics, thiazides) with their dosages
    - Document length of hospitalization
    - Document and assess adverse events (section 9)

- At Readmission:
    - HF readmissions are defined as either a hospital admission because of decompensated HF or an unscheduled contact at the emergency department if the patient is treated with intravenous loop diuretics.
    - During any readmission, EQ-5D questionnaire needs to be completed by the patient as soon as possible during the admission

- Long term follow-up

  Patients will be followed for a maximum of three months for secondary/tertiary endpoint analysis. This follow-up should not differ from standard of care for such patients. During one outpatient follow-up appointment 3 months (+ 14 days) after hospital discharge, standard of care data will be collected. The only study related tests will be a BNP / NT-ProBNP test and the collection of the EQ-5D patient questionnaire.

  During this follow –up visit the following data will need to be collected:
    - Collect vital signs including body weight, arterial blood pressure, heart rate
    - EQ-5D patient questionnaire to be completed by the patient (Appendix 2 and 3)
    - Perform volume assessment by 1 heart failure cardiologist who has been trained in the study volume assessment (Figure 3 in section 8)
    - Collect blood sample for local laboratory assessment: serum hemoglobin, hematocrit, electrolytes (Na, K, Cl, HCO_3), serum urea, serum Cr, serum albumin, and plasma BNP or NT-proBNP. In a subset of randomized patients participating in the laboratory sub-study blood collected on this visit will be shipped to the University Biobank Limburg (see section 7.10)
    - Record neurohumoral blockers (ace-I, ARB, beta-blockers, spironolactone, eplerenone, sacubutril/valsartan) and diuretics (loop diuretics, thiazides) with their dosages
    - Document and assess adverse events (section 9)

  We do not foresee that a significant amount of patients will be lost to follow-up as the follow-up period is short (max 3 months). The investigator should make every effort to contact participants

who are lost to follow-up. Attempts to contact such participants must be documented in the participant's records.

7.9 Table of trial procedures

	Screening phase	Treatment phase				Follow up phase		
		Study Day 1	Morning of Study Day 2	Morning of Study Day 3	Morning of Study Day 4	Discharge	Re-admission	3 Months after study start dose
Informed consent	X							
In- and exclusion criteria	X							
Randomization	X							
Demographics[1]	X							
Medical history	X							
Vitals[2]	X		X	X	X			X
Weight[12]	X		X	X	X	X		X
EQ5D	X				X		X[11]	X
Volume assessment	X		X	X	X	X		X
Study treatment		X[3]	X[4]	X[4]				
Urinary collection[5]		X	X					
Local lab	X[6]		X[7]	X[7]	X[7]			X[7]
Laboratory sub-study[13] blood	X				X[14]			X
Laboratory sub-study[13] Urine		X	X					
Plasma BNP or NT-proBNP[8]	X				X			X
Urine pregnancy testing[9]	X							
Dose of neurohumoral blockers	X				X	X		X
Dose of diuretics	X					X		X
Concomitant medication	X	X	X	X	X			
Adverse Events[10]	X	X	X	X	X	X	X	X

1) Age, race and ethnicity
2) Arterial blood pressure and heart rate
3) Start dose (IV) = 2 x orally daily maintenance dose of loop diuretics and 500 mg acetazolamide or placebo (see section 8)
4) As long as patient is volume overloaded, Treatment dose (IV) = half of start dose of loop diuretics and 500 mg acetazolamide or placebo between 8:00 and 12:00 and a second dose minimum 6 hours later with half of the start dose of loop diuretics (see section 8)
5) See appendix 4
6) Serum hemoglobin, hematocrit, electrolytes (Na, K, Cl, HCO_3), serum osmolality, serum urea, serum Cr, total protein, serum albumin, Fe, ferritin, TSAT, LDH and troponin
7) Serum hemoglobin, hematocrit, electrolytes (Na, K, Cl, HCO_3), serum urea, serum Cr and serum albumin
8) Protocol requires that plasma levels of BNP or NT-proBNP are collected. In case the patient is on succubutril/valsartan, it is mandatory that NT-proBNP plasma levels are determined on the blood sample.
9) Only pre- menopausal women who are not surgically sterile, as well as women of childbearing potential.
10) Safety reporting flow is documented in section 9
11) EQ5-D needs to be collected once during any HF readmission (as soon as possible during readmission))
12) Measurement of body weight should be performed as consistently as poss ble using a standardized scale, preferably with a precision of 50 g, in the morning, post-void, prior to eating, prior to the medication dose, and with patients wearing the same clothing. The scales should stand on a flat, solid surface rather than carpets unless specifically designed for use in that setting
13) Blood and urine will be collected in a subset of randomized patients participating in the laboratory sub-study. Blood will be collected on screening, day 4 and 3 month FU visit. Urine sample will be collected from Urinary Collection period 1 and Urinary collection period 2 (see also appendix 4).
14) In case day 4 falls into a weekend or public holiday, blood collection for sub-study can also be done on day 5 or day 6.

7.10 Laboratory sub-study

Blood samples and urine samples from centers who agreed to participate in the Laboratory sub-study will be collected during the Advor trial. All potential patients at these centers will be asked to participate in the laboratory sub-study. An additional signed and dated informed consent must be obtained before the additional blood and urine samples will be collected.

The right of a participant to refuse participation without giving reasons must be respected. The participant must remain free to withdraw at any time from this sub-study without giving reason and without prejudicing his/her further treatment and will be provided with a contact point where he/she may obtain further information about the sub-study. Where a participant is required to re-consent or new information is required to be provided to a participant it is the responsibility of the PI to ensure this is done in a timely manner.

All patients enrolled in the laboratory sub-study will have blood collected on screening, day 4 and during the 3 month follow up visit. The collected blood samples will be shipped to the University Biobank Limburg for storage and additional research. A urine samples collected from the Urinary Collection period 1 and from the Urinary Collection period 2 (see appendix 4) will be shipped to the University Biobank Limburg for storage and additional research.

Biomarkers related to cardiac and renal function/injury will be obtained from blood and urine in the subset of patients participating in the laboratory sub-study. Biomarkers will be used to elucidate the effect of acetazolamide and to explore drug effect versus baseline biomarkers of risk. Biomarkers of potential interest are NT-proBNP, Galectin 3, ST2, Cystatin C and NGAL. The list of potential biomarkers may be changed or expand further as it is recognized that more relevant or novel biomarkers may be discovered. Biomarkers may be measured during the process of this study or after its completion. No genetic analysis will be performed with the collect sub-study samples. Detailed sample handling instructions will be provided in a separate laboratory manual.

7.11 Withdrawal criteria

All subjects will be encouraged to remain on treatment and under observation for the full duration of the study. However, at any time during the study and without giving reasons, subjects may withdraw from the study at their own request or at the request of their legally acceptable representative. The subject will not suffer any disadvantage as a result.

It is important to note that discontinuation of study treatment (see section 8) is not the equivalent to withdrawal of informed consent. In cases where subjects indicate they do not want to "continue", investigators must determine whether this refers to discontinuation of study treatment (the most common expected scenario), unwillingness to attend the follow-up visit, unwillingness to have telephone contact, unwillingness to have any contact with study personnel, or unwillingness to allow contact with a third party (e.g., family member, doctor). Every effort must be made to continue to follow the subject until the end of the study.

In all cases, the reason for discontinuation (including "at the subject's request") must be recorded in the case report form (CRF) and in the subject's medical records.

No subject replacements are permitted in the study.

7.12 End of trial

The Sponsor's CTU ZOL will notify the FAMHP and main EC of the end of a clinical trial within 90 days of its completion date (last patient last visit).

8 TRIAL INTERVENTION / MEDICATION

The ADVOR study is a phase IV randomized, multi-center, double-blind study, comparing monotherapy with high-dose loop diuretics and placebo (standard of care - SOC) versus combination therapy with acetazolamide and high-dose loop diuretics in patients with decompensated heart failure and clinical signs of volume overload.

8.1 Name and description of intervention(s)

At randomization

At the moment of randomization, oral loop diuretics are stopped and the patient receives an IV bolus of loop diuretics at a dose equal to the double of his oral daily maintenance dose* with a maximal dose of 5 mg bumetanide (=200 mg furosemide). Bumetanide is the preferred loop diuretic agent to be used in this trial.

Conversion factor:

1 mg bumetanide po = 1 mg bumetanide IV

40 mg furosemide po = 40 mg furosemide IV

20 mg torsemide po = 40 mg furosemide IV = 1 mg bumetanide IV

Some examples are listed below:

- *if a patient takes 1x1 mg bumetanide po, the patient will receive 2x1x1 mg = 2 mg bumetanide IV*
- *if a patient takes 2x2,5 mg bumetanide po, the patient will receive 2x2x2,5 mg = 5 mg bumetanide IV*
- *if a patient takes 1x40 mg furosemide po, the patient will receive 2x1x40 mg = 80 mg furosemide IV*
- *If a patient takes 1x80 mg furosemide po, the patient will receive 2x1x80 mg = 160 mg furosemide IV*
- *If a patient takes 1x20 mg torsemide po, the patient will receive 2x1x1 mg bumetanide IV= 2 mg bumetanide IV or 2x1x40 furosemide IV = 80 mg furosemide IV*

Together with this initial dose of loop diuretics patients will receive an intravenous bolus of 500 mg of acetazolamide or placebo.

START DOSE (IV) = 2 x orally daily maintenance dose* of loop diuretics (max. 5 mg of bumetanide)
+
500 mg acetazolamide or placebo

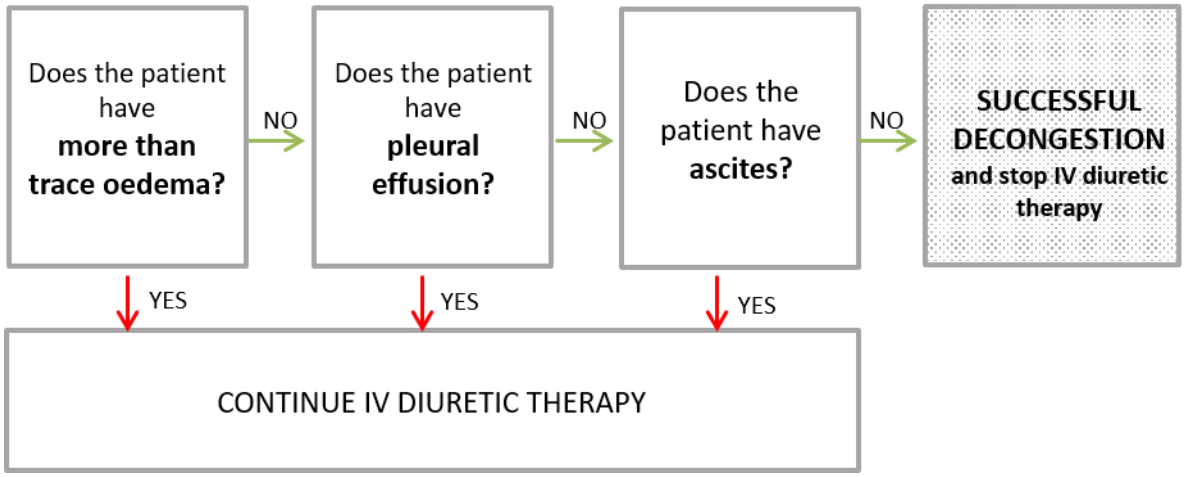
**If the oral daily maintenance dose has changed over the week prior to admission, it will be defined as the highest orally administered daily dose that the patient has received in an outpatient context 7 days prior to randomization.*

During the treatment phase

Between administering the start dose and next treatment dose a minimum of 6 hours is required. During the remaining part of the treatment phase, the patient will continue to receive 2 treatment doses every day provided that the treating physician has concluded during the morning rounds that the patient is still volume overloaded (see Figures 2+3). The dose will be half of the start dose given at randomization, administered between 8:00 and 12:00 am together with an intravenous bolus of 500 mg of acetazolamide or placebo. The second dose of loop diuretics, again half the start dose of loop diuretics, will be given 6 hours after the morning dose. Any patient with more than trace oedema, residual pleural effusion (to be confirmed only if present at study inclusion), or residual ascites (to be confirmed only if present at study inclusion) would be considered to be still volume overloaded (see Figures 2 + 3). If the patient is not volume overloaded anymore, the intravenous administration of diuretics should be stopped and changed to an oral regimen.

Between 8:00 and 12:00 am TREATMENT DOSE (IV) = START DOSE/2 (max. 5 mg of bumetanide)
+
500 mg acetazolamide or placebo
6 hours later TREATMENT DOSE (IV) = START DOSE/2

Figure 2. Flow chart to guide study.



After treatment phase

After the treatment phase, treating physicians are recommended not to prescribe oral acetazolamide as a maintenance diuretic therapy after decongestion; instead, they are encouraged to restart the original oral maintenance dose of loop diuretics of when the patient was still stable. Patients can be discharged as early as 24 hours after the physician concluded that the volume overload is no longer present. An

outpatient follow-up appointment is scheduled at least 3 months (with a window of +14 days) after discharge and according to preferences of the treating physician.

STOP TREATMENT

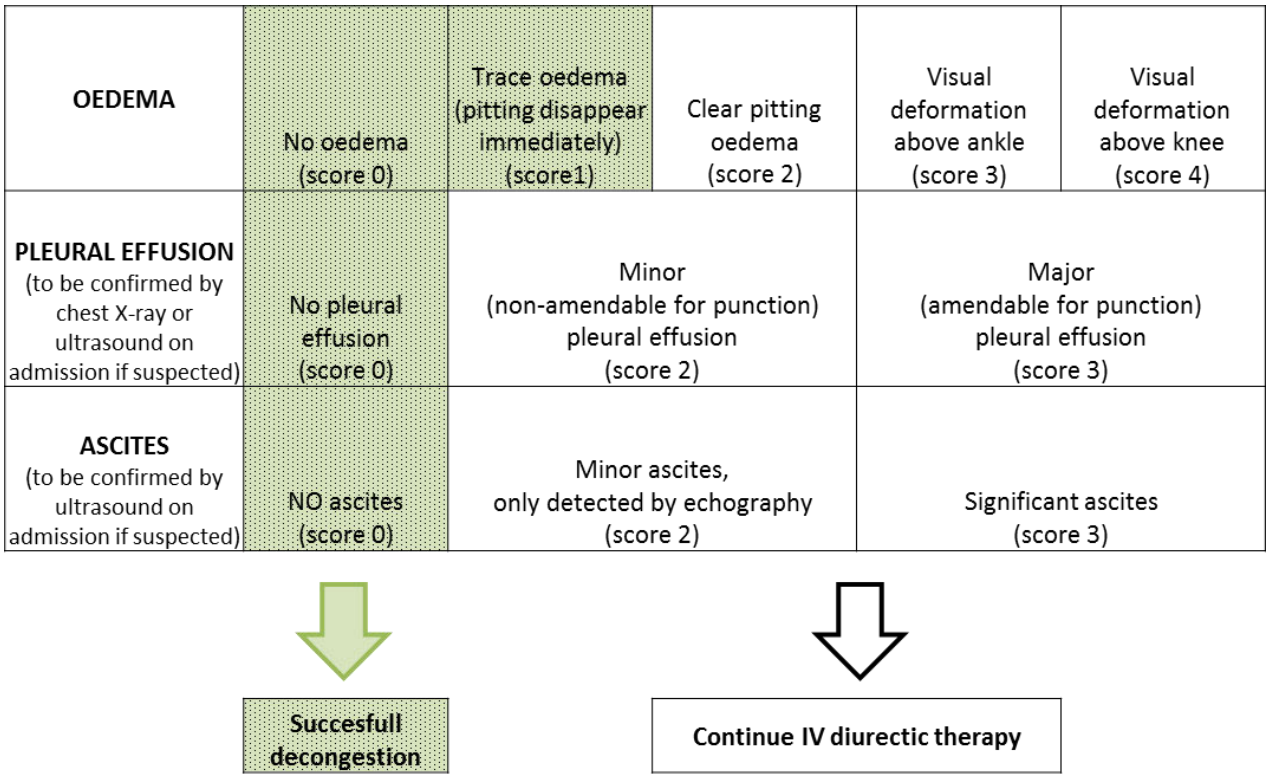
The treating physician is allowed to stop the study treatment, which counts as treatment failure **in case of persistent volume-overload** in following cases:

- symptomatic hypotension with a systolic blood pressure <100 mmHg
- asymptomatic hypotension with a systolic blood pressure <90 mmHg
- an increase of serum Cr levels x 1.5 of the serum Cr level compared to admission value.
- Occurrence of metabolic acidosis (ph < 7.2)

If any of these events occur when the patient is judged to be euvolemic, the study treatment is stopped and stopping is not considered a treatment failure.

Freedom from volume-overload (i.e.congestion) on the morning of day 4 will be defined as not more than trace oedema, no residual pleural effusion, and no residual ascites (Figure 3)

Figure 3: Volume assessment



Treatment DOSE ADJUSTMENTS in case of an inappropriate diuretic response

If urinary output (see Figure 1 and Appendix 4) on morning of day 3 is < 3500 mL and the patient is still volume overloaded, an escalation of decongestive treatment is mandatory. Three options can be chosen at the discretion of the treating physician.

Escalation therapy options:

- doubling of the IV dose of the loop diuretics (equal to the study start dose bid)
- add oral chlorthalidone 50 mg once daily
- ultrafiltration or renal replacement therapy might be considered

The decision to proceed with escalation therapy needs to be collected in the case report form as the patient needing escalation cannot reach the primary endpoint.

Background therapy

24h oral intake of fluid and sodium will be restricted to 1500 mL and 1.5 g, respectively. All patients receive the same maintenance infusion with 500 mL glucose 5% and 3g $MgSO_4$ administered over 24h time interval, until complete decongestion or end of the study treatment phase. All non-protocol fluids administered (including those for administration of intravenous medication) should be limited.

In case of **serum potassium levels <4 mmol/L,** 40 mmol of KCl is added to the maintenance infusion. Oral potassium supplements may be used at the discretion of the treating physician, but their use will be prospectively registered.

In case of **metabolic acidosis** with serum bicarbonate levels <20 mmol/L, it is recommended to administered intravenously 100 ml of $NaHCO_3$ 8.4%.

Treatment with **neurohumoral blockers** (e.g. renin-angiotensin system blockers saccubutril/valsartan, beta-blockers, and mineralocorticoid receptor antagonists) may be continued at the same or lower dosage at the discretion of the treating physician, until the end of the treatment phase (max 4 days) or until complete decongestion is achieved, whatever comes first. Dose increases for any of these medications are not allowed during the screening and treatment phase with the exception of mineralocorticoid receptor antagonists in case of hypokalaemia despite intravenous potassium supplement. In addition, starting an SGLT2 inhibitor and a switch from renin-angiotensin system blockers to saccubutril/valsartan is not allowed during the screening and treatment phase, but might be pursued after decongestion is achieved. After decongestion, it is strongly recommended to up-titrate doses of neurohumoral blockers according to the guidelines in the HFrEF patients. Dosages of neurohumoral blockers are collected on admission, on discharge and at three months follow-up.

8.2 Legal status of the intervention

The IMP (acetazolamide) is licensed for use in Belgium for the treatment of HF.

8.3 Summary of Product Characteristics (SmPC)

Information about common side effects already known about the investigational drug can be found in the SmPC filed in the Investigator Site File (ISF).

SmPC in Dutch:
http://bijsluiters.fagg-afmps.be/registrationSearchServlet?key=BE004137&leafletType=skp

SmPC in French:
http://bijsluiters.fagg-afmps.be/registrationSearchServlet?key=BE004137&leafletType=rcp

8.4 Drug storage and supply

The investigational medical product (IMP) (acetazolamide and placebo) will be shipped free of charge to the participating centers. The IMP must be received by a designated person in the pharmacy at the study center, handled and stored safely and properly, and kept in a secured location to which only the investigator and designees have access. Upon receipt, the PI or qualified delegated person will confirm the date of receipt of IMP. Receipt, distribution, return and destruction (if applicable) of the study drug must be properly documented according to the agreed and specified procedures. Specific instructions for the study drug recordkeeping are provided in the Manual of Operations.

All IMP should be stored according to the instructions specified on the labels (room temperature below 25°C).

8.5 Preparation and labelling of Investigational Medicinal Product

The IMPs (acetazolamide and placebo) will be presented as a white to off-white 500 mg powder for solution for injection in a sterile vial. Each study center will be supplied with study medication kits containing 3 vials of acetazolamide or placebo. Each kit will have an unique study kit number. The study medication supply will have appropriate labelled packaging according to national law of Good Manufacture Practices (GMP) ruling.

Each IMP need to be reconstitute prior to use. Specific instructions will be provided in the Manual of Operations. The reconstituted solution is clear and colourless and does not contain an antimicrobial preservative. Any unused solution can be stored in a refrigerator for up to 24 hours but any solution not used within this period must be discarded.

The direct intravenous route of administration is preferred. Intramuscular injection may be employed but is painful due to the alkaline pH of the solution. (preparation according to the SmPC see section 8.3)

8.6 Dosage schedules

Cfr point 8.1 for specific dosage schedules and routes of administration.

8.7 Dosage modifications

Dosage modifications of the study drug are not possible as there will only be one dose (and the administration of the IMP vs placebo is blinded).

8.8 Assessment of compliance

As the study drug will be administered intravenously during the treatment phase (72 hours) by the nurse taking care of the patient, no compliance issues with regards to the study medication are foreseen.

9 SAFETY REPORTING

As this is a pragmatic trial, the intervention will be used within the label and therefore safety reporting can be limited to the safety reporting which is necessary in routine care.

Timely, accurate, and complete reporting of clinical events is of crucial importance for success of the study. Additionally, reporting and review of safety information for clinical studies are crucial for the protection of subjects.

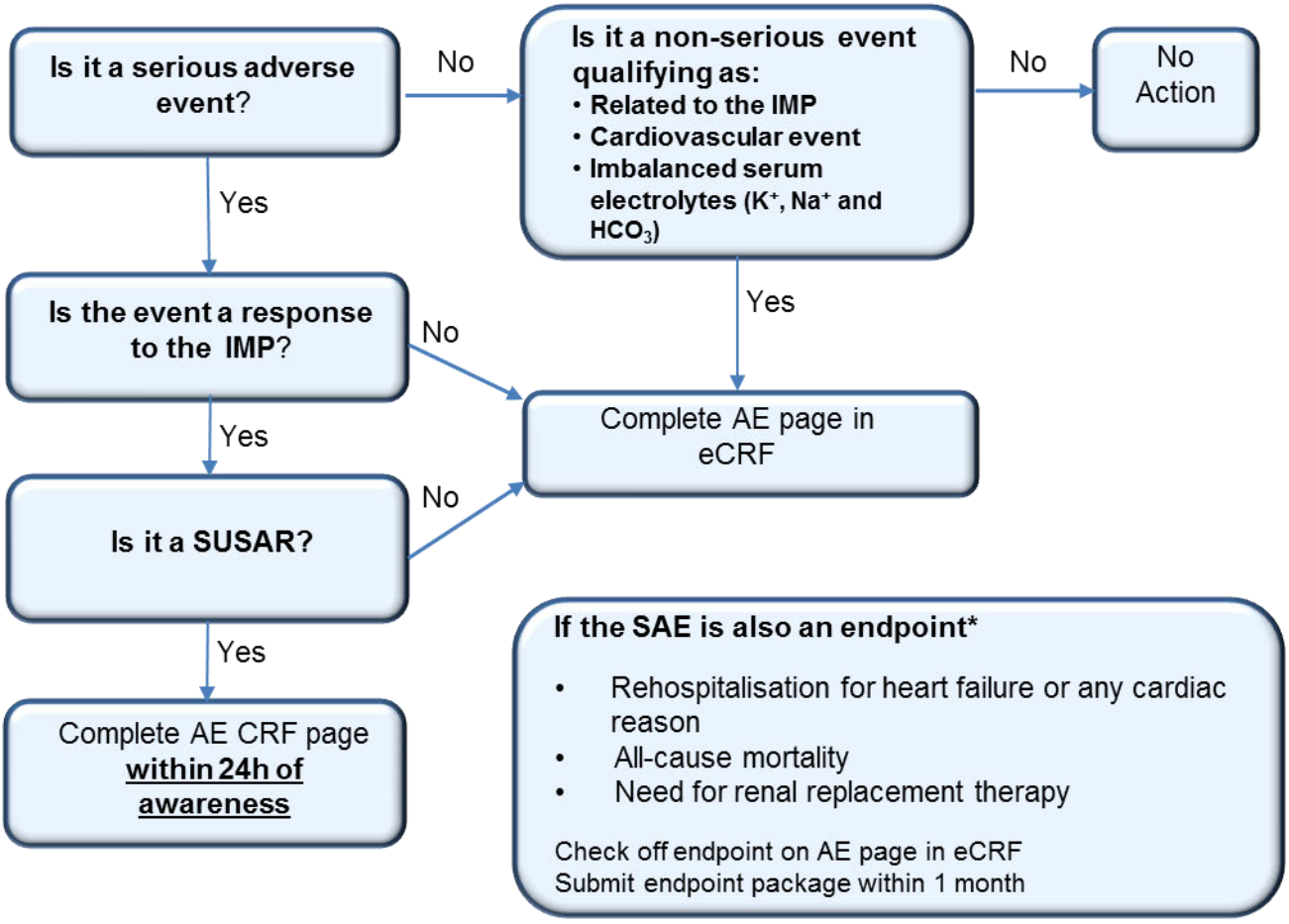
9.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product • in the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question

9.2 Recording and reporting Safety Information

During the course of this study, i.e. from signing the informed consent onwards until the end of the last follow-up visit, all SAEs, Endpoint Events and certain non-serious AEs which occurred until 3 months after study start dose are to be collected, documented, and reported by the Investigator in the applicable eCRF's following below "safety reporting flow chart":

Figure 4: Safety reporting flow chart



How to assess Safety Events (AEs, SAEs and SUSARs)

Seriousness, severity and causality need to be asses by the Principal Investigator or the physician to whom this activity is delegated to.

An adverse event is defined as a serious adverse event if the event is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect, or
- Is an important medical event.

Note: The term “life-threatening” in the definition of serious refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe

The following definitions should be used ***to assess intensity of adverse events***:

- Mild: Awareness of sign or symptom, but easily tolerated, i.e., does not interfere with subject's usual function.
- Moderate: Discomfort enough to cause interference with usual activity.
- Severe: Incapacitating with inability to work or do usual activity, i.e., interferes significantly with subject's usual function.

Severity vs. Seriousness: Severity is used to describe the intensity of a specific event whereas the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "seriousness," which is based on subject/event outcome at the time of the event.

The Investigator should ***assess causal relationship between an adverse event and the study drug on the basis of his/her clinical judgment, the latest SmPC (see section 8.3) and the following definitions.*** The causality assessment must be made based on the available information and can be updated as new information becomes available.

Related:

The AE follows a reasonable temporal sequence from study drug administration, and cannot be reasonably explained by the subject's clinical state or other factors (e.g., disease under study, concurrent diseases, and concomitant medications).

or

The AE follows a reasonable temporal sequence from study drug administration, and is a known reaction to the drug under study or its chemical group, or is predicted by known pharmacology.

Not Related:

The AE does not follow a reasonable sequence from study drug administration, or can be reasonably explained by the subject's clinical state or other factors (e.g., disease under study, concurrent diseases, and concomitant medications).

Recording and reporting non-serious Adverse Events (AEs)

AEs may be directly observed, reported spontaneously by the subject or by questioning the subject at each study visit. The Investigator must assess these AEs to determine seriousness, severity, and causality, in accordance with below definitions. The Investigator's assessment must be clearly documented in the site's source documentation. For the purposes of this study **non-serious AEs that are related to the IMP or non-serious AEs that that occur in the cardiovascular system or non-serious AEs events involving imbalance of serum electrolytes (restricted to K^+, Na^+, HCO_3) will be collected in the eCRF throughout the study**.

Recording and reporting of SAEs AND SUSARs

All SAEs an SUSARs will be collected throughout the study duration. Reporting of these events to the sponsor will occur on the eCRF AE page.

Reporting of SAEs and SUSARs is mandatory and should start;

- For SAEs, from consent
- For SUSARs, from 1st IMP dose

If the SAE is unexpected, i.e., the event is not previously documented as 'expected', and is thought to be related to the study drug, this event is considered a Suspected Unexpected Serious Adverse Reaction (SUSAR).

Where a participant withdraws consent for further processing of data, this does not preclude the reporting of SAEs and SUSARs which are required to continue being reported according to the protocol for regulatory purposes.

In all cases SAEs should be reported to the Sponsor in the eCRF. **Only in case the investigator is of the opinion that the SAE is a SUSAR, the investigator needs to inform the sponsor within 24 hours after awareness of the event.** Assessment of seriousness, causality and expectedness for trials involving IMPs will be made by the PI or another authorised doctor. If an authorised doctor from the reporting site is unavailable, initial reports without causality and expectedness assessment should be submitted to the Sponsor, but must be followed-up by medical assessment as soon as possible thereafter.

For each SUSAR the following information will need to be collected:

- full details in medical terms and case description
- event duration (start and end dates, if applicable)
- action taken
- outcome
- seriousness criteria
- causality (i.e. relatedness to trial drug / investigation), in the opinion of the investigator
- whether the event would be considered expected or unexpected.

All SAEs assigned by the PI or delegate (or following central review) as both suspected to be related to IMP-treatment and unexpected will be classified as SUSARs and will be subject to expedited reporting to the Federal agency for medicines and health products (FAMHP). The Sponsor will inform the FAMHP, the EC and the Marketing Authorisation Holder of SUSARs within the required expedited reporting timescales.

Endpoints

The following events will need to be marked as an “endpoint event” in the eCRF:

- All-cause mortality: All deaths from any cause. This includes all cardiovascular and non-cardiovascular deaths.

  Cardiovascular death: Death fulfilling any of the following criteria:
  - Death due to proximate cardiac cause (e.g. myocardial infarction, cardiac tamponade, worsening heart failure)
  - Death caused by non-coronary vascular conditions such as neurological events, pulmonary embolism, ruptured aortic aneurysm, dissecting aneurysm, or other vascular disease
  - All procedure-related deaths, including those related to a complication of the procedure or treatment for a complication of the procedure (e.g. surgical or non-surgical revascularisation)
  - All valve-related deaths including structural or non-structural valve dysfunction or other valve related adverse events
  - Sudden death or unwitnessed death
  - Death of unknown cause

  Non-cardiovascular death: any death that is not thought to be due to a cardiovascular cause

- Rehospitalisation for heart failure is defined as either a hospital admission or an unscheduled contact at the emergency department for worsening HF if the patient is treated with intravenous loop diuretics.
- Rehospitalisation for a cardiac event
- Need for Renal replacement therapy: ultrafiltration or dialysis

Submit complete endpoint package within 30 days of awareness to the sponsor. This package should Include anonymized source documents (SDs) relevant to the endpoint reported (e.g. discharge letter, emergency room notes, etc). The SD should include at least the reason for admission/death and the received treatment for the event (if applicable). The original SD need to be retained at the site.

Identifying a pregnancy

All pre- menopausal women who are not surgically sterile, as well as women of childbearing potential will have pregnancy urine tests performed at screening phase (Day 1). A positive pregnancy test at day 1 constitutes in screen failure.

9.3 Responsibilities

Principal Investigator (PI):

Checking for AEs when participants attend for treatment / follow-up.

1. Using medical judgement in assigning seriousness, causality and expectedness using the Reference Safety Information approved for the trial.
2. Ensuring that all SAEs and SUSARs are recorded in the eCRF. Ensure that only SUSARs are reported to the Sponsor and the Chief Investigator within 24 hours of becoming aware of the event and provide further follow-up information as soon as available.
3. Ensuring that AEs are recorded and reported to the Sponsor and the Chief Investigator in line with the requirements of the protocol.

Chief Investigator (CI) / delegate or independent clinical reviewer:

1. Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.
2. Using medical judgement in assigning seriousness, causality and expectedness of SAEs where it has not been possible to obtain local medical assessment.
3. Immediate review of all SUSARs.
4. Review of specific SAEs in accordance with the trial risk assessment and protocol as detailed in the Trial Monitoring Plan.
5. Assigning Medical Dictionary for Regulatory Activities (MedDRA) or Body System coding to all SAEs.
6. Preparing the clinical sections and final sign off of the Development Safety Update Report (DSUR).

Sponsor:

1. Central data collection and verification of AEs, SAEs, and SUSARs according to the trial protocol onto a safety database.
2. Reporting safety information to the CI, delegate or independent clinical reviewer for the ongoing assessment of the risk / benefit according to the Trial Monitoring Plan.
3. Reporting safety information to the independent oversight committee identified for the trial (Trial Steering Committee (TSC)) according to the Trial Monitoring Plan.
4. Expedited reporting of SUSARs to the Competent Authority (FAMHP IN be) and EC within required timelines.
5. Notifying Investigators of SUSARs that occur within the trial.
6. The unblinding of a participant for the purpose of expedited SUSAR reporting.
7. Checking for (annually) and notifying PIs of updates to the Reference Safety Information for the trial.

8. Preparing standard tables and other relevant information for the DSUR in collaboration with the CI and ensuring timely submission to the FAMHP and EC.

Trial Steering Committee (TSC):

In accordance with the Trial Terms of Reference for the TSC, periodically reviewing safety data.

9.4 Notification of deaths

All deaths will be reported to the sponsor within 5 working days of the research staff becoming aware of the event with coding of the reason of death (HF related, non-HF related, unknown). In case the death is deemed related to the IMP the event will need to be reported within 24 hours of awareness of the event.

9.5 Reporting urgent safety measures

If any urgent safety measures are taken the CI/Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the FAMHP and the relevant EC of the measures taken and the circumstances giving rise to those measures.

9.6 The type and duration of the follow-up of subjects after adverse events

Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

All SUSARs occurring from the time of the start of trial treatment until end of follow-up study phase (3 months) must be recorded in the eCRF within 24 hours of the investigational staff becoming aware of the event.

9.7 Development safety update reports

The CI will provide (in addition to the expedited reporting above) DSURs once a year throughout the clinical trial, or on request, to the Competent Authority (FAMHP in Belgium), Ethics Committee and Sponsor.

The report will be submitted within 60 days of the Developmental International Birth Date (DIBD) of the trial each year until the trial is declared ended

10 STATISTICS AND DATA ANALYSIS

The statistical analysis been planned together with Geert Molenberghs and Liesbeth Bruckers from CenStat - University Hasselt.

10.1 Sample size calculation

The ADVOR study is powered for primary end-point which is the most relevant end-point with respect to the study hypothesis and reliable data from large randomized clinical trials are available to make a formal power calculation.

In the DOSE trial, which recruited a similar study population as targeted in the ADVOR study, successful decongestion with a similar definition was approximately 11% vs 18% after 72 h in the low vs high-dose loop diuretics arm.[25] The high-dose loop diuretics arm of the DOSE trial is quite comparable to the standard of care group in the ADVOR study as the loop diuretic dose used in the latter is only slightly lower (2x instead of 2.5x the oral maintenance outpatient dose) and non-loop diuretics, which were infrequently used in the DOSE trial, are not allowed. Because of these slight differences, 15% is chosen as an estimate for occurrence of the primary end-point in the monotherapy with high-dose loop diuretics (SOC) group.

No reliable data are available from large clinical trials to estimate occurrence of the primary end-point in the acetazolamide arm of the ADVOR study. Therefore, after thorough discussion with the advisory board as well as with Frank Hulstaert / Leen Verleye (KCE Trials) a success rate of 25% was chosen, which represents a clear meaningful benefit of 10% more patients with appropriate decongestion after 72 h. Using both estimates, considering a type I error rate $\alpha=0.05$ and type II error rate $\beta=0.20$ (yielding a statistical power of 80%), the targeted sample size for the ADVOR study is calculated at n = 494. A 5% drop out has been calculated in order to estimate the total number of 519 patients to be enrolled in the study.

10.2 Planned recruitment rate

Patients will be recruited by approximately 24 Belgian sites within a period of approximately 24 months. The recruitment rate will start slow due to the site initiation activities at each center, which are performed in parallel during the first recruitment months. The inclusion criteria have been widened so almost all decompensated HF patients with volume overload might be suitable study candidates, which should lead to an easy recruitment of patients. In case recruitment would be lower than expected, the number of participating centers can be increased. Also, with the publication of three-monthly research letters to the participating sites and announcement through posters/leaflets of the ADVOR-study in the outpatient cardiology department, the inclusion process might be enhanced.

10.3 Statistical analysis plan

- The treatment effect for the primary end-point [Treatment success (decongestion achieved) on morning of day 4 without escalating diuretic strategy (doubling loop diuretic dose, addition of chlorthalidone, or ultrafiltration) during IV diuretic therapy on morning of day 3] is evaluated by means of a generalized linear mixed model. The statistical model will include a fixed treatment effect and random center effect.
- For the first secondary end-point [occurrence of the combined endpoint of all-cause mortality and heart failure readmission during 3 months of follow-up], a generalized linear mixed model for a binary outcome will be used. The model will incorporate a fixed treatment effect and random center effect. If the treatment effect on the composite endpoint of 'all-cause mortality and HF readmission' turns out to be statistically significant, both components will be evaluated separately in a hierarchical fashion with HF readmissions first and all-cause mortality second. For this analysis, HF readmission will include patient dying from HF during the 3 months of FU. As a sensitivity analysis the worst case scenario, assuming a HF readmission for all patients dying to non HF related causes during the 3 month follow-up will be executed.
- Length of index hospitalization and change in quality of life scores are compared among treatment arms with a linear mixed model (fixed treatment effect and random center effect). Transformation will be employed when the model assumptions (such normality) are violated.
- All hypothesis are 2-sided and tested with a significance level of $\alpha=0.05$.

The proposed statistical models all assume the missing data mechanism to be missing at random. To investigate the sensitivity of the conclusions with respect to this assumption, a sensitivity analysis by means of multiple imputation technique will be performed.

Secondary Analysis:

- All statistical models discussed in the secondary analysis include a fixed treatment effect and random effect for the center, and in case of longitudinal outcome random patient effects (intercept, slope).
- The mixed models for the primary and secondary end-point analyses will be extended with explanatory variables (such as gender, age, race, ethnicity, kidney function, neurohumoral blockers, diuretics and blood pressure)
- For the end-points collected repeatedly over time, the rate of change will be investigated using longitudinal data models.
- In case patients are re-hospitalized more than once for the same reason, i.e. HF readmission, models for recurrent events will be employed.
- To study the treatment effect on the evolution of a patient's weight a mixed model for repeated measurements data will be used. The two treatment arms will also be compared for the area under the weight curve using an ANOVA model.

- For the first secondary end-point [time till combined endpoint of all-cause mortality and heart failure readmission during 3 months of follow-up], the Kaplan-Meier method will also be used to construct survival curves for both treatment arms. A mixed effects Cox proportional hazard model, with a fixed treatment effect and random center effect is used to investigate the treatment effect for this endpoint. The Hazard ratio with a 95% confidence interval will be obtained. The appropriateness of the proportional hazard assumption will be examined.
- For the primary and secondary endpoint subgroups analysis, with subgroups defined on the basis of LVEF, will also be performed.

10.4 Data collection for economic evaluation

In Belgium 2% of the population has HF, with 15.000 new cases being diagnosed annually and 3% of the annual health care budget is spent on HF, of which most is related to recurrent hospitalization for decompensated HF. HF decompensation often with repeated hospitalization has a tremendous negative impact on quality of life of the patients. Additionally, patients compare an episode of decompensation with 'drowning' so they are in constant fear of recurrent decompensations.

Reducing the number of hospital admittances will have an impact on the health care budget but also reducing the length of hospital stay per admittance will have a positive impact.

According to the "Technische Cel voor de verwerking van de gegevens met betrekking tot ziekenhuizen" (MKG- MFG data of 2014, DRG 194 Hartfalen), 21784 patients were hospitalized for heart failure with an average hospital stay of 12 days in 2014. The average hospitalization costed 7015,44 €/patient, making up a total amount of 152.824.344 €. Of these patients, 10854 patients were hospitalized with advanced symptoms (NYHA III and NYHA IV) with a considerable longer hospitalization duration (17.55 days) and higher costs (10 873,77€/patient). Cost was inclusive of hospital-day-care-price, fees and pharmaceutical products.

According to the MZG department of hospital "Ziekenhuis Oost Limburg AV" HF patients with "majeure or extreme" HF (comparable with NYHA III and NYHA IV) have an average hospital stay of 9,12 days which is significant less then MKG- MFG data available. This reduction of the length of a hospital stay is a result of our HF care giver project as well as the clinical adoption of the use of more effective decongestive therapies often including Acetazolamide. The protocol has been scientifically validated and has been published also in Acta Cardiologica which is a peer-reviewed Belgian Cardiology Journal ((Determinants and impact of the natriuretic response to diuretic therapy in heart failure with reduced ejection fraction and volume overload. Acta Cardiol. 2015 Jun;70:265-73 + Implementation of Transmural Disease Management in Patients Admitted with Advanced Heart Failure. Acta Cardiol. 2014;69:145- 54).).

The ADVOR trial will evaluate whether adding a low cost medication (Diamox ® 8.28 € public price/vial) on top of the standard treatment will reduce the hospital stay of admitted HF patients with 25% as well as reduce the need for renal replacement strategy. In daily practice this would result in an additional cost of < 20€ to potentially reduce the average cost with at least 2500 € per hospitalised patient.This would also imply that acetazolamide, on top of usual care, has the potential to result in a direct cost saving of

38.203.690 € in Belgium (=25% reduction on the budget actually spent on HF hospitalization).
Additionally, it's estimated that a more thorough successful decongestion will also lead to a reduction in heart failure rehospitaliastion of 25% thereby further reducing health care cost. Therefore, a positive result of the ADVOR trial and wide adaptation of the therapeutic algorithm might translate into a reduction in expenses directly related to 1) a shorter hospitalization duration, 2) less costly 'majeur or extreem' HF, and 3) less HF hospitalizations.

While these estimations might be considered 'very optimistic', they are merely a reflection of the potential impact of a reduction of hospitalisation duration as well as readmission seen in the Ziekenhuis Oost Limburg AV after implementation of the aforementioned care pathways including a better and faster decongestion with acetazolamide. However, assuming that the primary endpoint is reached (25% decongestion in the treatment group vs 15% in the standard of care), this would translate in a 11.7% relative risk reduction. If this would translate into a 11,7% reduction in heart failure related expenditure, an annual considerable cost-saving of 17.880.408 € might still be reached.

Importantly, due to the off-patent status of the drug tested in ADVOR there will never be an industry sponsored trial to support this hypothesis. Therefore, ADVOR will lead to a revival of a "forgotten" medicine because it has the potential to significantly impact the way we treat congestion in heart failure patients.

Finally, it's expected that ADVOR will result in a novel innovative approach of treatment of decompensated HF which is focused at a different level of the nephron to achieve better, easier and safer decongestion. In that way ADVOR might pave the way towards a complete change in thinking with regards to treating decompensated heart failure.

This protocol has been designed with a later possible economic analysis in mind. Therefore, economical evaluation of the trial will be possible as several variables will be collected;

- HF readmission
- Length of hospital stay
- Need for renal replacement therapy
- Longitudinal assessment of QoL (EQ5D)…..

As for any further data analyses, an economic analysis can of course be conducted by the sponsor of the trial and the chief investigator, totally independent from KCE.

In addition, an economic analysis can be part of a KCE health technology assessment (HTA) project. HTA projects are conducted by KCE at its own costs as part of its annual work programme approved by the KCE board, and following the KCE processes. The decision for KCE to perform a HTA on the topic will depend on the trial results and the prioritisation of the topic among the topics introduced that year. Each KCE HTA project includes a literature review. Data from different studies may be included. A meta-analysis may be conducted for that purpose, including the results or the coded individual data of the funded trial. HTA projects are conducted internally at KCE or are outsourced to a certain extent using a public tender procedure. In any case, KCE uses external experts during the project.

For an HTA following a trial funded by KCE Trials, KCE would among others, invite the team of the chief investigator to act as external clinical experts to accompany the HTA project.

11 DATA HANDLING

11.1 Data collection tools and source document identification

It is the responsibility of the Principal Investigator at each site to maintain adequate and accurate source data, source documentation and CRFs to record all observations and other data pertinent to the clinical investigation in a timely manner.

Patient's personal data, which are included in the sponsor database shall be treated in compliance with all applicable laws and regulations. The data collected will be anonymized and the data will only be used for the purpose(s) of this trial.

Source Data are defined as "All information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies)."

Source Documents are defined as "Original documents, data and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries of evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial)."

Case report form (CRF) is a form on which individual patient data required by the trial protocol are recorded.

All data relating to the trial must be recorded in the eCRF prepared by the Sponsor. Data reported in the eCRF should be in English, consistent with the source data or the discrepancies should be explained. If information is not known, this must be clearly indicated in the eCRF. All missing and ambiguous data will be queried.

The study data will be transcribed by study personnel from the source documents onto an eCRF, within 5 working days of the subject's visit.

Worksheets may be used for the capture of some data to facilitate completion of the eCRF. Any such worksheets will become part of the subjects' source documentation..

Every effort should be made to ensure that all subjective assessments to be recorded in the eCRF are performed by the same individual who made the initial screening assessment.

The Investigator must verify that all data entries in the eCRF are accurate and correct. All eCRF entries, corrections, and alterations must be made by the Investigator or other authorized study-site personnel. The Investigator or an authorized member of the investigational staff must adjust the eCRF (if applicable) and complete the query.

ADVOR uses an eCRF which will be used to perform statistical analysis for the trial. The CRF will be constructed to ensure:

- adequate data collection
- proper trails will be kept to demonstrate the validity of the trial (both during and after the trial)
- that only the data required by the protocol are captured in the CRF

An annotated CRF is developed with coding convention as will be used in the database.

The Principal investigator is responsible to keep records of all participating patients (sufficient information to link records e.g., CRFs, hospital records and samples), all original signed informed consent forms and copies of the CRF pages.

11.2 Data handling and record keeping

All collected study data will be recorded in the CRF created with the software of CASTOR EDC.

CASTOR EDC complies with all applicable medical data privacy laws and regulations: GCP, 21 CFR Part 11, EU Annex 11, the European Data Protection Directive, ISO9001, and ISO27001/NEN7510.

Once the PI and delegated member(s) of the investigational staff have been trained, they will receive the link of the eCRF together with a log-in account and password. Detailed information regarding the eCRF is provided in the Manual of Operations.

Besides the data entered in the CRF, source documentation will need to be transferred to the sponsor if an endpoint (see section 9) has been reported. Only anonymised sourced data and in accordance with the Belgian Privacy Act of 8 December 1992 and the European Data Protection Act should be transferred to the sponsor. Detailed information regarding the transfer of source documents to the sponsor is provided in the Manual of Operations.

11.3 Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

11.4 Archiving

- archiving will be authorised by the Sponsor following submission of the end of study report
- It is the responsibility of the Principal Investigator at each site to ensure all essential trial documentation and source records (e.g. signed Informed Consent Forms, Investigator Site Files, patients' hospital notes, etc.) at their site are securely retained for at least 20 years
- The sponsor will be responsible for archiving all CRF documents and trial database for at least 20 years
- Therefore, all essential documents will be archived for a minimum period after completion of trial as required by the applicable legislation

12 MONITORING, AUDIT & INSPECTION

A Trial Monitoring Plan will be developed and agreed by the TMG based on the trial risk assessment which will be done by exploring the trial dataset or performing site visits.

The Sponsor will perform on-site monitoring visits as frequently as necessary. The monitor will record dates of the visits in a study center visit log that will be kept at the site. The first post-initiation visit will be made as soon as possible after enrollment at the center has begun. Monitoring might be initially conducted across all sites, and subsequently conducted using a risk based approach that focuses, for example, on sites that have the highest enrolment rates, large numbers of withdrawals, or atypical (low or high) numbers of reported adverse events. At these visits, the monitor will compare the data entered into the CRFs with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the CRF are known to the Sponsor, Chief Investigator and investigational staff and are accessible for verification by the Sponsor site contact. If electronic records are maintained at the investigational site, the method of verification must be discussed with the investigational staff. The processes reviewed can relate to participant enrolment, consent, eligibility, and allocation to trial groups; adherence to trial interventions and policies to protect participants, including reporting of harm and completeness, accuracy, and timeliness of data collection

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data, patients informed consent will be obtained hereto. Findings from this review of CRFs and source documents will be discussed with the investigational staff. The Sponsor expects that, during monitoring visits, the relevant investigational staff will be available, the source documentation will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the Investigator on a regular basis during the study to provide feedback on the study conduct.

13 ETHICAL AND REGULATORY CONSIDERATIONS

13.1 Ethics Committee (EC) review & reports

- Before the start of the trial, approval will be sought from a EC for this trial protocol, informed consent forms and other relevant documents e.g. insurance documents, advertisements and GP information letters
- Substantial amendments that require review by EC will not be implemented until the EC grants a favourable opinion for the study (note that amendments may also need to be reviewed and accepted by the FAMHP before they can be implemented in practice at sites)
- All correspondence with the EC will be retained in the Trial Master File/Investigator Site File
- An annual progress report (APR) will be submitted to the EC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended
- It is the Chief Investigator's responsibility to produce the annual reports as required.
- The Chief Investigator will notify the EC of the end of the study
- If the study is ended prematurely, the Chief Investigator will notify the EC, including the reasons for the premature termination
- Within one year after the end of the study, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the EC

13.2 Peer review

The protocol has been reviewed by KCE (the funder).

In addition, ADVOR has undergone a high quality peer review by two individual experts who have knowledge of the relevant discipline to consider the clinical and/or service based aspects of the protocol, and/or have the expertise to assess the methodological and statistical aspects of the study.

13.3 Public and Patient Involvement

At least 2 % of the Belgian population has heart failure (HF), with 15.000 new cases being diagnosed annually and 3% of the annual health care budget spent on HF, the most of which is related to recurrent hospitalization for decompensated HF. These hospitalizations have a tremendous negative impact on the quality of life of patients.

Therefore, ADVOR will be a RCT with patient and public involvement at all stages of the clinical research. Indeed, ADVOR actually is a result of an active partnership between patients, members of the public including KCE / health care administrators, and researchers in the research process. More specifically. all endpoints of ADVOR are patient-centric measures (i.e.primary endpoint of decongestion) with a formal power calculation. Unfortunately, powering a study for hard cardiovascular endpoints would need

thousands of patients with corresponding budgets and would not be feasible within the Belgian context. Fortunately, successful decongestion (i.e. free of volume overload) has been proved study after study to be a relevant clinical outcome strongly associated with overall prognosis and was therefore chosen as the primary end-point for the ADVOR trial. It's also very patient relevant. In addition, secondary endpoints: all-cause mortality, HF readmission length of stay, and QoL are also very patient-centric.

In addition, the primary and secondary outcome measures are in line with the COMET (Core Outcome Measures in Effectiveness Trials) initiative. Indeed 'decongestion' (being dry is for the patient a very important improvement in symptomatology), heart failure readmission, all-cause mortality, length of stay, quality of life….can all be considered standardised relevant core outcomes sets. Therefore, the core outcomes relevant for any acute heart failure study will be collected and reported, making it easier for the results of ADVOR to be compared, contrasted and combined as appropriate with other trials (Zannad F et al, European Journal of Heart Failure, 2013;15:1082-1094). Additionally, we will continue to explore other tertiary outcomes, which are often mechanistically very interesting, as well.

The protocol has also been discussed with members of "Mon Coeur Entre Parenthèses" which is a patient association representing HF patients and their family.

We will also collect the dosages of neurohumoral blocker therapy throughout the study period, thereby hopefully facilitating a better implementation of guideline recommended therapy. As guidelines recommend a specific dosage for each of these drugs, it's easy to standardize the intake of such medications to be used in an accurate analysis. This will be done as an exploratory tertiary end-point.

Finally, strategies that reduce the number of hospital admittances / length of stay will have an immediate impact on the health care budget.

13.4 Regulatory Compliance

The trial will not commence until a Clinical Trial Authorisation (CTA) is obtained from the FAMPH and EC.

The protocol and trial conduct shall be governed and construed in accordance with the laws of Belgium. The Trial will for instance comply with the Belgian law of May 7th 2004 regarding experiments on the human person and any relevant amendments.

The validity, interpretation and performance of this Protocol shall be governed and construed in accordance with the laws of Belgium. Belgian courts have the exclusive jurisdiction.

13.5 Protocol compliance

Protocol deviations, non-compliances, or breaches are departures from the approved protocol.

- Prospective, planned deviations or waivers to the protocol are not allowed and must not be used e.g. it is not acceptable to enrol a subject if they do not meet the eligibility criteria or restrictions specified in the trial protocol

- Accidental protocol deviations can happen at any time. They must be adequately documented and explained on the relevant forms and reported to the Chief Investigator and Sponsor immediately.
- Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

13.6 Notification of Serious Breaches to GCP and/or the protocol

A “serious breach” is a breach which is likely to effect to a significant degree

- the safety or physical or mental integrity of the subjects of the trial; or
- the scientific value of the trial

The sponsor and the Chief Investigator will be notified immediately of any case where the above definition applies during the trial conduct phase.

The sponsor of the clinical trial will notify the licensing authority in writing of any serious breach of the conditions and principles of GCP in connection with that trial; or the protocol relating to that trial, as amended from time to time, within 7 days of becoming aware of that breach.

13.7 Data protection and patient confidentiality

All investigators and trial site staff must comply with the requirements of the Regulation (EU) 2016/679 of April 27, 2016 of the European Parliament and the Council Concerning the protection of individuals with regard to the processing of personal data and the free movement of such data and repealing Directive 95/46 / EC (general data Protection Regulation) and the Belgian Privacy Act of 8 December 1992 on the protection of privacy in relation to the processing of personal data and the European Data Protection Act. with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act’s core principles.
Therefore,

- personal information will be collected, kept secure, and maintained in a way that is conform all regulation concerning privacy
- the creation of coded, depersonalised data where the participant’s identifying information is replaced by an unrelated sequence of characters
- secure maintenance of the data and the linking code in separate locations using encrypted digital files within password protected folders and storage media
- limiting access to the minimum number of individuals necessary for quality control, audit, and analysis with a list of persons who have access to data, and all this conform the regulation concerning privacy
- the confidentiality of data will be preserved when the data are transmitted to sponsors and co-investigators
- the data will be stored for at least 20 years

- The data custodians are are prof. dr. Mullens, Evi Theunissen and Joke Vanlangenaeker.

13.8 Financial and other competing interests for the chief investigator, PIs at each site and committee members for the overall trial management

As acetazolamide is an off-patent drug, no competing interests that might influence trial design, conduct, or reporting are present for any of the chief investigator, PIs at each site and committee members for the overall trial management.

13.9 Indemnity

1. The Sponsor will ensure appropriate insurance to meet the potential legal liability of the Sponsor(s) for harm to participants arising from the management of the research. Before the start of the trial, approval will be sought from the EC.
2. The Sponsor will ensure appropriate insurance for legal liability of the Sponsor(s) or employer(s) for harm to participants arising from the design of the research. Before the start of the trial, approval will be sought from the EC.
3. The participating sites will ensure appropriate insurance to meet the potential legal liability of investigators/collaborators arising from harm to participants in the conduct of the research.

13.10 Access to the final trial dataset

Only the steering group has access to the full trial dataset in order to ensure that the overall results are not disclosed by an individual trial site prior to the main publication.

However, site investigators will be allowed to access the full dataset if a formal request describing their plans is approved by the steering group.

14 DISSEMINATION POLICY

14.1 Dissemination policy

Upon completion,

- the data arising from the trial will be owned by the sponsor
- the data will be analysed and tabulated and a Final Study Report prepared
- the full study report can be accessed on the website of KCE as well as on ClinicalTrials.gov
- participating investigators will have rights to publish any of the trial data upon approval of the steering committee
- The publication containing the primary study results should be finalized within 6 months of the statistical analysis. There are no time limits or review requirements on the additional publications.
- Funding by KCE will be acknowledged within the publications
- The participants of the trial will be notified by a letter containing the outcome of the trial by provision of the publication and/or via a specifically designed newsletter
- The participant might specifically request results from their PI upon completion of the trial, which might be provided once the results have been published
- It's foreseen that at the latest at publication, a machine readable electronic copy of the published version or final peer-reviewed manuscript accepted for publication in a repository for scientific publications will be deposit (open access). The research data needed to validate the results presented in the scientific publications will be deposited.

Upon completion, the study will also be submitted for presentation at the annual European Society of Cardiology in the late-breaking Clinical Trial Session as well as during the Annual European Heart Failure Association meeting.

The primary study results of ADVOR will be reported fully and made publicly available when the research has been completed. All researchers shall ensure that the outcome of the research is prepared as a research paper for publication in a suitable peer-reviewed, preferably open-access, journal. In addition, the database of the ADVOR study will be available for further sub-analysis per request of any of the sub-investigators. As a result, we feel that at least 10 other publications might be possible based on the data collected in ADVOR study which might all help to treat decompensated HF patients better. The Consort Guidelines and checklist will be reviewed prior to generating any publications for the trial to ensure they meet the standards required for submission to high quality peer reviewed journals etc. *http://www.consort-statement.org/*

All participating investigators will also try to disseminate their research findings to the broader public as well as to the research participants when the study has completed.

Endpoints were meticulously chosen based on consensus of all participating investigators including European Heart Failure Association as well as HF patient organisation (Mon Coeur Entre Parenthèses). Therefore, a positive result will also have the potential to be adopted soon in the national and international guidelines to treat decompensated HF patients.

In conclusion, it's felt that a positive endpoint might lead to a fast adoption of the use of acetazolamide in the treatment of ADHF patients because of

1) Publication in top ranked cardiology journal
2) Presentation of study results in national and international cardiology meetings
3) Adoption in guidelines
4) Internationally recognized expert study team

14.2 Authorship eligibility guidelines and any intended use of professional writers

For ADVOR, the Steering Committee will comprise the Publication Committee. KCE may serve as members of the committee. This committee will manage study publications with the goal of publishing findings from the data. The Publication Committee will develop the final Publication Plan as a separate document. In addition, the committee will apply and reinforce the authorship guidelines set forth in the Publication Plan. Membership in the Publication Committee does not guarantee authorship. The committee will meet at regular intervals.

Publications will adhere to authorship criteria defined by the International Committee of Medical Journal Editors (ICMJE, Uniform requirements for manuscripts submitted to biomedical journals, www.icmje.org). Individual authorship criteria defined by the target journal or conference will be followed when it differs from ICMJE criteria. Authors, including KCE representatives, must at a minimum meet all of the conditions below:

- Substantial contribution to conception and design, or acquisition of data, or analysis and interpretation of data; AND
- Drafting the article or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Decisions regarding authorship and contributorship will be made by the committee. The selected authors will be responsible for drafting the publication. All selected authors must fulfill the authorship conditions stated above to be listed as authors, and all contributors who fulfill the conditions must be listed as authors.

All investigators not listed as co-authors will be acknowledged as the "ADVOR Study Investigators" and will be individually listed according to the guidelines of the applicable scientific journal when possible. Any other contributors will be acknowledged by name with their specific contribution indicated. Based on the recruitment, site investigators might also be part of the Authorship.

A methods paper describing the ADVOR study, as well as the publication containing the primary study results will be drafted by the Chief Investigator, and submitted for publication after approval of the members of the steering committee.

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■ APPENDICES

16 APPENDIX 1: AUTHORISATION OF PARTICIPATING SITES

Appendix 1.1. Required documentation

Prior to submitting the trial to the Ethics Committee, the Principal Investigator (PI) is required to sign a protocol signature page confirming his/her agreement to conduct the trial in accordance with these documents and all of the instructions and procedures found in this protocol.

Detailed information regarding the mandatory documentation which are required before the trial can start at the participating sites can be found in the Manual of Operations.

Appendix 1.2. Procedure for initiating/opening a new site

Once all start-up documentation (see Manual of Operations) from the participating site is available at the sponsor and the IMP/study material is available at the participating site, the sponsor will send confirmation by e-mail to the PI that the study can start. Only upon receipt of this site activation confirmation the site can screen/enrol patients.

Appendix 1.3. Principal Investigator responsibilities

The PI is the responsible leader of the investigational team of the participating site. The PI is responsible that he/she and his/her investigational team conducts the trial according the instructions and procedures documented in this protocol. Full list of PI's legal responsibilities are listed in the Clinical Trial Agreement.

The PI has the primary responsibility to protect the rights and welfare of the patient in the trial. The PI's primary responsibilities also include the following:

- Delegation of Responsibilities

  PI must personally perform or delegate to qualified sub-investigator or investigational staff all of the necessary tasks to carry out this trial. Even when specific tasks are delegated, the PI remains ultimately responsible for proper conduct of the trial and fulfilment of all associated obligations.

- Oversight of Investigational Team

  The PI must provide members of the investigational team with sufficient oversight, training and information to facilitate appropriate safety procedures and protocol adherence. In addition, the EC must be informed if a PI is no longer able to 67ulfil his or her duties for any reason including, but not limited to, traveling for a prolonged period of time.

- Evaluation of Adequacy of Resources

  Pis must ensure that adequate resources (facilities, equipment, supplies, and personnel) exist to conduct the research, protect subjects and ensure the integrity of the research.

- Document Retention

The PI must ensure adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial patients. Source data should be attributable, legible, contemporaneous, original, accurate and complete. PI must ensure that this source data is reported to the sponsor in the CRF and in the required reports according to the timelines defined in this protocol.

17 APPENIX 2: EQ-5D QUESTIONNAIRE (DUTCH)

Vink onder elke titel het ENE vakje aan dat het best uw gezondheid VANDAAG beschrijft.

MOBILITEIT

Ik heb geen problemen met rondwandelen ❑
Ik heb een beetje problemen met rondwandelen ❑
Ik heb matige problemen met rondwandelen ❑
Ik heb ernstige problemen met rondwandelen ❑
Ik ben niet in staat om rond te wandelen ❑

ZELFZORG

Ik heb geen problemen met mijzelf te wassen of aan te kleden ❑
Ik heb een beetje problemen met mijzelf te wassen of aan te kleden ❑
Ik heb matige problemen met mijzelf te wassen of aan te kleden ❑
Ik heb ernstige problemen met mijzelf te wassen of aan te kleden ❑
Ik ben niet in staat mijzelf te wassen of aan te kleden ❑

DAGELIJKSE ACTIVITEITEN *(bijv. werk, studie, huishouden, gezins- en vrijetijdsactiviteiten)*

Ik heb geen problemen met mijn dagelijkse activiteiten ❑
Ik heb een beetje problemen met mijn dagelijkse activiteiten ❑
Ik heb matige problemen met mijn dagelijkse activiteiten ❑
Ik heb ernstige problemen met mijn dagelijkse activiteiten ❑
Ik ben niet in staat mijn dagelijkse activiteiten uit te voeren ❑

PIJN / ONGEMAK

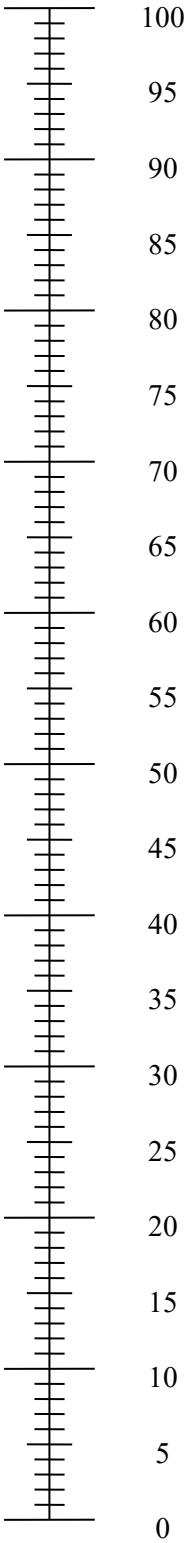
Ik heb geen pijn of ongemak ❑
Ik heb een beetje pijn of ongemak ❑
Ik heb matige pijn of ongemak ❑
Ik heb ernstige pijn of ongemak ❑
Ik heb extreme pijn of ongemak ❑

ANGST / DEPRESSIE

Ik ben niet angstig of depressief ❑
Ik ben een beetje angstig of depressief ❑
Ik ben matig angstig of depressief ❑
Ik ben erg angstig of depressief ❑
Ik ben extreem angstig of depressief ❑

- We willen weten hoe goed of slecht uw gezondheid VANDAAG is.
- Deze meetschaal (te vergelijken met een thermometer) is genummerd van 0 tot 100.
- 100 staat voor de beste gezondheid die u zich kunt voorstellen.
  0 staat voor de slechtste gezondheid die u zich kunt voorstellen.
- Plaats een X op de meetschaal om aan te geven hoe uw gezondheid VANDAAG is.
- Noteer nu het getal dat u aangeduid hebt op de meetschaal in het onderstaande vakje.

UW GEZONDHEID VANDAAG = ☐



18 APPENDIX 3 : EQ-5D QUESTIONNAIRE (FRENCH)

Pour chaque rubrique, veuillez cocher UNE case, celle qui décrit le mieux votre santé AUJOURD'HUI.

Mobilité

Je n'ai aucun problème pour me déplacer à pied ❑

J'ai des problèmes légers pour me déplacer à pied ❑

J'ai des problèmes modérés pour me déplacer à pied ❑

J'ai des problèmes sévères pour me déplacer à pied ❑

Je suis incapable de me déplacer à pied ❑

Autonomie de la personne

Je n'ai aucun problème pour me laver ou m'habiller tout(e) seul(e) ❑

J'ai des problèmes légers pour me laver ou m'habiller tout(e) seul(e) ❑

J'ai des problèmes modérés pour me laver ou m'habiller tout(e) seul(e) ❑

J'ai des problèmes sévères pour me laver ou m'habiller tout(e) seul(e) ❑

Je suis incapable de me laver ou de m'habiller tout(e) seul(e) ❑

Activités courantes *(exemples: travail, études, travaux ménagers, activités familiales ou loisirs)*

Je n'ai aucun problème pour accomplir mes activités courantes ❑

J'ai des problèmes légers pour accomplir mes activités courantes ❑

J'ai des problèmes modérés pour accomplir mes activités courantes ❑

J'ai des problèmes sévères pour accomplir mes activités courantes ❑

Je suis incapable d'accomplir mes activités courantes ❑

Douleurs / gêne

Je n'ai ni douleur ni gêne ❑

J'ai des douleurs ou une gêne légère(s) ❑

J'ai des douleurs ou une gêne modérée(s) ❑

J'ai des douleurs ou une gêne sévère(s) ❑

J'ai des douleurs ou une gêne extrême(s) ❑

Anxiété / Dépression

Je ne suis ni anxieux(se), ni déprimé(e) ❑

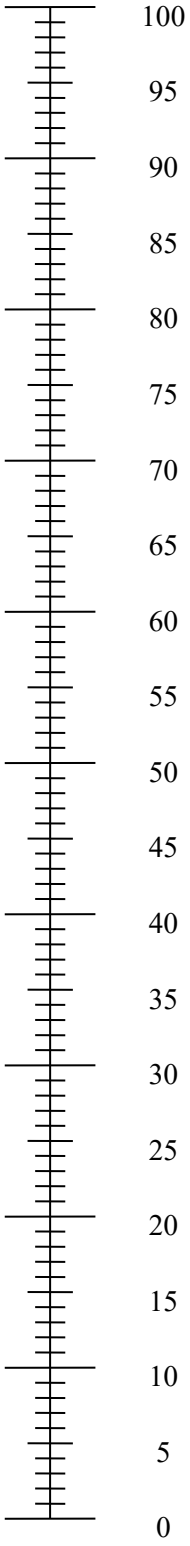
Je suis légèrement anxieux(se) ou déprimé(e) ❑

Je suis modérément anxieux(se) ou déprimé(e) ❑

Je suis sévèrement anxieux(se) ou déprimé(e) ❑

Je suis extrêmement anxieux(se) ou déprimé(e) ❑

- Nous aimerions savoir dans quelle mesure votre santé est bonne ou mauvaise AUJOURD'HUI.
- Cette échelle est numérotée de 0 à 100.
- 100 correspond à la meilleure santé que vous puissiez imaginer.
  0 correspond à la pire santé que vous puissiez imaginer.
- Veuillez faire une croix (X) sur l'échelle afin d'indiquer votre état de santé AUJOURD'HUI.
- Maintenant, veuillez noter dans la case ci-dessous le chiffre que vous avez coché sur l'échelle.



VOTRE SANTÉ AUJOURD'HUI = ☐

19 APPENDIX 4 : URINARY COLLECTION PROCEDURE

Urinary collection procedure:

Patients need to empty their bladder before the administration of the start dose of loop diuretics.

The urinary collection need to start immediately after first bolus administration of loop diuretics and acetazolamide or placebo and the collection will stop the latest as close to but prior to the morning bolus of study medication on day 3. This collection will be defined as the Total Urinary Collection in the study. Importantly, the total volume needs to be written down in the study files as this is will be used for tertiary end-point analysis and is needed for the clinician to decide if escalation of therapy is needed.

Total Urinary Collection equals the sum of Urinary Collection period 1 + Urinary Collection period 2:

Urinary Collection period 1 = Urine collection that starts immediately after first bolus administration until the morning of day 2. This Urinary Output 1 value will be reported in the CRF together with start- and stop date/time of this first collection period.

Urinary Collection period 2 = Urine collection that starts with the end of Urinary Output 1 until the morning of day 3 prior to the morning bolus of study medication. This Urinary Output 2 value will be reported in the CRF together with start- and stop date/time of this second collection period.

How to collect the urine?

Urinary catheter insertion is strongly recommended but not mandatory to achieve an optimal urine collection. However, in case of urinary incontinence, placement of a urinary catheter is mandatory.

Alternatively, the patient need to use a urinary container(s) to collect ALL urine. Prior to the stop of urinary collection period the patient need to be instructed to empty their bladder. Care should be taken to ensure ALL urine is collected.

At the end of a Urinary Collection period all urine of this period needs to be collected in the urinary container(s).

Which measurements need to be done?

For each urinary collection period the container(s) or a sample (if multiple containers are kept, they need to be mixed before taking a sample) will need to be sent to the local lab for analysis of the volume, Cr, total protein and Na (and bumetanide level, if available).

20 APPENDIX 5 : AMENDEMENT HISTORY

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made

List details of all protocol amendments here whenever a new version of the protocol is produced.

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the EC committee or FAMHP.

Final Protocol

Version - 2.0

3rd of January 2019

FULL/LONG TITLE OF THE TRIAL

A multi-center, randomized, double-blind, phase IV clinical trial on the diuretic effects of Acetazolamide (Diamox ®) in patients with Decompensated heart failure and Volume OveRload

SHORT STUDY TITLE / ACRONYM

Acetazolamide (Diamox ®) in Decompensated heart failure with Volume OveRload (ADVOR)

PROTOCOL VERSION NUMBER AND DATE

Version number: version 2.0
Version date: 03 January, 2019

RESEARCH REFERENCE NUMBERS

EudraCT Number:	2018-001345-14
Clinical trials.gov Number:	NCT03505788
SPONSOR Number:	ZOLCAR17001
KCE Trial Number:	KCE-17001

TABLE OF CONTENTS

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the requirements for the conduct of clinical trials in the EU as provided for in " Directive 2001/20/EC", and any subsequent amendments, GCP guidelines, the Belgian law of May 7th 2004 regarding experiments on the human person, the Sponsor's SOPs, and other regulatory requirements as amended.

The undersigned agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

The undersigned also confirm that they will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature: [redacted]

Date: 3/1/2019

General Director **Ziekenhuis Oost-Limburg AV**

Signature: [redacted]

Date: 4/1/2019

Chairman **Ziekenhuis Oost-AV**

[redacted]

Date: 3/1/2019.

Medical Director **Ziekenhuis Oost-Limburg AV**

Ziekenhuis Oost-Limburg

Chief Investigator:
Signature: [redacted]

Date: 08/01/2019

Date:

Statistician:
Signature: [redacted]

Date: 3/1/2019

Head of CenStat - Hasselt University

Acknowledged by **Funder (KCE)**
Signature: [redacted]

Date: 3/1/2019

Print name: [redacted]

KEY TRIAL CONTACTS

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Sponsor	Ziekenhuis Oost Limburg Autonome Verzorgingsinstelling Schiepse bos 6, 3600 Genk, Belgium Main contact person: Evi Theunissen Phone: [redacted] / E-mail: [redacted] Legal contact persons: Joke Vanlangenaeker and Martine Verplancke Phone: [redacted] / E-mail: [redacted] Although KCE provides funding for the trial, KCE shall under no circumstances be considered as sponsor of the Study or assume any responsibilities or liabilities in connection therewith, and sponsor or co-sponsor shall make no representations whatsoever in this respect.
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Project team	ADVOR study team Clinical Trial Unit, Ziekenhuis Oost-Limburg, Autonome Verzorgingsinstelling

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Committees	Trial Steering Committee Trial Management Group Endpoint Adjudication Committee *See below for more information regarding these committees*

TRIAL SUMMARY

Trial Title	A multi-center, randomized, double-blind, phase IV clinical trial on the diuretic effects of Acetazolamide (Diamox ®) in patients with Decompensated heart failure and Volume OveRload
Short title	Acetazolamide (Diamox ®) in Decompensated heart failure with Volume OveRload
Clinical Phase	IV
Study type	Interventional
Planned sample size	Approximately 519 patients
Trial duration	Approximately 27 months (3 months follow-up / patient)
Planned Trial Period	2018-2021
Purpose and rationale	To investigate if combination therapy with acetazolamide improves loop diuretic efficacy to increase diuresis in decompensated heart failure (HF) patients, allowing for a better/faster decongestion and potentially resulting in improved clinical outcome and increased quality of life.
Primary endpoint	Treatment success (decongestion achieved) on the morning of day 4 without the need for escalating diuretic strategy (doubling loop diuretic dose, addition of chlorthalidone, or ultrafiltration) on the morning of day 3
Secondary endpoints	1. Combined end-point of all-cause mortality and heart failure readmission during 3 months of follow-up 2. Length of index hospital admission 3. Longitudinal changes in EuroQoL five dimensions questionnaire (EQ-5D) (baseline, the morning of day 4 or at discharge (whatever comes first), any readmission, and 3 months).
Trial Design	The ADVOR study is a phase IV randomized, multi-center, double-blind study, comparing monotherapy with high-dose loop diuretics (standard of care - SOC) versus combination therapy with acetazolamide and high-dose loop diuretics in patients with decompensated heart failure and clinical signs of volume overload. The study consists of 3 phases: • screening phase: starting from identifying a study subject prior to / during hospitalization until the first dose of study medication will be given • treatment phase: starting from the first dose of study medication administration until the morning of day 4 or earlier in case of successful decongestion sooner. • follow-up phase: starting when the treatment phase ends until 3 months after the study start dose.
Trial Participants	The study population will consist of patients hospitalized with decompensated HF and demonstrating at least one clinical sign of volume overload.
Main Inclusion Criteria	• An elective or emergency hospital admission with clinical diagnosis of ADHF and at least one clinical sign of volume overload (e.g. oedema, ascites or pleural effusion) • Maintenance therapy with oral loop diuretics at a dose of at least 1 mg bumetanide or 40 mg furosemide or 20 mg torsemide for at least 1 month before hospital admission • Plasma NT-proBNP levels >1000 ng/L or BNP levels >250 ng/L at screening

Main Exclusion Criteria	• Concurrent diagnosis of an acute coronary syndrome defined as typical chest pain in addition to a troponin rise above the 99th percentile and electrocardiographic changes suggestive of cardiac ischemia • History of congenital heart disease requiring surgical correction • History of cardiac transplantation and/or ventricular assist device • Systolic blood pressure <90 mmHg or mean arterial pressure <65 mmHg at screening • Expected use of intravenous inotropes, vasopressors or nitroprusside during the study. Use of nitrates and/or molsidomine is allowed at the discretion of the treating physician • Estimated glomerular filtration rate (eGFR) <20 mL/min/1.73m² at screening • Use of renal replacement therapy or ultrafiltration at any time before study inclusion • Treatment with intravenous loop diuretics > 2 mg bumetanide or an equivalence of another loop diuretic during the index hospitalization before randomization • Treatment with acetazolamide within 1 month prior to randomization • Exposure to nephrotoxic agents (i.e. contrast dye) anticipated within the next 3 days • Use of any non-protocol defined diuretic agent with the exception of mineralocorticoid receptor antagonists during the treatment phase of the study. Thiazides, metolazone, indapamide and amiloride should be stopped upon study inclusion. If patient is taking a combination drug including a thiazide-type diuretic, the thiazide-type diuretic should be stopped upon study inclusion. • Current use of sodium-glucose transporter-2 inhibitors • Subjects who are pregnant or breastfeeding • Subjects with urinary incontinence who are not willing to receive a bladder catheter
Exploratory tertiary end-points	• Body weight change after day 1, 2, 3, 4 and discharge compared to screening • All-cause mortality during first 3 months after study start dose • Heart failure readmissions during first 3 months after study start dose • All cause rehospitalisations during first 3 months after study start dose • Total urinary volume and natriuresis starting from first intravenous (IV) diuretic administration at randomization until the morning of day 3 (urinary output, see figure 1 and appendix 4) • Relative plasma BNP or NT-proBNP change from screening until the assessment on day 4 or at discharge (whatever comes first) and at 3 months • Total dose of IV loop diuretics used during treatment phase. • Changes in doses of neurohumoral blockers from baseline to discharge and after 3 months • Need for renal replacement therapy or ultrafiltration during first 3 months after study start dose • Incidence of hyponatremia during treatment phase • Hypokalaemia during treatment phase • Incidence of metabolic acidosis requiring NaHCO3 supplements during first 4 days • WRF defined as a >0.3 mg/dL increase in serum Cr, or a >20% decrease in eGFR by the CKD-EPI formula during treatment phase • Liver dysfunction at screening • Plasma volume changes during treatment phase (assessed by albumin and hematocrit) • Occurrence of iron deficiency at screening

	Optional laboratory sub-study in participating centers: • Change from baseline in selected biomarkers from baseline through 3 months after study start dose in a subset of randomized patients
Safety assessments	• Adverse events • Laboratory values (including monitoring hypokalaemia, metabolic acidosis, substantial increase in creatinine (Cr), substantial decrease in eGFR)
Data analysis	• The treatment effect for the primary endpoint is evaluated by means of a generalized linear mixed model. The statistical model will include a fixed treatment effect and random center effect. • For the first secondary end-point [occurrence of the combined endpoint of all-cause mortality and heart failure re-admission during 3 months of follow-up], a generalized linear mixed model for a binary outcome will be used. The model will incorporate a fixed treatment effect and random center effect. If the treatment effect on the composite endpoint of 'all-cause mortality and HF readmission' turns out to be statistically significant, both components will be evaluated separately in a hierarchical fashion with HF readmissions first and all-cause mortality second. • Length of index hospitalization and change in quality of life scores are compared among treatment arms with a linear mixed model (fixed treatment effect and random center effect). Transformation will be employed when the model assumptions (such normality) are violated. • All hypothesis are 2-sided and tested with a significance level of α=0.05

ROLE OF STUDY SPONSOR AND FUNDER

Ziekenhuis Oost Limburg autonome verzorginsinstelling (ZOL AV), as mentioned in KEY TRIAL CONTACT shall act as sponsor of the Study, as defined in the Law of 2004, and in accordance with the Law of 2004, the sponsor shall assume, even without fault, the responsibility of any damage incurred by a study patient or, in the case of death, his rightful claimants sustained that arises either in direct or indirect connection with the experiments and shall provide compensation therefore. The Sponsor shall enter into an insurance contract in accordance with article 29 of the Law of 2004. ZOL AV shall ensure that it shall be mentioned in the Protocol, the Informed Consent Forms and in other relevant communication with the Study Subjects or the Regulatory Authorities as sponsor of the Study.

ZOL AV has designed the trial together with the Trial Steering Committee (TSC). ZOL AV and the TSC will be responsible for the data analysis (with assistance of the “center of statistics”, University Hasselt), interpretation, manuscript writing, and dissemination of results. ZOL AV will have the final decision regarding any of these aspects of the trial. However, publication of the main study results will be the responsibility of the TSC. Even in case of a negative study result, the data will be published.

ZOL AV acknowledges and agrees for the avoidance of doubt that KCE shall under no circumstances be considered as sponsor of the Study or assume any responsibilities or liabilities in connection therewith, and ZOL AV shall make no representations whatsoever in this respect.

ROLES AND RESPONSIBILITIES OF TRIAL MANAGEMENT COMMITEES

Trial Steering Committee (TSC)

The role of the Trial Steering Committee (TSC) is to provide the overall supervision of the trial. The TSC includes members who are independent of the investigators, their employing organisations or institutions, funders and sponsors. The TSC monitors trial progress, conduct and advise on scientific credibility. The TSC will consider and act, as appropriate, and ultimately carries the responsibility for deciding whether a trial needs to be stopped on grounds of safety or efficacy. The TSC shall oversee the performance of the study and discuss important topics in relation thereto.

The TSC will meet on average 3 times per year or as necessary when adapted to the stage of the trial (set-up, conduct, analysis). The TSC is composed of the CI, trial statistician, the trial coordinator, 7 independent experts, minimum 2 representatives of other participating centers with at least one representative of the French speaking sites and one representative of the Dutch speaking sites, up to 2 representatives of patients or the general public, 1 representative of the sponsor and 1 representative of the funder. The TSC will send reports to the sponsor and the funder. KCE shall have the right (but not the obligation) to be present at each TSC meeting.

Details of the final members of the TSC, their responsibilities, number of meetings and reporting procedures can be found in the TSC charter.

Trial Management Group (TMG)

The TMG includes those individuals responsible for the day-to-day management of the trial, such as the Chief Investigator, statistician, trial coordinator, and data manager. The role of the group is to monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself.

Chief Investigator: Wilfried Mullens (ZOL AV, Genk, Belgium)

Statistician: Liesbeth Bruckers (University Hasselt, Belgium)

Trial Coordinator: Katrien Tartaglia (ZOL AV, Genk, Belgium)

Data Manager: Liesbet Van Brussel (ZOL AV, Genk, Belgium)

Data Safety Monitoring Committee (DSMC)

The DSMC is not needed as this is a low-risk pragmatic interventional trial with a short inclusion period studying an old drug with a well-known safety profile within an accepted clinical indication.

Endpoint Adjudication Committee (EAC)

The EAC will adjudicate the HF-related endpoints. Throughout the course of the study the EAC will assess events, and determine whether these events should contribute to the secondary endpoints of the ADVOR trial. HF readmissions are defined as either a hospital admission because of decompensated HF or an unscheduled contact at the emergency department for worsening HF if the patient is treated with

intravenous loop diuretics. The EAC will have at least two members, specialized in heart failure management. None of the EAC members will be participating investigators in the ADVOR trial.

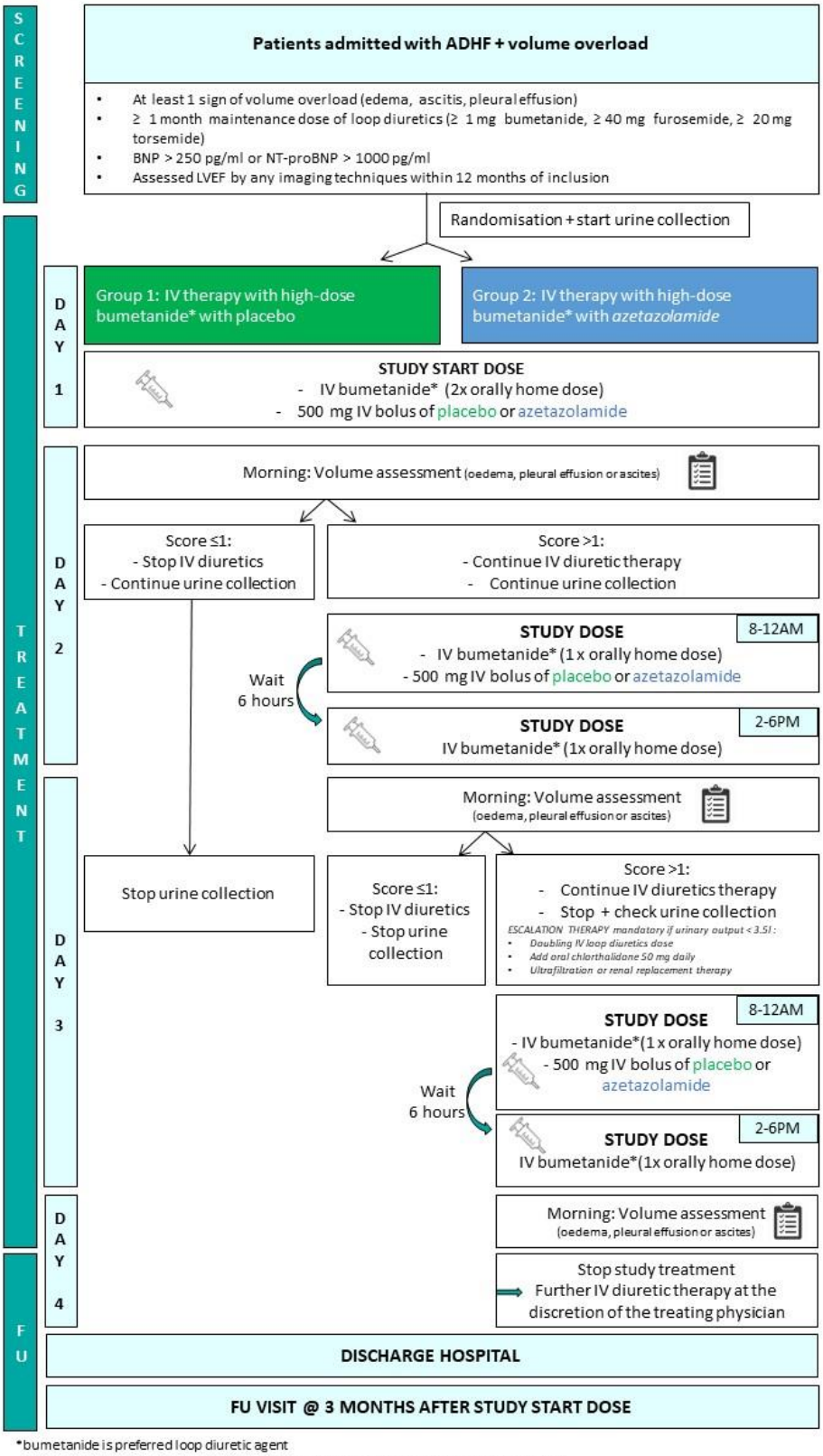
LIST OF ABBREVIATIONS

ADHF	Acute Decompensated Heart Failure
AE	Adverse Event
BID	“Bis in die” (twice a day)
BWGHF	Belgian Working Group of Heart Failure
BNP	B-type natriuretic peptide
CI	Chief Investigator
Cr	Creatinine
EAC	Endpoint Adjudication Committee
eCRF	Electronic Case Report Form
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
EQ-5D	EuroQol five dimension (questionnaire)
ESC	European society of Cardiology
GCP	Good Clinical Practice
GMP	Good Manufacture Practice
HFA	Heart Failure Association
HF	Heart Failure
HFpEF	Heart Failure with preserved Ejection Fraction
HFrEF	Heart Failure with reduced Ejection Fraction
HTA	Health technology assessment
IEC	Independent ethics committee
IMP	Investigational medicinal product
IRT	Interactive response technology
ISF	Investigator Site File
IV	Intravenous
NT-proBNP	N-terminal of pro-B-type natriuretic peptide
MG	milligram
PI	Principal Investigator
PM	Project Manager
QoL	Question of Life
RCT	Randomised Clinical Trial
RRT	Renal replacement therapy
SD	Source document
SI	Sub-investigator
SOC	Standard of care
TMG	Trial Management Group
TSC	Trial Steering Committee

VAS	Visual analogue scale
WRF	Worsening renal function
ZOL AV	Ziekenhuis Oost Limburg Autonome Verzorgingsinstelling

TRIAL FLOW CHART

Figure 1. Trial flow Chart.



*bumetanide is preferred loop diuretic agent
Conversion factor is 1 mg bumetanide = 20mg torsemide = 40 mg furosemide (IV and oral)
Bolus of bumetanide is limited to 5 mg bumetanide

1 BACKGROUND

Aging of the population and prolongation of the lifespan of cardiac patients by modern therapeutic innovations have led to an increased incidence of HF (1). During the last two decades, important progress has been made in the treatment of ambulatory HFrEF patients. Renin-angiotensin system blockers, β-blockers, mineralocorticoid receptor antagonists, ivabradine, neprilysin inhibition, and cardiac resynchronization therapy have all been demonstrated to reduce morbidity and/or mortality in ambulatory HFrEF patients (2-18).

Despite these important advances, many patients are still hospitalized frequently with decompensated HF demonstrating most often signs and symptoms of systemic congestion and volume overload, which is associated with worse outcome (19). Treatment in these cases mainly focuses on symptomatic relief through administration of diuretics, although clear evidence on the optimal agent, dosing schedule, and administration route is lacking. Coexisting renal dysfunction often complicates decongestive treatment and worsening renal function (WRF), often defined as a 0.3 mg/dL rise in serum Cr, is a common finding in this context (20). However, the prognostic impact of WRF defined as Cr change is unsure as it might be associated with worse, neutral or even better outcome (21-23). In contrast, persistent congestion and volume overload, as a reflection of the renal inability to preserve sodium homeostasis, has been more consistently associated with higher mortality and more frequent readmissions in HF (24). This suggests that achieving a net negative fluid balance might be an attractive treatment target in decompensated HF.

Loop diuretics are by far the most commonly used agents to achieve decongestion in decompensated HF. Especially in diuretic-naïve patients, they are often very effective to relief dyspnoea and congestive symptoms. However, in the Diuretic Optimization Strategies Evaluation (DOSE) trial, which is the only randomized clinical trial on diuretic therapy for decompensated HF patients, no differences in patients' global assessment of symptoms or change in renal function were observed when loop diuretics were administered by bolus as compared with continuous infusion or at high versus low dose during a hospitalization for decompensated HF (25). Also, only a minority of patients (15%) were adequately decongested after 72 h in the DOSE trial, thereby indicating the urgent need for more effective decongestive therapies. Furthermore, guidelines from international cardiac societies lack high-quality data on the optimal dosing, timing and method of delivery of diuretic agents. Importantly, there are several reasons why loop diuretics might be less effective or even harmful in HF. First, loop diuretics directly stimulate renin production by inhibiting the $Na^+/K^+/2Cl^-$-cotransporter on the luminal side of the macula densa, which depletes intracellular chloride levels in the macula densa. The consequence is an increased cyclo-oxygenase-2 and nitric oxide synthase I activity in macula densa cells, leading to paracrine prostaglandin E_2 and nitric oxide secretion (26). Both prostaglandin E_2 and nitric oxide work in concert to stimulate renin release by granulosa cells of the afferent arteriole and further detrimental activation of the renin-angiotensin-aldosterone axis. Second, impaired secretion of loop diuretics in the proximal tubules of HF patients, especially when there is concomitant renal dysfunction, results in lower concentrations at the place where these agents act – the luminal side of the thick ascending limb of Henle's loop. Third, increased sodium reabsorption in the proximal tubules might result in less sodium offered to the thick ascending limb

of Henle's loop, especially if glomerular filtration is concomitantly impaired, hampering the efficacy of loop diuretics.

Recent advances in heart failure (HF) biomarker studies suggest promise from markers enhancing traditional method of assessing affected patients. B-type natriuretic peptide (BNP) and its biologically inert, amino-terminal pro-peptide counterpart (NT-proBNP)[32,33] have quickly become an essential component in the diagnosis and determining prognosis in HF. With a large number of biomarkers now or soon to be available, an understanding of the role that biomarkers may play in HF care is necessary.

2 RATIONALE

From a pathophysiological point of view, targeting sodium reabsorption in the proximal tubules has several potential benefits in HF. First, it is the place where most sodium is reabsorbed, especially in decompensated HF. Second, greater delivery of chloride to macula densa cells decreases renin production, ceasing neurohumoral activation. Third, endogenous natriuretic peptides (acting in the distal nephron) will possibly regain their effects (27). The carbonic anhydrase inhibitor acetazolamide (Diamox®), which is approved for the treatment of mountain sickness, inhibits sodium reabsorption in the proximal tubules. Despite the pathophysiological rationale for inhibition of proximal sodium reabsorption in decompensated HF, acetazolamide is now a largely forgotten diuretic. One observational study in patients with decompensated HF and marked volume overload found that the addition of acetazolamide improved loop diuretic efficacy with ~100 mmol Na+ excreted per 40 mg of furosemide equivalent dose (28). Thus, although the diuretic and natriuretic capacity of acetazolamide is poor on its own, it might well be a very efficient booster of diuretic efficacy in combinational diuretic therapy with loop diuretics. This concept is further supported by one small randomized trial including 24 patients with volume overload refractory to loop diuretic therapy (29). All these patients demonstrated a greatly reduced fractional sodium excretion, which was easily overcome by the addition of acetazolamide. We've conducted a small two-center trial to see if improved diuretic efficacy with acetazolamide in a patient population with heart failure and cardio-renal syndrome at high risk for diuretic resistance translates into better natriuresis. (Clinical Trial NCT01973335). The study has just been finished with analysis of the results ongoing. Importantly, the promising concept of blocking proximal nephron sodium absorption with acetazolamide has been published over the last couple of years (30).

The ADVOR study has an innovative primary end-point. As abundant evidence has consistently linked persistent volume overload after decongestive therapy in decompensated HF with poor outcomes, decongestion itself is a valid surrogate end-point (24). It has been demonstrated from reanalysis of the DOSE trial and CARRESS that the persistent oedema has excellent prognostic ability to predict death, readmissions or unscheduled medical contacts (31). In ADVOR, a more exhaustive congestion score with the emphasis on relief of volume overload will be used. Secondary end-points in ADVOR will be very clinically relevant. A more thorough decongestion should also translate into less readmissions for recurrent decompensation, better renal preservation, and eventually lower mortality. This will be assessed as a key secondary end-point after hospital stay. Additionally, time to decongestion is a major determinant of hospital stay and combinational therapy with acetazolamide might significantly shorten this. Finally, improved quality

of life for patients is expected with better decongestion and will be the last secondary end-point. Importantly, acetazolamide is an easy and cheap drug to use (add on, bolus infusion, no special monitoring required), with a potentially favourable cost-efficiency profile. Important health economic data, specifically for the Belgian situation, will be obtained through the ADVOR study.

During the ADVOR study centers can decide to participate in the optional laboratory sub-study. The urine and blood samples collected through this laboratory sub-study will be stored in the University Biobank of Limburg and it will become the biggest databank of its kind within diuretic studies for patients with decompensated heart failure.

This laboratory sub-study will investigate more in detail the mechanistic and potential favourable effect of acetazolamide and loop diuretics. Furthermore, this laboratory sub-study will provide new insights into the pathophysiology of decompensated HF and potentially will allow for identifying a high risk patient population. This could ultimately lead to improved and patient tailored treatment strategies. Biomarker sub-studies have become a valuable source of data for such analysis, and offer unique insights into mechanistic and pathophysiologic pathways in a well selected and phenotyped patient population.

2.1 Assessment and management of risk

The study will examine if the addition of acetazolamide will lead to a better decongestion in decompensated HF patients with volume overload. It's expected that the better decongestion will lead to less HF readmissions, reduced all-cause mortality, improved quality of life, reduced hospital stay duration and significant reduction in HF related health care expenditure.

The ADVOR trial will investigate if adding acetazolamide, which can be very easily administered, in every hospital, without additional extra testing or invasive monitoring, through a bolus infusion in acute decompensated heart failure (ADHF) patients might lead to faster, safer and easier decongestion. If proven beneficial, this approach can easily be adopted by every hospital in a quick manner with considerable cost-savings with regards to health care expenditure and improvements of quality of life for patients.

Importantly, ADVOR will examine if an improved application of existing decongestive therapies (not novel drugs), based on strong scientific reasoning, will result in a better outcome for patients and society. Therefore, data from the study will provide information regarding the safety and efficacy of acetazolamide treatment in above mentioned patient population.

As such, the ADVOR study was specifically designed to have maximum benefit without additional risk for this frail patient population. The study will:

1) Be conducted with limited additional testing
2) Have minimal or no additional expected risk for the patient (comparison of standard diuretic regimen with standard diuretic regimen + addition of acetazolamide)
3) Have very clinically meaningful endpoints. Achieving a faster decongestion with reduced risk for escalation of therapy, which often increases complication rates as well as length of hospital stay.

This will be beneficial for the patients in the short-term. Additionally, a more thorough decongestion should also translate into less readmissions for recurrent decompensation and improved quality of care

Therefore, ADVOR can be considered a 'Low-intervention clinical trial' as:

1) Acetazolamide, the investigational medicinal products, which already has been authorised, has a very low risk profile and is well-known to the general cardiologist
2) According to the protocol of the clinical trial, Acetazolamide the investigational medicinal product, will be used in accordance with the terms of the marketing authorisation
3) The additional diagnostic or monitoring procedures do not pose more than minimal additional risk or burden to the safety of the subjects compared to normal clinical practice

3 OBJECTIVES AND ENDPOINTS / OUTCOME MEASURES

ADVOR will investigate if combination therapy with acetazolamide improves loop diuretic efficacy to increase diuresis in decompensated HF patients with volume overload, allowing for a better/faster decongestion and a lower total dose of loop diuretics. A better / faster decongestion should lead to less HF readmission, reduced all-cause mortality, shorter length of stay and improved quality of life.

3.1 Primary objective

The ADVOR study is a phase IV randomized, multi-center, double-blind study, comparing monotherapy with high-dose loop diuretics (standard of care - SOC) versus combination therapy with acetazolamide and high-dose loop diuretics in patients with decompensated HF and clinical sign(s) of volume overload. While the SOC results in 15% effective decongestion, it's estimated - based on strong scientific reasons - that the combination therapy should have a success rate of 25%, which represents a clear meaningful benefit of 10% more patients with appropriate decongestion after 72 h.

- Population; patients hospitalized with decompensated HF and demonstrating signs of volume overload.
- Intervention: combination therapy with high-dose loop diuretics + acetazolamide
- Comparison: monotherapy with high-dose loop diuretics + placebo
- Outcome: % decongestion, need for escalating diuretic therapy, HF readmission, all-cause mortality, length of stay, QoL
- Time: 72 h for primary endpoint, 3 months for secondary endpoint

3.2 Secondary objectives

1. Combination therapy with acetazolamide improves loop diuretic efficacy to increase diuresis in decompensated HF patients, allowing for a better/faster decongestion and a lower total dose of loop diuretics.
2. Combination therapy with acetazolamide leads to lower occurrence of diuretic resistance and escalating diuretic therapy in decompensated HF
3. Combination therapy with acetazolamide will potentially lead to improved clinical outcome in decompensated HF (less heart failure readmissions, lower all-cause mortality)
4. Combination therapy with acetazolamide will shorten the length of stay in patients with decompensated HF, which is expected to reduce health care expenditure
5. Combination therapy with acetazolamide will potentially lead to improved quality of life

3.3 Endpoints

The ADVOR study has an innovative primary end-point. As abundant evidence has consistently linked persistent volume overload after decongestive therapy in decompensated HF with poor outcomes, decongestion itself is a valid surrogate end-point. It has been demonstrated from reanalysis of the DOSE trial and CARRESS that the persistent oedema has excellent prognostic ability to predict death, readmissions or unscheduled medical contacts. In ADVOR, a more exhaustive yet easy to use – 'volume

assessment' score - will be used. Importantly, ADVOR will be a double blind randomized trial thereby excluding any potential for bias in the clinical judgement of the treating physician for any of the endpoints including the primary endpoint of decongestion.

Secondary endpoints in ADVOR will be very clinically relevant. A more thorough decongestion should also translate into less readmissions for recurrent decompensation, and eventually lower mortality. This will be assessed as a key secondary end-point after hospital stay. Additionally, time to decongestion is a major determinant of hospital stay and combinational therapy with acetazolamide might significantly shorten this. Finally, improved quality of life for patients is expected with better decongestion and will be the last secondary endpoint. Importantly, acetazolamide is an easy drug and very cheap drug to use (add on, bolus infusion, no special monitoring required), which will therefore be easily adopted by the health care. Endpoints have also been discussed with "Mon Coeur Entre Parenthèses" which is a HF patient association (VZW) representing HF patients and their peers and deemed to be important by them.

In addition, the primary and secondary outcome measures are in line with the COMET (Core Outcome Measures in Effectiveness Trials) initiative. Indeed 'decongestion' (being dry is for the patient a very important improvement in symptomatology), heart failure readmission, all-cause mortality, length of stay and quality of life can all be considered standardised relevant core outcomes sets. Therefore, the core outcomes relevant for any acute heart failure study will be collected and reported, making it easier for the results of ADVOR to be compared, contrasted and combined as appropriate with other trials (Zannad F et al, European Journal of Heart Failure, 2013;15:1082-1094). Additionally, we will continue to explore other tertiary outcomes, which are often mechanistically very interesting, as well.

We will also collect the dosages of neurohumoral blocker therapy throughout the study period, thereby hopefully facilitating a better implementation of guideline recommended therapy. As guidelines recommend a specific dosage for each of these drugs in HFrEF, it's easy to standardize the intake of such medications to be used in an accurate analysis. This will be done as an exploratory tertiary end-point.

3.4 Primary endpoint

Treatment success (decongestion achieved) on the morning of day 4 without the need for escalating diuretic strategy (doubling loop diuretic dose, addition of chlorthalidone, or ultrafiltration) on the morning of day 3.

3.5 Secondary endpoints

1. Combined end-point of all-cause mortality and heart failure readmission during 3 months of follow-up
2. Length of index hospital admission
3. Longitudinal changes in EuroQoL five dimensions questionnaire (EQ-5D) (as soon as possible after screening up to day 1, the morning of day 4 or at discharge (whatever comes first), at any readmission, 3 months)

3.6 Exploratory tertiary endpoints

1. Body weight change after day 1, 2, 3, 4 and discharge compared to screening
2. All-cause mortality during first 3 months after study start dose
3. Heart failure readmissions during first 3 months after study start dose
4. All cause rehospitalisation during first 3 months after study start dose
5. Total urinary volume and natriuresis starting from first intravenous (IV) diuretic administration at randomization until the morning of day 3
6. Relative plasma BNP or NT-proBNP change from screening until day 4 or at discharge (whatever comes first) and at 3 months follow up visit
7. Total dose of IV loop diuretics used during first 4 days
8. Changes in doses of neurohumoral blockers from baseline to discharge and after 3 months.
9. Need for renal replacement therapy or ultrafiltration during first 3 months after study start dose
10. Hyponatremia during treatment phase
11. Hypokalaemia during treatment phase
12. Incidence of metabolic acidosis requiring NaHCO3 supplements during first 4 days
13. WRF defined as a >0.3 mg/dL increase in serum Cr, or a >20% decrease in eGFR by the CKD-EPI formula during treatment phase
14. Liver dysfunction at screening
15. plasma volume changes during treatment phase (assessed by albumin and hematocrit)
16. Occurrence of iron deficiency at screening
17. Optional laboratory sub-study in participating centers: Change from screening in selected biomarkers from baseline through 3 months after study start dose in a subset of randomized patients

4 TRIAL DESIGN

The ADVOR study is a phase IV randomized, multi-center, double-blind study, comparing monotherapy of high-dose loop diuretics (SOC) versus combination therapy of acetazolamide with high-dose loop diuretics in patients with decompensated HF and volume overload. Data from the study will provide information regarding the safety and efficacy of acetazolamide treatment in the above-mentioned patient population. Randomized clinical trial with 2 treatment arms; therapy with high-dose loop diuretics and placebo vs therapy with high-dose loop diuretics and acetazolamide. An automated web-based system is used to randomly assign patients in a 1:1 ratio with variable blocks sizes, stratified for LVEF according to study center. To ensure an equal proportion of HFpEF versus HFrEF patients in both study arms, the population will be stratified at inclusion according to LVEF ≤40% versus >40%. Permuted block randomisation, at center and LVEF stratum level will be used to achieve this. Objective is to demonstrate superiority of the combination therapy with regards to achieving decongestion at 72 hours.

The study consists of 3 phases (cfr Figure 1 Flow Chart):

- screening phase: starting from identifying a study subject prior / during hospitalization until the first dose of study medication will be given
- treatment phase: starting from the first dose of study medication administration until the morning of day 4 or earlier in case of successful decongestion sooner
- follow up phase: starting when the treatment phase ends until 3 months after the study start dose

5 STUDY SETTING

The study population will consist of patients hospitalized for decompensated HF with clinical sign(s) of volume overload. The goal is to randomize approximately 519 patients in approximately 24 centers in Belgium. Importantly, participating centers are located in Flanders, Wallonia and Brussels, which also ensures that the study population is representative of the real-life patient population in which the study drug will be used in case the study is positive.

The study is supported by the members of the Belgian Working Group of Heart Failure (BWGHF). This is a national scientific non-profit group which was established in 2004 as one of the official working groups of the Belgian Society of Cardiology. Therefore, a positive result will almost immediately be adopted by the Belgian cardiology community as all members of the BWGHF are considered leaders in the field.

Finally, only centers who have fulfilled all the duties with regards to study selection and training will be allowed to randomize patients.

6 ELIGIBILITY CRITERIA

Decompensated HF patients with volume overload independent of ejection fraction might be included. This is important as 50% of decompensated HF patients are HFpEF patients (HF with preserved ejection fraction) and 50% of the decompensated HF patients are HFrEF patients (HF with reduced ejection fraction). In addition, patients do not need to have an echocardiogram at study inclusion to establish left ventricular ejection fraction which further simplifies the inclusion procedure. Finally, the DOSE trial (only other RCT studying the effects of diuretic therapy) also included patients without pre-specification of EF. To ensure an equal proportion of HFpEF versus HFrEF patients in both study arms, the population will be stratified at inclusion according to LVEF ≤40% versus >40% (assessed within 12 months before inclusion). Permuted block randomisation according to center and LVEF stratum will be used to achieve this.

Importantly, though the diagnosis of HF sometimes is difficult to establish in HFpEF, the main inclusion criteria are; 1) clinical diagnosis of decompensated HF and at least one clinical sign of volume overload (e.g. oedema, ascites or pleural effusion), 2) increased BNP / NT-ProBNP to ensure the volume overload is secondary to congestion/heart failure and 3) maintenance therapy with oral loop diuretics at a dose of ≥ 1 mg bumetanide or equivalent dose for ≥ 1 month before hospital admission will be remained. The combination of these three criteria will guarantee that only decompensated HF patients with volume overload will be included.

6.1 Inclusion criteria

- Signed written informed consent must be obtained before any study assessment is performed
- Male or female patients of 18 years of age or older
- An elective or emergency hospital admission with clinical diagnosis of decompensated HF with at least one clinical sign of volume overload (e.g. oedema (score 2 or more), ascites confirmed by echography or pleural effusion confirmed by chest X-ray or echography)
- Maintenance therapy with oral loop diuretics at a dose of at least 1 mg bumetanide or an equivalent dose for at least 1 month before hospital admission (Conversion: 1 mg bumetanide = 40 mg furosemide = 20 mg torsemide)
- Plasma NT-proBNP levels >1000 ng/L or BNP levels >250 ng/L at the time of screening.
- Assessed LVEF by any imaging technique; i.e. echocardiography, catheterization, nuclear scan or magnetic resonance imaging within 12 months of inclusion

6.2 Exclusion criteria

- Concurrent diagnosis of an acute coronary syndrome defined as typical chest pain in addition to a troponin rise above the 99th percentile and electrocardiographic changes suggestive of cardiac ischemia
- History of congenital heart disease requiring surgical correction
- History of a cardiac transplantation and/or ventricular assist device
- Systolic blood pressure <90 mmHg or mean arterial pressure <65 mmHg at screening

- Expected use of intravenous inotropes, vasopressors or nitroprusside during the study. The use of nitrates and/or molsidomine is allowed at the discretion of the treating physician.
- Estimated glomerular filtration rate <20 mL/min/1.73m² at screening
- Use of renal replacement therapy or ultrafiltration at any time before study inclusion
- Treatment with intravenous loop diuretics > 2 mg bumetanide or an equivalence of another loop diuretic during the index hospitalization and prior to randomization
- Treatment with acetazolamide within 1 month prior to randomization
- Exposure to nephrotoxic agents (i.e. contrast dye) anticipated within the next 3 days
- Use of any non-protocol defined diuretic agent with the exception of mineralocorticoid receptor antagonists during the treatment phase of the study.. Thiazides, metolazone, indapamide and amiloride should be stopped upon study inclusion. If patient is taking a combination drug including a thiazide-type diuretic, the thiazide-type diuretic should be stopped.
- Current use of sodium-glucose transporter-2 inhibitors
- Subjects who are pregnant or breastfeeding
- Subjects with urinary incontinence who are not willing to receive a bladder catheter.

7 TRIAL PROCEDURES

7.1 Recruitment

Patients might be enrolled during an admission of decompensated HF with volume overload, on the condition of fulfilling inclusion and not fulfilling exclusion criteria. In addition, patients might already be informed that they could participate in the study during an outpatient visit (if they need to be hospitalized for decompensated HF). Patients will be recruited by the sites within a period of approximately 24 months.

7.1.1 Patient identification

It is recommended to inform the medical team (cardiology and the health care providers) who might have first contact with potential study subjects (e.g. emergency room physicians) about the trial. They should inform the subject regarding the trial. Only a member of the patient's existing clinical care team should have access to patients' records without explicit consent in order to identify potential participants and check whether they meet the inclusion criteria or make the initial approach to patients. In case the treating physician is not a member of the ADVOR trial (a principal investigator (PI) or sub-investigator (SI)), he/she could refer the patient to the ADVOR investigator. The screening process can start only if a written informed consent is obtained. The investigator of the ADVOR trial should confirm eligibility of the subject.

Potential participants might also be recruited through publicity (posters, leaflets) which can be made publically available in cardiology outpatient departments only if approval has been obtained by the ethics committee.

7.1.2 Screening

Following screening technical requirements are necessary to meet any noted inclusion or exclusion criteria:

- laboratory tests (BNP or NT-proBNP part of inclusion criteria and eGFR part of exclusion criteria)
- chest X ray or chest ultrasound in case pleural effusion is used as inclusion criteria. Importantly, a chest X-ray is considered standard of care in case of decompensated HF (guidelines of the European Society of Cardiology (ESC))
- abdominal ultrasound in case ascites is used as inclusion criteria

No other technical procedure need to be performed as part of routine care. However, the LVEF assessed by any technical exam (echocardiogram, nuclear scan, MRI, catheterization) within the last 12 months will need to be recorded. As only patients already treated with a loop diuretic are allowed in the study, we expect that every patient has a LVEF recorded within 12 months preceding study entry.

We don't expect screen failures i.e. patients who do not meet eligibility criteria at time of screening are not able to enter the study at a later stage as we expect them to have received already more than 2 mg loop diuretics intravenously (which will count as an exclusion criterium).

Patients will not receive any special incentives or compensation through participation in the study. No specific study visits are needed. The 3 month FU visit is standard of care as recommended by the guidelines of the ESC. Study related exams (BNP or NT-proBNP) will be reimbursed by the study.

7.2 Consent

The Principal Investigator (PI) retains overall responsibility for the informed consent of participants at their site and must ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained and competent to participate according to the ethically approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki. If delegation of consent is acceptable then details should be provided.

Informed consent must be obtained prior to the participant undergoing procedures that are specifically for the purposes of the trial and are outside of standard routine care at the participating site (including the collection of identifiable participant). In case the patient agrees to participate in the optional laboratory sub-study, an additional signed and dated informed consent must be obtained before the laboratory sub-study blood and urine samples will be collected.

The right of a participant to refuse participation without giving reasons must be respected.

The participant must remain free to withdraw at any time from the trial without giving reasons and without prejudicing his/her further treatment and will be provided with a contact point where he/she may obtain further information about the trial. Where a participant is required to re-consent or new information is required to be provided to a participant it is the responsibility of the PI to ensure this is done in a timely manner.

The PI takes responsibility for ensuring that all vulnerable patients are protected and participate voluntarily in an environment free from coercion or undue influence.

Eligible patients may only be included in the study after providing written (witnessed, where required by law or regulation), Independent Ethics Committee (IEC) approved informed consent or if patient is incapable of doing so, after such consent has been provided by a legally acceptable representative(s) of the patient. In cases the patient's representative gives consent, the patient should be informed about the study to the extent possible given his/her understanding. If the patient is capable of doing so, he/she should indicate assent by personally signing and dating the written informed consent document.

The process of obtaining informed consent should be documented in the patient source documents.

Once the informed consent has been signed by the patient and patient is randomised, a new patient file should be created in the electronic Case Report Form (eCRF) by the PI or the qualified person to whom this task has been delegated. Once the patient file is created, the eCRF system will automatically generate a trial number for this patient. Once a number is assigned to a patient, the patient number can't be re-used.

7.3 The randomisation scheme

Randomized clinical trial with 2 treatment arms; therapy with high-dose loop diuretics and placebo vs therapy with high-dose loop diuretics and acetazolamide. An automated web-based system is used to randomly assign patients in a 1:1 ratio with variable blocks sizes, stratified for LVEF according to study center. To ensure an equal proportion of HFpEF versus HFrEF patients in both study arms, the population will be stratified at inclusion according to LVEF ≤40% versus >40%. Permuted block randomization according to center and LVEF stratum will be used to achieve this.

Only the PI or qualified person to whom he/she has delegated this study task can randomize the patient in the automated web-based system.

7.4 Blinding

This is a double-blind study. Therefore, after randomization, the study team and patient will not be aware of which treatment (acetazolamide or placebo) is administrated to the trial participant.

Once a patient is assigned to a study group (treatment group or control group), he/she will remain in that arm and all efforts will be made to provide the optimal therapy specified for that treatment assignment. In the unforeseen circumstance that this is clinically not feasible, the patient will remain in the assigned treatment arm for statistical analysis based on the intention-to-treat principle, as it represents a normal medical situation of success and failure of delivering the planned medical therapy.

7.5 Unblinding

Patient, site personnel, sponsor personnel and data analysts will remain blinded to the identity of the treatment from the time of randomization until database lock. Though we do not foresee serious adverse events related to the study drug, the study code should only be broken for valid medical or safety reasons e.g. in the case of a severe adverse event where it is necessary for the investigator or treating health care professional to know which treatment the patient is receiving before the participant can be treated. It is not mandatory but strongly encouraged to contact the chief investigator before unblinding any patients' treatment assignment. Patient and members of the research team should remain blinded.

Following rules apply for unblinding;

- Rapid unblinding of a patient can be performed by a physician of the study team. Detailed information concerning the unblinding procedures is provided in the Manual of Operations.
- On receipt of the treatment allocation details, the PI or treating health care professional will continue to deal with the participant's medical emergency as appropriate.
- The PI/Investigation team documents the breaking of the code and the reasons for doing so on the medical notes and eCRF. It will also be documented at the end of the trial in any final study report and/or statistical report.

Unblinded data are to be kept strictly confidential until the time of unblinding of the trial and will not be accessible by anyone else involved in the trial with the following exceptions: (1) the PM of the company responsible for the labelling and packaging of the IMP, (2) the IRT system programmers who work on the randomization and drug management system; and (3) the data manager who prepares reports required for regulatory reporting (suspected unexpected serious adverse reactions [SUSAR] reporting). These individuals will not be involved in the day-to-day running of the study.

7.6 Screening phase assessments

Screening testing will occur after the consent process has been finalized. The screening data gathering needs to be performed prior to the administration of the study diuretic agents. The following assessments are required and data need to be collected:

- Obtain written informed consent
- Check inclusion and exclusion criteria
- Collect demographics (collect age, gender, race and ethnicity)
- Collect medical history and notification of LVEF
- Collect concomitant medication (see Appendix 5)
- Collect vital signs including body weight, arterial blood pressure and heart rate
- EQ-5D patient questionnaire to be completed by the patient (Appendix 2 and 3)
- Perform volume assessment by the Principal Investigator or a Sub-investigator who has been trained in the study volume assessment. Volume assessment is based on the presence of oedema, ascites, pleural effusion (Figure 3 in section 8).
- Collect blood sample for local laboratory assessment: serum hemoglobin, hematocrit, electrolytes (Na, K, Cl, HCO_3), serum osmolality, serum urea, serum Cr, total protein, serum albumin, Fe, ferritin, TSAT, LDH, troponin, eGFR and plasma BNP or NT-proBNP
    - In a subset of randomized patients participating in the laboratory sub-study blood collected during screening will be shipped to the University Biobank Limburg (see section 7.10)
- Perform pregnancy urine test (applicable for all pre- menopausal women who are not surgically sterile)
- Randomization (see section 7.3)

7.7 Treatment phase assessments

7.7.1 Day 1

- Request patient to empty their bladder before administration of the first dose of loop diuretics.
- Administer the first bolus of loop diuretics and acetazolamide or placebo to the patient.
- Start the urinary collection immediately after first bolus of loop diuretics and acetazolamide or placebo is administered. The collection will stop the latest as close to but prior to the morning bolus of study medication on day 3. Urinary catheter insertion is strongly recommended but not mandatory to achieve an optimal urine collection. However, in case of urinary incontinence, placement of a urinary catheter is mandatory. Care should be taken to ensure ALL urine is collected. For more details regarding the study urinary collection see appendix 4.

If the patient is enrolled between midnight and 6 a.m., day 1 assessments and day 2 assessment can be performed on the same day but only if there is a difference of minimum 6 hours between the start of the study medication on day 1 and study medication on day 2.

7.7.2 Day 2, Day 3 and Day 4

- Collect vital signs including body weight, arterial blood pressure, heart rate
- As long as patient is assessed as volume overloaded during the previous volume assessment, perform volume assessment by the Principal Investigator or a Sub-investigator who has been trained in the study volume assessment (Figure 3 in section 8)
- Collect morning blood sample for local laboratory assessment: serum haemoglobin, haematocrit, electrolytes (Na, K, Cl, HCO_3), serum urea, serum Cr, serum albumin.
- On the morning of Day 2 and Day 3, collect an urine sample from urine collection period 1 and period 2 respectively for analysis in local lab. In case patient is participating in the optional laboratory sub-study, collect an additional urine sample for each urine collection period for shipment to the University Biobank Limburg for storage and additional research (see section 7.10). Urinary collection will stop at the morning of day 3 (appendix 4).
- In case patient is still volume overloaded continue study treatment as described in section 8
- Collect concomitant medication (see Appendix 5)
- Document and assess adverse events (section 9)
- In addition, on the morning of day 4 or at discharge (whatever comes first):
    - EQ-5D to be completed by the patient.
    - Collect blood sample for local laboratory assessment of the BNP or NT-ProBNP level. In a subset of randomized patients participating in the laboratory sub-study blood collected on day 4 will be shipped to the University Biobank Limburg (see section 7.10)

7.8 Follow-up phase assessments

- At Discharge:
  - Perform volume assessment by the Principal Investigator or a Sub-investigator who has been trained in the study volume assessment (Figure 3 in section 8)
  - Collect weight
  - Collect concomitant medication (see Appendix 5)
  - Document length of hospitalization
  - Document and assess adverse events (section 9)

- At Readmission:
  - HF readmissions are defined as either a hospital admission because of decompensated HF or an unscheduled contact at the emergency department if the patient is treated with intravenous loop diuretics.
  - During any readmission, EQ-5D questionnaire needs to be completed by the patient as soon as possible during the admission

- Long term follow-up

  Patients will be followed for a maximum of three months for secondary/tertiary endpoint analysis. This follow-up should not differ from standard of care for such patients. During one outpatient follow-up appointment 3 months (90 days) (+ 14 days) after the start of study medication, standard of care data will be collected. The only study related tests will be a BNP / NT-ProBNP test and the collection of the EQ-5D patient questionnaire.

  During this follow –up visit the following data will need to be collected:
  - Collect vital signs including body weight, arterial blood pressure, heart rate
  - EQ-5D patient questionnaire to be completed by the patient (Appendix 2 and 3)
  - Perform volume assessment by Principal Investigator or Sub-investigator who has been trained in the study volume assessment (Figure 3 in section 8)
  - Collect blood sample for local laboratory assessment: serum hemoglobin, hematocrit, electrolytes (Na, K, Cl, HCO_3), serum urea, serum Cr, serum albumin, and plasma BNP or NT-proBNP. In a subset of randomized patients participating in the laboratory sub-study blood collected on this visit will be shipped to the University Biobank Limburg (see section 7.10)
  - Collect concomitant medication (see Appendix 5)
  - Document and assess adverse events (section 9)

  We do not foresee that a significant amount of patients will be lost to follow-up as the follow-up period is short (max 3 months). The investigator should make every effort to contact participants who are lost to follow-up. Attempts to contact such participants must be documented in the participant's records.

7.9 Table of trial procedures

	Screening phase	Treatment phase				Follow up phase		
		Study Day 1	Morning of Study Day 2	Morning of Study Day 3	Morning of Study Day 4[15]	Discharge	Re-admission	3 Months after study start dose
Informed consent	X							
In- and exclusion criteria	X							
Randomization	X							
Demographics[1]	X							
Medical history	X							
Vitals[2]	X		X	X	X			X
Weight[12]	X		X	X	X	X		X
EQ5D	X				X		X[11]	X
Volume assessment	X		X[16]	X[16]	X[16]	X		X
Study treatment		X[3]	X[4]	X[4]				
Urinary collection[5]		X	X	X				
Local lab	X[6]		X[7]	X[7]	X[7]			X[7]
Laboratory sub-study[13] blood	X				X[14]			X
Laboratory sub-study[13] Urine			X	X				
Plasma BNP or NT-proBNP[8]	X				X			X
Urine pregnancy testing[9]	X							
Concomitant medication[17]	X	X	X	X	X	X		X
Adverse Events[10]	X	X	X	X	X	X	X	X

1) Age, race and ethnicity
2) Arterial blood pressure and heart rate
3) Start dose (IV) = 2 x orally daily maintenance dose of loop diuretics and 500 mg acetazolamide or placebo (see section 8)
4) As long as patient is volume overloaded, Treatment dose (IV) = 1x orally daily maintenance dose of loop diuretics and 500 mg acetazolamide or placebo between 8:00 and 12:00 and a second dose minimum 6 hours later with again 1x orally daily maintenance dose (see section 8)
5) See appendix 4
6) Serum hemoglobin, hematocrit, electrolytes (Na, K, Cl, HCO_3), serum osmolality, serum urea, serum Cr, total protein, serum albumin, Fe, ferritin, TSAT, LDH, troponin and eGFR
7) Serum hemoglobin, hematocrit, electrolytes (Na, K, Cl, HCO_3), serum urea, serum Cr and serum albumin

8) Protocol requires that plasma levels of BNP or NT-proBNP are collected. In case the patient is on succubutril/valsartan, it is mandatory that NT-proBNP plasma levels are determined on the blood sample.
9) Only pre- menopausal women who are not surgically sterile, as well as women of childbearing potential.
10) Safety reporting flow is documented in section 9
11) EQ5-D needs to be collected once during any readmission (as soon as poss ble during readmission))
12) Measurement of body weight should be performed as consistently as poss ble using a standardized scale, preferably with a precision of 50 g, in the morning, post-void, prior to eating, prior to the medication dose, and with patients wearing the same clothing. The scales should stand on a flat, solid surface rather than carpets unless specifically designed for use in that setting
13) Blood and urine will be collected in a subset of randomized patients participating in the laboratory sub-study. Blood will be collected on screening, day 4 or at discharge (whatever comes first) and 3 month FU visit. Urine sample will be collected from Urinary Collection period 1 and Urinary collection period 2 (see also appendix 4).
14) In case day 4 falls into a weekend or public holiday, blood collection for sub-study can also be done on day 5 or day 6. If a patient is being discharged before the morning of day 4, blood collection for the sub-study should occur at discharge.
15) In case discharge is earlier than the morning of day 4, all assessments of day 4 should be performed at discharge.
16) As long as patient was assessed volume overloaded during the previous volume assessment a volume assessment needs to be performed
17) See appendix 5

7.10 Laboratory sub-study

Blood samples and urine samples from centers who agreed to participate in the Laboratory sub-study will be collected during the Advor trial. All potential patients at these centers will be asked to participate in the laboratory sub-study. An additional signed and dated informed consent must be obtained before the additional blood and urine samples will be collected.

The right of a participant to refuse participation without giving reasons must be respected. The participant must remain free to withdraw at any time from this sub-study without giving reason and without prejudicing his/her further treatment and will be provided with a contact point where he/she may obtain further information about the sub-study. Where a participant is required to re-consent or new information is required to be provided to a participant it is the responsibility of the PI to ensure this is done in a timely manner.

All patients enrolled in the laboratory sub-study will have blood collected on screening, day 4 and during the 3 month follow up visit. In case the patient is being discharged before the morning of day 4, blood samples should be collected at discharge. The collected blood samples will be shipped to the University Biobank Limburg for storage and additional research. Urine samples collected from the Urinary Collection period 1 and from the Urinary Collection period 2 (see appendix 4) will be shipped to the University Biobank Limburg for storage and additional research.

Biomarkers related to cardiac and renal function/injury will be obtained from blood and urine in the subset of patients participating in the laboratory sub-study. Biomarkers will be used to elucidate the effect of acetazolamide and to explore drug effect versus baseline biomarkers of risk. Biomarkers of potential interest are NT-proBNP, Galectin 3, ST2, Cystatin C and NGAL. The list of potential biomarkers may be changed or expand further as it is recognized that more relevant or novel biomarkers may be discovered. Biomarkers may be measured during the process of this study or after its completion. No genetic analysis will be performed with the collected sub-study samples. Detailed sample handling instructions will be provided in a separate laboratory manual.

7.11 Withdrawal criteria

All subjects will be encouraged to remain on treatment and under observation for the full duration of the study. However, at any time during the study and without giving reasons, subjects may withdraw from the study at their own request or at the request of their legally acceptable representative. The subject will not suffer any disadvantage as a result.

It is important to note that discontinuation of study treatment (see section 8) is not the equivalent to withdrawal of informed consent. In cases where subjects indicate they do not want to "continue", investigators must determine whether this refers to discontinuation of study treatment (the most common expected scenario), unwillingness to attend the follow-up visit, unwillingness to have telephone contact, unwillingness to have any contact with study personnel, or unwillingness to allow contact with a third party (e.g., family member, doctor). Every effort must be made to continue to follow the subject until the end of the study.

In all cases, the reason for discontinuation (including "at the subject's request") must be recorded in the case report form (CRF) and in the subject's medical records.

No subject replacements are permitted in the study.

7.12 End of trial

The Sponsor's CTU ZOL will notify the FAMHP and main EC of the end of a clinical trial within 90 days of its completion date (last patient last visit).

8 TRIAL INTERVENTION / MEDICATION

The ADVOR study is a phase IV randomized, multi-center, double-blind study, comparing monotherapy with high-dose loop diuretics and placebo (standard of care - SOC) versus combination therapy with acetazolamide and high-dose loop diuretics in patients with decompensated heart failure and clinical signs of volume overload.

8.1 Name and description of intervention(s)

At randomization

At the moment of randomization, oral loop diuretics are stopped and the patient receives an IV bolus of loop diuretics at a dose equal to the double of his oral daily maintenance dose* with a maximal dose of 5 mg bumetanide (=200 mg furosemide). Bumetanide is the preferred loop diuretic agent to be used in this trial.

Conversion factor:

1 mg bumetanide po = 1 mg bumetanide IV

40 mg furosemide po = 40 mg furosemide IV

20 mg torsemide po = 40 mg furosemide IV = 1 mg bumetanide IV

Some examples are listed below:

- *if a patient takes 1x1 mg bumetanide po, the patient will receive 2x1x1 mg = 2 mg bumetanide IV*
- *if a patient takes 2x2,5 mg bumetanide po, the patient will receive 2x2x2,5 mg = 5 mg bumetanide IV*
- *if a patient takes 1x40 mg furosemide po, the patient will receive 2x1x40 mg = 80 mg furosemide IV*
- *If a patient takes 1x80 mg furosemide po, the patient will receive 2x1x80 mg = 160 mg furosemide IV*
- *If a patient takes 1x20 mg torsemide po, the patient will receive 2x1x1 mg bumetanide IV = 2 mg bumetanide IV or 2x1x40 furosemide IV = 80 mg furosemide IV*

Together with this initial dose of loop diuretics patients will receive an intravenous bolus of 500 mg of acetazolamide or placebo.

START DOSE (IV) = 2 x orally daily maintenance dose* of loop diuretics (max. 5 mg of bumetanide)
+
500 mg acetazolamide or placebo

**If the oral daily maintenance dose has changed over the week prior to randomization, it will be defined as the highest orally administered daily dose that the patient has received in an outpatient context 3 days prior to randomization.*

During the treatment phase

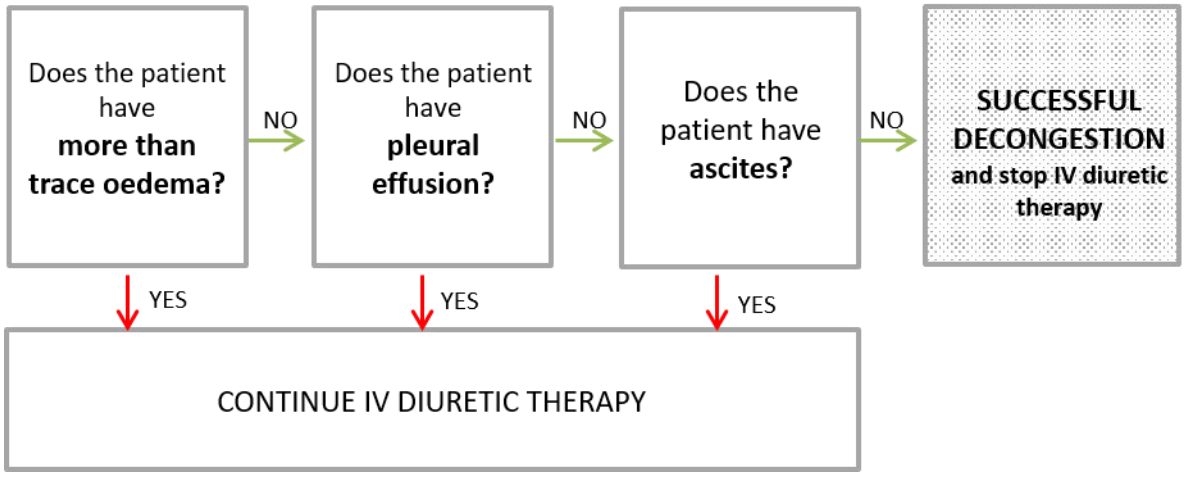
Between administering the start dose and next treatment dose a minimum of 6 hours is required. During the remaining part of the treatment phase, the patient will continue to receive 2 treatment doses every day provided that the treating physician has concluded during the morning rounds that the patient is still volume overloaded (see Figures 2+3). The dose will be the orally daily maintenance dose, administered between 8:00 and 12:00 am, together with an intravenous bolus of 500 mg of acetazolamide or placebo. The second dose of loop diuretics, again the orally daily maintenance dose, will be given 6 hours after the morning dose.

Any patient with more than trace oedema, residual pleural effusion or residual ascites would be considered to be still volume overloaded (see Figures 2 + 3). Residual pleural effusion and/or ascites should always be confirmed by chest X-ray or echography. If pleural effusion/ascites is used as an inclusion criteria, chest X-ray and/or echography should be repeated until decongestion has been achieved (volume assessment score ≤ 1). If not present at inclusion, new evidence of pleural effusion and/or ascites may arise during the treatment phase, but if scored, it should be confirmed by a chest X-ray or echography. If the patient is not volume overloaded anymore, the intravenous administration of study medication should be stopped. Once decongestion is achieved (volume assessment score ≤ 1) during the treatment phase, no volume assessment should be performed the following morning.

Between 8:00 and 12:00 am TREATMENT DOSE (IV) = 1x orally daily maintenance dose (max. 5 mg of bumetanide)
+
500 mg acetazolamide or placebo

6 hours later TREATMENT DOSE (IV) = 1x orally daily maintenance dose

Figure 2. Flow chart to guide study.



After treatment phase

After the treatment phase, treating physicians are recommended not to prescribe oral acetazolamide as a maintenance diuretic therapy after decongestion; instead, they are encouraged to restart the original oral maintenance dose of loop diuretics when the patient was still stable. Patients can be discharged as early as 24 hours after the physician concluded that the volume overload is no longer present. An outpatient follow-up appointment is scheduled at least 3 months (90 days) (with a window of + 14 days) after the start of study medication. It is mandatory to perform a volume assessment at discharge and at follow-up. It is recommended to perform a chest X-ray or echography in case of clinical signs of pleural effusion or ascites, yet, it will be left to the discretion of the treating physician.

STOP TREATMENT

The treating physician is allowed (but not obliged) to stop the study treatment, which counts as treatment failure **in case of persistent volume-overload** in following cases:

- symptomatic hypotension with a systolic blood pressure <100 mmHg
- asymptomatic hypotension with a systolic blood pressure <90 mmHg
- an increase of serum Cr levels x 1.5 of the serum Cr level compared to admission value.
- Occurrence of metabolic acidosis (ph < 7.2)

If any of these events occur when the patient is judged to be euvolemic, the study treatment is stopped and stopping is not considered a treatment failure.

Freedom from volume-overload (i.e. congestion) on the morning of day 4 will be defined as not more than trace oedema, no residual pleural effusion, and no residual ascites (Figure 3)

Figure 3: Volume assessment

OEDEMA	No oedema (score 0)	Trace oedema (pitting disappear immediately) (score1)	Clear pitting oedema (score 2)	Visual deformation above ankle (score 3)	Visual deformation above knee (score 4)
PLEURAL EFFUSION (to be confirmed by chest X-ray or ultrasound on admission if suspected)	No pleural effusion (score 0)	Minor (non-amendable for punction) pleural effusion (score 2)		Major (amendable for punction) pleural effusion (score 3)	
ASCITES (to be confirmed by ultrasound on admission if suspected)	NO ascites (score 0)	Minor ascites, only detected by echography (score 2)		Significant ascites (score 3)	

Succesfull decongestion

Continue IV diurectic therapy

Treatment DOSE ADJUSTMENTS in case of an inappropriate diuretic response

If the total urinary output (see Figure 1 and Appendix 4) on the morning of day 3 is < 3500 mL and the patient is still volume overloaded, an escalation of decongestive treatment is mandatory. One of the outlined three options can be chosen at the discretion of the treating physician.

Escalation therapy options:

- doubling of the IV dose of the loop diuretics
- add oral chlorthalidone 50 mg once daily
- ultrafiltration or renal replacement therapy might be considered

The decision to proceed with escalation therapy needs to be collected in the case report form as the patient needing escalation cannot reach the primary endpoint.

Background therapy

24h oral intake of fluid and sodium will be restricted to 1500 mL and 1.5 g, respectively. It is recommended that all patients receive the same maintenance infusion with 500 mL glucose 5% and 3g $MgSO_4$ administered over 24h time interval, until complete decongestion or end of the study treatment phase. All non-protocol fluids administered (including those for administration of intravenous medication) should be limited.

In case of **serum potassium levels <4 mmol/L,** 40 mmol of KCl is added to the maintenance infusion. Oral potassium supplements may be used at the discretion of the treating physician, but their use will be prospectively registered.

In case of **metabolic acidosis** with serum bicarbonate levels <20 mmol/L, it is recommended to administer intravenously 100 ml of $NaHCO_3$ 8.4%.

Treatment with **neurohumoral blockers** (e.g. renin-angiotensin system blockers, sacubitril/valsartan, beta-blockers, and mineralocorticoid receptor antagonists) may be continued at the same or lower dosage at the discretion of the treating physician, until the end of the treatment phase (max 4 days) or until complete decongestion is achieved, whatever comes first. Dose increases for any of these medications are not allowed during the screening and treatment phase with the exception of mineralocorticoid receptor antagonists in case of hypokalaemia despite intravenous potassium supplement. In addition, starting an SGLT2 inhibitor and a switch from renin-angiotensin system blockers to sacubitril/valsartan is not allowed during the screening and treatment phase, but might be pursued after decongestion is achieved. After decongestion, it is strongly recommended to up-titrate doses of neurohumoral blockers according to the guidelines in the HFrEF patients. Dosages of neurohumoral blockers are collected at screening, at discharge and at three months follow-up.

8.2 Legal status of the intervention

The IMP (acetazolamide) is licensed for use in Belgium for the treatment of HF.

8.3 Summary of Product Characteristics (SmPC)

Information about common side effects already known about the investigational drug can be found in the SmPC filed in the Investigator Site File (ISF).

SmPC in Dutch:
http://bijsluiters.fagg-afmps.be/registrationSearchServlet?key=BE004137&leafletType=skp

SmPC in French:
http://bijsluiters.fagg-afmps.be/registrationSearchServlet?key=BE004137&leafletType=rcp

8.4 Drug storage and supply

The investigational medical product (IMP) (acetazolamide and placebo) will be shipped free of charge to the participating centers. The IMP must be received by a designated person in the pharmacy at the study center, handled and stored safely and properly, and kept in a secured location to which only the investigator and designees have access. Upon receipt, the PI or qualified delegated person will confirm the date of receipt of IMP. Receipt, distribution, return and destruction (if applicable) of the study drug must be properly documented according to the agreed and specified procedures. Specific instructions for the study drug recordkeeping are provided in the Manual of Operations.

All IMP should be stored according to the instructions specified on the labels (room temperature below 25°C).

8.5 Preparation and labelling of Investigational Medicinal Product

The IMPs (acetazolamide and placebo) will be presented as a white to off-white 500 mg powder for solution for injection in a sterile vial. Each study center will be supplied with study medication kits containing 3 vials of acetazolamide or placebo. Each kit will have an unique study kit number. The study medication supply will have appropriate labelled packaging according to national law of Good Manufacture Practices (GMP) ruling.

Each IMP need to be reconstituted prior to use. Specific instructions will be provided in the Manual of Operations. The reconstituted solution is clear and colourless and does not contain an antimicrobial preservative. Any unused solution can be stored in a refrigerator for up to 24 hours but any solution not used within this period must be discarded.

The direct intravenous route of administration is preferred. Intramuscular injection may be employed but is painful due to the alkaline pH of the solution. (preparation according to the SmPC see section 8.3)

8.6 Dosage schedules

Cfr point 8.1 for specific dosage schedules and routes of administration.

8.7 Dosage modifications

Dosage modifications of the study drug are not possible as there will only be one dose (and the administration of the IMP vs placebo is blinded).

8.8 Assessment of compliance

As the study drug will be administered intravenously during the treatment phase by the nurse taking care of the patient, no compliance issues with regards to the study medication are foreseen.

9 SAFETY REPORTING

As this is a pragmatic trial, the intervention will be used within the label and therefore safety reporting can be limited to the safety reporting which is necessary in routine care.

Timely, accurate, and complete reporting of clinical events is of crucial importance for success of the study. Additionally, reporting and review of safety information for clinical studies are crucial for the protection of subjects.

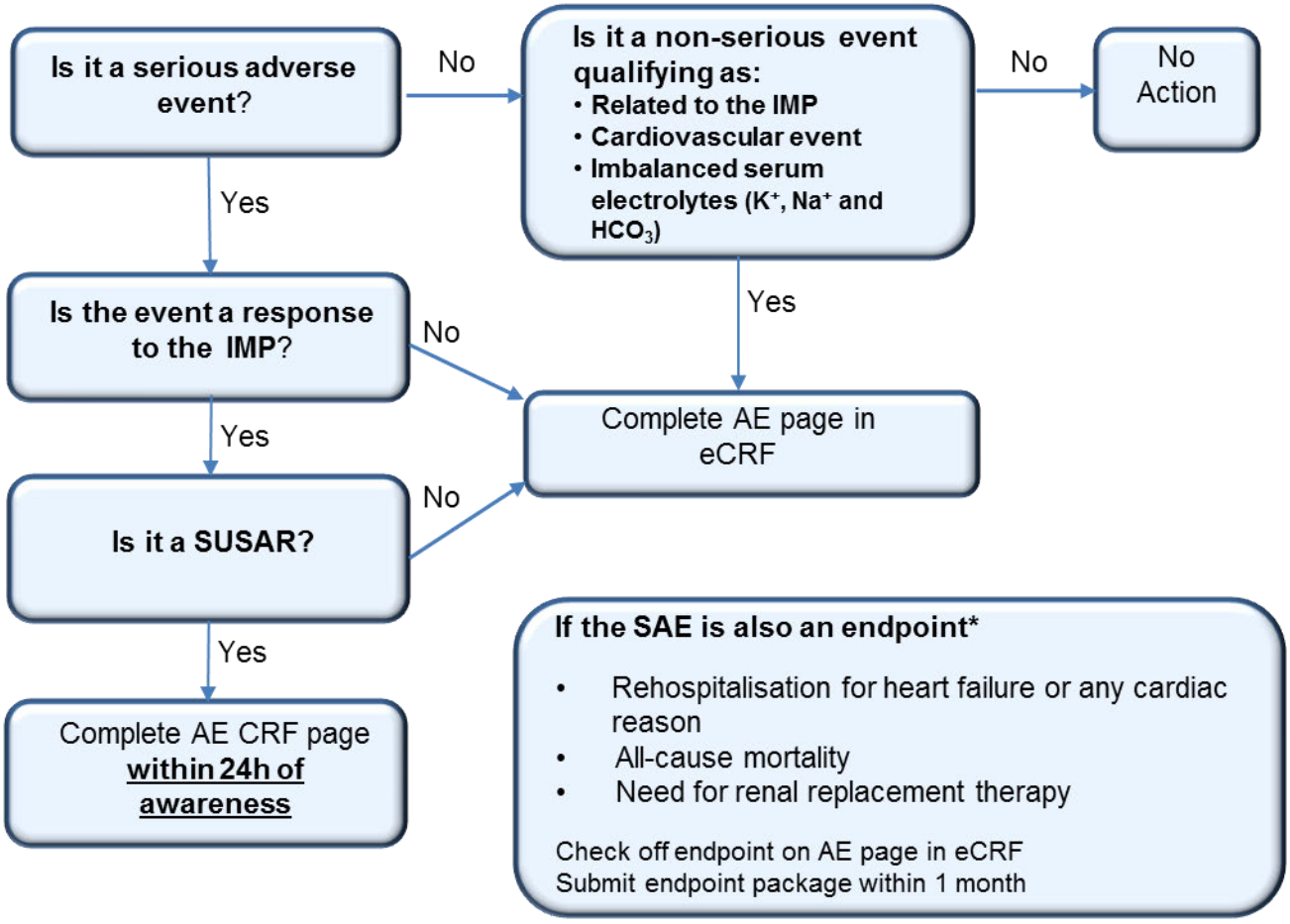
9.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product • in the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question

9.2 Recording and reporting Safety Information

During the course of this study, i.e. from signing the informed consent onwards until the end of the last follow-up visit, all SAEs, Endpoint Events and certain non-serious AEs which occurred until 3 months after study start dose are to be collected, documented, and reported by the Investigator in the applicable eCRF's following below "safety reporting flow chart":

Figure 4: Safety reporting flow chart



How to assess Safety Events (AEs, SAEs and SUSARs)

Seriousness, severity and causality need to be assessed by the Principal Investigator or the physician to whom this activity is delegated to.

An adverse event is defined as a serious adverse event if the event is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect, or
- Is an important medical event.

Note: The term "life-threatening" in the definition of serious refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe

The following definitions should be used ***to assess intensity of adverse events***:

- Mild: Awareness of sign or symptom, but easily tolerated, i.e. does not interfere with subject's usual function.
- Moderate: Discomfort enough to cause interference with usual activity.
- Severe: Incapacitating with inability to work or do usual activity, i.e. interferes significantly with subject's usual function.

Severity vs. Seriousness: Severity is used to describe the intensity of a specific event whereas the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "seriousness," which is based on subject/event outcome at the time of the event.

The Investigator should ***assess causal relationship between an adverse event and the study drug on the basis of his/her clinical judgment, the latest SmPC (see section 8.3) and the following definitions.*** The causality assessment must be made based on the available information and can be updated as new information becomes available.

Related:

The AE follows a reasonable temporal sequence from study drug administration, and cannot be reasonably explained by the subject's clinical state or other factors (e.g. disease under study, concurrent diseases, and concomitant medications).

or

The AE follows a reasonable temporal sequence from study drug administration, and is a known reaction to the drug under study or its chemical group, or is predicted by known pharmacology.

Not Related:

The AE does not follow a reasonable sequence from study drug administration, or can be reasonably explained by the subject's clinical state or other factors (e.g. disease under study, concurrent diseases, and concomitant medications).

Recording and reporting non-serious Adverse Events (AEs)

AEs may be directly observed, reported spontaneously by the subject or by questioning the subject at each study visit. The Investigator must assess these AEs to determine seriousness, severity, and causality, in accordance with below definitions. The Investigator's assessment must be clearly documented in the site's source documentation. For the purposes of this study **non-serious AEs that are related to the IMP or non-serious AEs that that occur in the cardiovascular system or non-serious AEs events involving imbalance of serum electrolytes (restricted to K^+, Na^+, HCO_3) will be collected in the eCRF throughout the study**.

Recording and reporting of SAEs AND SUSARs

All SAEs an SUSARs will be collected throughout the study duration. Reporting of these events to the sponsor will occur on the eCRF AE page.

Reporting of SAEs and SUSARs is mandatory and should start;

- For SAEs, from consent
- For SUSARs, from 1st IMP dose

If the SAE is unexpected, i.e., the event is not previously documented as 'expected', and is thought to be related to the study drug, this event is considered a Suspected Unexpected Serious Adverse Reaction (SUSAR).

Where a participant withdraws consent for further processing of data, this does not preclude the reporting of SAEs and SUSARs which are required to continue being reported according to the protocol for regulatory purposes.

In all cases SAEs should be reported to the Sponsor in the eCRF. **Only in case the investigator is of the opinion that the SAE is a SUSAR, the investigator needs to inform the sponsor within 24 hours after awareness of the event.** Assessment of seriousness, causality and expectedness for trials involving IMPs will be made by the PI or another authorised doctor. If an authorised doctor from the reporting site is unavailable, initial reports without causality and expectedness assessment should be submitted to the Sponsor, but must be followed-up by medical assessment as soon as possible thereafter.

For each SUSAR the following information will need to be collected:

- full details in medical terms and case description
- event duration (start and end dates, if applicable)
- action taken
- outcome
- seriousness criteria
- causality (i.e. relatedness to trial drug / investigation), in the opinion of the investigator
- whether the event would be considered expected or unexpected.

All SAEs assigned by the PI or delegate (or following central review) as both suspected to be related to IMP-treatment and unexpected will be classified as SUSARs and will be subject to expedited reporting to the Federal agency for medicines and health products (FAMHP). The Sponsor will inform the FAMHP, the EC and the Marketing Authorisation Holder of SUSARs within the required expedited reporting timescales.

Endpoints

The following events will need to be marked as an "endpoint event" in the eCRF and will be reviewed by the Endpoint Adjudication Committee (EAC):

- All-cause mortality: All deaths from any cause. This includes all cardiovascular and non-cardiovascular deaths.

  Cardiovascular death: Death fulfilling any of the following criteria:
  - Death due to proximate cardiac cause (e.g. myocardial infarction, cardiac tamponade, worsening heart failure)
  - Death caused by non-coronary vascular conditions such as neurological events, pulmonary embolism, ruptured aortic aneurysm, dissecting aneurysm, or other vascular disease
  - All procedure-related deaths, including those related to a complication of the procedure or treatment for a complication of the procedure (e.g. surgical or non-surgical revascularisation)
  - All valve-related deaths including structural or non-structural valve dysfunction or other valve related adverse events
  - Sudden death or unwitnessed death
  - Death of unknown cause

  Non-cardiovascular death: any death that is not thought to be due to a cardiovascular cause

- Rehospitalisation for heart failure is defined as either a hospital admission or an unscheduled contact at the emergency department for worsening HF if the patient is treated with intravenous loop diuretics.
- Need for Renal replacement therapy: ultrafiltration or dialysis

The site will need to submit a complete endpoint package within 30 days upon request of the sponsor to advor@zol.be. This package should include anonymized source documents (SDs) relevant to the endpoint reported (e.g. discharge letter, emergency room notes, etc). The SD should include at least the reason for admission/death and the received treatment for the event (if applicable). The original SDs need to be retained at the site.

Identifying a pregnancy

All pre- menopausal women who are not surgically sterile will have pregnancy urine tests performed at screening phase (Day 1). A positive pregnancy test at day 1 constitutes in a screening failure.

9.3 Responsibilities

Principal Investigator (PI):

Checking for AEs when participants attend for treatment / follow-up.

1. Using medical judgement in assigning seriousness, causality and expectedness using the Reference Safety Information approved for the trial.
2. Ensuring that all SAEs and SUSARs are recorded in the eCRF. Ensuring that SUSARs are reported to the Sponsor and the Chief Investigator within 24 hours of becoming aware of the event and provide further follow-up information as soon as available.
3. Ensuring that AEs are recorded and reported to the Sponsor and the Chief Investigator in line with the requirements of the protocol.

Chief Investigator (CI) / delegate or independent clinical reviewer:

1. Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.
2. Using medical judgement in assigning seriousness, causality and expectedness of SAEs where it has not been possible to obtain local medical assessment.
3. Immediate review of all SUSARs.
4. Review of specific SAEs in accordance with the trial risk assessment and protocol.
5. Assigning Medical Dictionary for Regulatory Activities (MedDRA) or Body System coding to all SAEs.
6. Preparing the clinical sections and final sign off of the Development Safety Update Report (DSUR).

Sponsor:

1. Central data collection and verification of AEs, SAEs, and SUSARs according to the trial protocol.
2. Reporting safety information to the CI, delegate or independent clinical reviewer for the ongoing assessment of the risk / benefit according to the Trial Monitoring Plan.
3. Reporting safety information to the independent oversight committee identified for the trial (Trial Steering Committee (TSC)).
4. Expedited reporting of SUSARs to the Competent Authority (FAMHP IN be) and EC within required timelines.
5. Notifying Investigators of SUSARs that occur within the trial.
6. The unblinding of a participant for the purpose of expedited SUSAR reporting.
7. Checking for (annually) and notifying PIs of updates to the Reference Safety Information for the trial.
8. Preparing standard tables and other relevant information for the DSUR in collaboration with the CI and ensuring timely submission to the FAMHP and EC.

Trial Steering Committee (TSC):

In accordance with the Trial Terms of Reference for the TSC, periodically reviewing safety data.

9.4 Notification of deaths

All deaths will be reported to the sponsor within 5 working days of the research staff becoming aware of the event with coding of the reason of death (HF related, non-HF related, unknown). In case the death is deemed related to the IMP the event will need to be reported within 24 hours of awareness of the event.

9.5 Reporting urgent safety measures

If any urgent safety measures are taken the CI/Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the FAMHP and the relevant EC of the measures taken and the circumstances giving rise to those measures.

9.6 The type and duration of the follow-up of subjects after adverse events

Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

All SUSARs occurring from the time of the start of trial treatment until end of follow-up study phase (3 months) must be recorded in the eCRF within 24 hours of the investigational staff becoming aware of the event.

9.7 Development safety update reports

The CI will provide (in addition to the expedited reporting above) DSURs once a year throughout the clinical trial, or on request, to the Competent Authority (FAMHP in Belgium), Ethics Committee and Sponsor.

The report will be submitted within 60 days of the Developmental International Birth Date (DIBD) of the trial each year until the trial is declared ended

10 STATISTICS AND DATA ANALYSIS

The statistical analysis been planned together with Geert Molenberghs and Liesbeth Bruckers from CenStat - University Hasselt.

10.1 Sample size calculation

The ADVOR study is powered for primary end-point which is the most relevant end-point with respect to the study hypothesis and reliable data from large randomized clinical trials are available to make a formal power calculation.

In the DOSE trial, which recruited a similar study population as targeted in the ADVOR study, successful decongestion with a similar definition was approximately 11% vs 18% after 72 h in the low vs high-dose loop diuretics arm[25]. The high-dose loop diuretics arm of the DOSE trial is quite comparable to the standard of care group in the ADVOR study as the loop diuretic dose used in the latter is only slightly lower (2x instead of 2.5x the oral maintenance outpatient dose) and non-loop diuretics, which were infrequently used in the DOSE trial, are not allowed. Because of these slight differences, 15% is chosen as an estimate for occurrence of the primary end-point in the monotherapy with high-dose loop diuretics (SOC) group.

No reliable data are available from large clinical trials to estimate occurrence of the primary end-point in the acetazolamide arm of the ADVOR study. Therefore, after thorough discussion with the advisory board as well as with Frank Hulstaert / Leen Verleye (KCE Trials) a success rate of 25% was chosen, which represents a clear meaningful benefit of 10% more patients with appropriate decongestion after 72 h. Using both estimates, considering a type I error rate $\alpha=0.05$ and type II error rate $\beta=0.20$ (yielding a statistical power of 80%), the targeted sample size for the ADVOR study is calculated at n = 494. A 5% drop out has been calculated in order to estimate the total number of 519 patients to be enrolled in the study.

10.2 Planned recruitment rate

Patients will be recruited by approximately 24 Belgian sites within a period of approximately 24 months. The recruitment rate will start slow due to the site initiation activities at each center, which are performed in parallel during the first recruitment months. The inclusion criteria have been widened so almost all decompensated HF patients with volume overload might be suitable study candidates, which should lead to an easy recruitment of patients. In case recruitment would be lower than expected, the number of participating centers can be increased. Also, with the publication of three-monthly research letters to the participating sites and announcement through posters/leaflets of the ADVOR-study in the outpatient cardiology department, the inclusion process might be enhanced.

10.3 Statistical analysis plan

- The treatment effect for the primary end-point [Treatment success (decongestion achieved) on the morning of day 4 without escalating diuretic strategy (doubling loop diuretic dose, addition of chlorthalidone, or ultrafiltration) during IV diuretic therapy on morning of day 3 is evaluated by means

of a generalized linear mixed model. The statistical model will include a fixed treatment effect and random center effect.

- For the first secondary end-point [occurrence of the combined endpoint of all-cause mortality and heart failure readmission during 3 months of follow-up], a generalized linear mixed model for a binary outcome will be used. The model will incorporate a fixed treatment effect and random center effect. If the treatment effect on the composite endpoint of 'all-cause mortality and HF readmission' turns out to be statistically significant, both components will be evaluated separately in a hierarchical fashion with HF readmissions first and all-cause mortality second. For this analysis, HF readmission will include patient dying from HF during the 3 months of FU. As a sensitivity analysis the worst case scenario, assuming a HF readmission for all patients dying to non HF related causes during the 3 month follow-up will be executed.
- Length of index hospitalization and change in quality of life scores are compared among treatment arms with a linear mixed model (fixed treatment effect and random center effect). Transformation will be employed when the model assumptions (such normality) are violated.
- All hypothesis are 2-sided and tested with a significance level of $\alpha=0.05$.

The proposed statistical models all assume the missing data mechanism to be missing at random. To investigate the sensitivity of the conclusions with respect to this assumption, a sensitivity analysis by means of multiple imputation technique will be performed.

Secondary Analysis:

- All statistical models discussed in the secondary analysis include a fixed treatment effect and random effect for the center, and in case of longitudinal outcome random patient effects (intercept, slope).
- The mixed models for the primary and secondary end-point analyses will be extended with explanatory variables (such as gender, age, race, ethnicity, kidney function, neurohumoral blockers, diuretics and blood pressure)
- For the end-points collected repeatedly over time, the rate of change will be investigated using longitudinal data models.
- In case patients are re-hospitalized more than once for the same reason, i.e. HF readmission, models for recurrent events will be employed.
- To study the treatment effect on the evolution of a patient's weight a mixed model for repeated measurements data will be used. The two treatment arms will also be compared for the area under the weight curve using an ANOVA model.
- For the first secondary end-point [time till combined endpoint of all-cause mortality and heart failure readmission during 3 months of follow-up], the Kaplan-Meier method will also be used to construct survival curves for both treatment arms. A mixed effects Cox proportional hazard model, with a fixed treatment effect and random center effect is used to investigate the treatment effect for this endpoint. The Hazard ratio with a 95% confidence interval will be obtained. The appropriateness of the proportional hazard assumption will be examined.

- For the primary and secondary endpoint subgroups analysis, with subgroups defined on the basis of LVEF, will also be performed.

10.4 Data collection for economic evaluation

In Belgium 2% of the population has HF, with 15.000 new cases being diagnosed annually and 3% of the annual health care budget is spent on HF, of which most is related to recurrent hospitalization for decompensated HF. HF decompensation often with repeated hospitalization has a tremendous negative impact on quality of life of the patients. Additionally, patients compare an episode of decompensation with 'drowning' so they are in constant fear of recurrent decompensations.

Reducing the number of hospital admittances will have an impact on the health care budget but also reducing the length of hospital stay per admittance will have a positive impact.

According to the "Technische Cel voor de verwerking van de gegevens met betrekking tot ziekenhuizen" (MKG- MFG data of 2014, DRG 194 Hartfalen), 21784 patients were hospitalized for heart failure with an average hospital stay of 12 days in 2014. The average hospitalization costed 7015,44 €/patient, making up a total amount of 152.824.344 €. Of these patients, 10854 patients were hospitalized with advanced symptoms (NYHA III and NYHA IV) with a considerable longer hospitalization duration (17.55 days) and higher costs (10 873,77€/patient). Cost was inclusive of hospital-day-care-price, fees and pharmaceutical products.

According to the MZG department of hospital "Ziekenhuis Oost Limburg AV" HF patients with "majeure or extreme" HF (comparable with NYHA III and NYHA IV) have an average hospital stay of 9,12 days which is significant less then MKG- MFG data available. This reduction of the length of a hospital stay is a result of our HF care giver project as well as the clinical adoption of the use of more effective decongestive therapies often including Acetazolamide. The protocol has been scientifically validated and has been published also in Acta Cardiologica which is a peer-reviewed Belgian Cardiology Journal ((Determinants and impact of the natriuretic response to diuretic therapy in heart failure with reduced ejection fraction and volume overload. Acta Cardiol. 2015 Jun;70:265-73 + Implementation of Transmural Disease Management in Patients Admitted with Advanced Heart Failure. Acta Cardiol. 2014;69:145- 54).).

The ADVOR trial will evaluate whether adding a low cost medication (Diamox ® 8.28 € public price/vial) on top of the standard treatment will reduce the hospital stay of admitted HF patients with 25% as well as reduce the need for renal replacement strategy. In daily practice this would result in an additional cost of < 20€ to potentially reduce the average cost with at least 2500 € per hospitalised patient. This would also imply that acetazolamide, on top of usual care, has the potential to result in a direct cost saving of 38.203.690 € in Belgium (=25% reduction on the budget actually spent on HF hospitalization).
Additionally, it's estimated that a more thorough successful decongestion will also lead to a reduction in heart failure rehospitalisation of 25% thereby further reducing health care cost. Therefore, a positive result of the ADVOR trial and wide adaptation of the therapeutic algorithm might translate into a reduction in expenses directly related to 1) a shorter hospitalization duration, 2) less costly 'majeur or extreem' HF, and 3) less HF hospitalizations.

While these estimations might be considered 'very optimistic', they are merely a reflection of the potential impact of a reduction of hospitalisation duration as well as readmission seen in the Ziekenhuis Oost Limburg AV after implementation of the aforementioned care pathways including a better and faster decongestion with acetazolamide. However, assuming that the primary endpoint is reached (25% decongestion in the treatment group vs 15% in the standard of care), this would translate in a 11.7% relative risk reduction. If this would translate into a 11,7% reduction in heart failure related expenditure, an annual considerable cost-saving of 17.880.408 € might still be reached.

Importantly, due to the off-patent status of the drug tested in ADVOR there will never be an industry sponsored trial to support this hypothesis. Therefore, ADVOR will lead to a revival of a "forgotten" medicine because it has the potential to significantly impact the way we treat congestion in heart failure patients.

Finally, it's expected that ADVOR will result in a novel innovative approach of treatment of decompensated HF which is focused at a different level of the nephron to achieve better, easier and safer decongestion. In that way ADVOR might pave the way towards a complete change in thinking with regards to treating decompensated heart failure.

This protocol has been designed with a later possible economic analysis in mind. Therefore, economical evaluation of the trial will be possible as several variables will be collected;

- HF readmission
- Length of hospital stay
- Need for renal replacement therapy
- Longitudinal assessment of QoL (EQ5D)…..

As for any further data analyses, an economic analysis can of course be conducted by the sponsor of the trial and the chief investigator, totally independent from KCE.

In addition, an economic analysis can be part of a KCE health technology assessment (HTA) project. HTA projects are conducted by KCE at its own costs as part of its annual work programme approved by the KCE board, and following the KCE processes. The decision for KCE to perform a HTA on the topic will depend on the trial results and the prioritisation of the topic among the topics introduced that year. Each KCE HTA project includes a literature review. Data from different studies may be included. A meta-analysis may be conducted for that purpose, including the results or the coded individual data of the funded trial. HTA projects are conducted internally at KCE or are outsourced to a certain extent using a public tender procedure. In any case, KCE uses external experts during the project. For an HTA following a trial funded by KCE Trials, KCE would among others, invite the team of the chief investigator to act as external clinical experts to accompany the HTA project.

11 DATA HANDLING

11.1 Data collection tools and source document identification

It is the responsibility of the Principal Investigator at each site to maintain adequate and accurate source data, source documentation and CRFs to record all observations and other data pertinent to the clinical investigation in a timely manner.

Patient's personal data, which are included in the sponsor database shall be treated in compliance with all applicable laws and regulations. The data collected will be anonymized and the data will only be used for the purpose(s) of this trial.

Source Data are defined as "All information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies)."

Source Documents are defined as "Original documents, data and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries of evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial)."

Case report form (CRF) is a form on which individual patient data required by the trial protocol are recorded.

All data relating to the trial must be recorded in the eCRF (electronic CRF) prepared by the Sponsor. Data reported in the eCRF should be in English, consistent with the source data or the discrepancies should be explained. If information is not known, this must be clearly indicated in the eCRF. All missing and ambiguous data will be queried.

The study data will be transcribed by study personnel from the source documents onto an eCRF, within 5 working days of the subject's visit.

Worksheets may be used for the capture of some data to facilitate completion of the eCRF. Any such worksheets will become part of the subjects' source documentation..

Every effort should be made to ensure that all subjective assessments to be recorded in the eCRF are performed by the same individual who made the initial screening assessment.

The Investigator must verify that all data entries in the eCRF are accurate and correct. All eCRF entries, corrections, and alterations must be made by the Investigator or other authorized study-site personnel. The Investigator or an authorized member of the investigational staff must adjust the eCRF (if applicable) and complete the query.

ADVOR uses an eCRF which will be used to perform statistical analysis for the trial. The CRF will be constructed to ensure:

- adequate data collection

- proper trails will be kept to demonstrate the validity of the trial (both during and after the trial)
- that only the data required by the protocol are captured in the CRF

An annotated CRF is developed with coding conventions that will be used in the database.

The Principal investigator is responsible to keep records of all participating patients (sufficient information to link records e.g., CRFs, hospital records and samples), all original signed informed consent forms and copies of the CRF pages.

11.2 Data handling and record keeping

All collected study data will be recorded in the CRF created with the software of CASTOR EDC.

CASTOR EDC complies with all applicable medical data privacy laws and regulations: GCP, 21 CFR Part 11, EU Annex 11, the European Data Protection Directive, ISO9001, and ISO27001/NEN7510.

Once the PI and delegated member(s) of the investigational staff have been trained, they will receive the link of the eCRF together with a log-in account and password. Detailed information regarding the eCRF is provided in the Manual of Operations.

Besides the data entered in the CRF, source documentation will need to be transferred to the sponsor if an endpoint (see section 9) has been reported. Only anonymised sourced data should be transferred to the sponsor. This transfer should be performed in accordance with the Belgian Privacy Act of 8 December 1992 related to the protection of the privacy in the processing of personal data and as of the 25th of May 2018 the Regulation (EU) 2016/679 of The European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation), and as of the 5th of September 2018 the Law of 30 July 2018 related to the protection of natural persons with regard to the processing of personal data, and the Law of 22 August 2002 related to the rights of patients, including their respective Royal Decrees). Detailed information regarding the transfer of source documents to the sponsor is provided in the Manual of Operations.

11.3 Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

11.4 Archiving

- archiving will be authorised by the Sponsor following submission of the end of study report
- It is the responsibility of the Principal Investigator at each site to ensure all essential trial documentation and source records (e.g. signed Informed Consent Forms, Investigator Site Files, patients' hospital notes, etc.) at their site are securely retained for at least 25 years
- The sponsor will be responsible for archiving all CRF documents and trial database for at least 25 years

- Therefore, all essential documents will be archived for a minimum period after completion of trial as required by the applicable legislation

12 MONITORING, AUDIT & INSPECTION

A Trial Monitoring Plan will be developed and agreed by the TMG based on the trial risk assessment which will be done by exploring the trial dataset or performing site visits.

The Sponsor will perform on-site monitoring visits as frequently as necessary. The monitor will record dates of the visits in a study center visit log that will be kept at the site. The first post-initiation visit will be made as soon as possible after enrollment at the center has begun. Monitoring might be initially conducted across all sites, and subsequently conducted using a risk based approach that focuses, for example, on sites that have the highest enrolment rates, large numbers of withdrawals, or atypical (low or high) numbers of reported adverse events. At these visits, the monitor will compare the data entered into the CRFs with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the CRF are known to the Sponsor, Chief Investigator and investigational staff and are accessible for verification by the Sponsor site contact. If electronic records are maintained at the investigational site, the method of verification must be discussed with the investigational staff. The processes reviewed can relate to participant enrolment, consent, eligibility, and allocation to trial groups; adherence to trial interventions and policies to protect participants, including reporting of harm and completeness, accuracy, and timeliness of data collection

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data, patients informed consent will be obtained hereto. Findings from this review of CRFs and source documents will be discussed with the investigational staff. The Sponsor expects that, during monitoring visits, the relevant investigational staff will be available, the source documentation will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the Investigator on a regular basis during the study to provide feedback on the study conduct.

13 ETHICAL AND REGULATORY CONSIDERATIONS

13.1 Ethics Committee (EC) review & reports

- Before the start of the trial, approval will be sought from a EC for this trial protocol, informed consent forms and other relevant documents e.g. insurance documents, advertisements and GP information letters
- Substantial amendments that require review by EC will not be implemented until the EC grants a favourable opinion for the study (note that amendments may also need to be reviewed and accepted by the FAMHP before they can be implemented in practice at sites)
- All correspondence with the EC will be retained in the Trial Master File/Investigator Site File
- An annual progress report (APR) will be submitted to the EC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended
- It is the Chief Investigator's responsibility to produce the annual reports as required.
- The Chief Investigator will notify the EC of the end of the study
- If the study is ended prematurely, the Chief Investigator will notify the EC, including the reasons for the premature termination
- Within one year after the end of the study, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the EC

13.2 Peer review

The protocol has been reviewed by KCE (the funder).

In addition, ADVOR has undergone a high quality peer review by two individual experts who have knowledge of the relevant discipline to consider the clinical and/or service based aspects of the protocol, and/or have the expertise to assess the methodological and statistical aspects of the study.

13.3 Public and Patient Involvement

At least 2 % of the Belgian population has heart failure (HF), with 15.000 new cases being diagnosed annually and 3% of the annual health care budget spent on HF, the most of which is related to recurrent hospitalization for decompensated HF. These hospitalizations have a tremendous negative impact on the quality of life of patients.

Therefore, ADVOR will be a RCT with patient and public involvement at all stages of the clinical research. Indeed, ADVOR actually is a result of an active partnership between patients, members of the public including KCE / health care administrators, and researchers in the research process. More specifically all endpoints of ADVOR are patient-centric measures (i.e.primary endpoint of decongestion) with a formal power calculation. Unfortunately, powering a study for hard cardiovascular endpoints would need

thousands of patients with corresponding budgets and would not be feasible within the Belgian context. Fortunately, successful decongestion (i.e. free of volume overload) has been proved study after study to be a relevant clinical outcome strongly associated with overall prognosis and was therefore chosen as the primary end-point for the ADVOR trial. It's also very patient relevant. In addition, secondary endpoints: all-cause mortality, HF readmission length of stay, and QoL are also very patient-centric.

In addition, the primary and secondary outcome measures are in line with the COMET (Core Outcome Measures in Effectiveness Trials) initiative. Indeed 'decongestion' (being dry is for the patient a very important improvement in symptomatology), heart failure readmission, all-cause mortality, length of stay, quality of life can all be considered standardized relevant core outcomes sets. Therefore, the core outcomes relevant for any acute heart failure study will be collected and reported, making it easier for the results of ADVOR to be compared, contrasted and combined as appropriate with other trials (Zannad F et al, European Journal of Heart Failure, 2013;15:1082-1094). Additionally, we will continue to explore other tertiary outcomes, which are often mechanistically very interesting, as well.

The protocol has also been discussed with members of "Mon Coeur Entre Parenthèses" which is a patient association representing HF patients and their family.

We will also collect the dosages of neurohumoral blocker therapy throughout the study period, thereby hopefully facilitating a better implementation of guideline recommended therapy. As guidelines recommend a specific dosage for each of these drugs, it's easy to standardize the intake of such medications to be used in an accurate analysis. This will be done as an exploratory tertiary end-point.

Finally, strategies that reduce the number of hospital admittances / length of stay will have an immediate impact on the health care budget.

13.4 Regulatory Compliance

The trial will not commence until a Clinical Trial Authorisation (CTA) is obtained from the FAMPH and EC.

The protocol and trial conduct shall be governed and construed in accordance with the laws of Belgium. The Trial will for instance comply with the Belgian law of May 7th 2004 regarding experiments on the human person and any relevant amendments.

The validity, interpretation and performance of this Protocol shall be governed and construed in accordance with the laws of Belgium. Belgian courts have the exclusive jurisdiction.

13.5 Protocol compliance

Protocol deviations, non-compliances, or breaches are departures from the approved protocol.

- Prospective, planned deviations or waivers to the protocol are not allowed and must not be used e.g. it is not acceptable to enrol a subject if they do not meet the eligibility criteria or restrictions specified in the trial protocol
- Accidental protocol deviations can happen at any time. They must be adequately documented and explained on the relevant forms and reported to the Chief Investigator and Sponsor immediately.

- Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

13.6 Notification of Serious Breaches to GCP and/or the protocol

A “serious breach” is a breach which is likely to effect to a significant degree

- the safety or physical or mental integrity of the subjects of the trial; or
- the scientific value of the trial

The sponsor and the Chief Investigator will be notified immediately of any case where the above definition applies during the trial conduct phase.

The sponsor of the clinical trial will notify the licensing authority in writing of any serious breach of the conditions and principles of GCP in connection with that trial; or the protocol relating to that trial, as amended from time to time, within 7 days of becoming aware of that breach.

13.7 Data protection and patient confidentiality

All investigators and trial site staff must comply with the requirements of the Regulation (EU) 2016/679 of April 27, 2016 of the European Parliament and the Council Concerning the protection of individuals with regard to the processing of personal data and the free movement of such data and repealing Directive 95/46 / EC (general data Protection Regulation), the Belgian Privacy Act of 8 December 1992 on the protection of privacy in relation to the processing of personal data and as of the 5th of September 2018 the Law of 30 July 2018 related to the protection of natural persons with regard to the processing of personal data, the Law of 22 August 2002 related to the rights of patients, including their respective Royal Decrees), with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act’s core principles.

Therefore,

- personal information will be collected, kept secure, and maintained in a way that is conform all regulation concerning privacy
- the creation of coded, depersonalised data where the participant’s identifying information is replaced by an unrelated sequence of characters
- secure maintenance of the data and the linking code in separate locations using encrypted digital files within password protected folders and storage media
- limiting access to the minimum number of individuals necessary for quality control, audit, and analysis with a list of persons who have access to data, and all this conform the regulation concerning privacy
- the confidentiality of data will be preserved when the data are transmitted to sponsors and co-investigators
- the data will be stored for at least 25 years

- The data custodians are prof. dr. Mullens, Evi Theunissen and Joke Vanlangenaeker.

13.8 Financial and other competing interests for the chief investigator, PIs at each site and committee members for the overall trial management

As acetazolamide is an off-patent drug, no competing interests that might influence trial design, conduct, or reporting are present for any of the chief investigator, PIs at each site and committee members for the overall trial management.

13.9 Indemnity

1. The Sponsor will ensure appropriate insurance to meet the potential legal liability of the Sponsor(s) for harm to participants arising from the management of the research. Before the start of the trial, approval will be sought from the EC.
2. The Sponsor will ensure appropriate insurance for legal liability of the Sponsor(s) or employer(s) for harm to participants arising from the design of the research. Before the start of the trial, approval will be sought from the EC.
3. The participating sites will ensure appropriate insurance to meet the potential legal liability of investigators/collaborators arising from harm to participants in the conduct of the research.

13.10 Access to the final trial dataset

Only the steering group has access to the full trial dataset in order to ensure that the overall results are not disclosed by an individual trial site prior to the main publication.

However, site investigators will be allowed to access the full dataset if a formal request describing their plans is approved by the steering group.

14 DISSEMINATION POLICY

14.1 Dissemination policy

Upon completion,

- the data arising from the trial will be owned by the sponsor
- the data will be analysed and tabulated and a Final Study Report prepared
- the full study report can be accessed on the website of KCE as well as on ClinicalTrials.gov
- participating investigators will have rights to publish any of the trial data upon approval of the steering committee
- The publication containing the primary study results should be finalized within 6 months of the statistical analysis. There are no time limits or review requirements on the additional publications.
- Funding by KCE will be acknowledged within the publications
- The participants of the trial will be notified by a letter containing the outcome of the trial by provision of the publication and/or via a specifically designed newsletter
- The participant might specifically request results from their PI upon completion of the trial, which might be provided once the results have been published
- It's foreseen that at the latest at publication, a machine readable electronic copy of the published version or final peer-reviewed manuscript accepted for publication in a repository for scientific publications will be deposit (open access). The research data needed to validate the results presented in the scientific publications will be deposited.

Upon completion, the study will also be submitted for presentation at the annual European Society of Cardiology in the late-breaking Clinical Trial Session as well as during the Annual European Heart Failure Association meeting.

The primary study results of ADVOR will be reported fully and made publicly available when the research has been completed. All researchers shall ensure that the outcome of the research is prepared as a research paper for publication in a suitable peer-reviewed, preferably open-access, journal. In addition, the database of the ADVOR study will be available for further sub-analysis per request of any of the sub-investigators. As a result, we feel that at least 10 other publications might be possible based on the data collected in ADVOR study which might all help to treat decompensated HF patients better. The Consort Guidelines and checklist will be reviewed prior to generating any publications for the trial to ensure they meet the standards required for submission to high quality peer reviewed journals etc. *http://www.consort-statement.org/*

All participating investigators will also try to disseminate their research findings to the broader public as well as to the research participants when the study has completed.

Endpoints were meticulously chosen based on consensus of all participating investigators including European Heart Failure Association as well as HF patient organisation (Mon Coeur Entre Parenthèses). Therefore, a positive result will also have the potential to be adopted soon in the national and international guidelines to treat decompensated HF patients.

In conclusion, it's felt that a positive endpoint might lead to a fast adoption of the use of acetazolamide in the treatment of ADHF patients because of

1) Publication in top ranked cardiology journal
2) Presentation of study results in national and international cardiology meetings
3) Adoption in guidelines
4) Internationally recognized expert study team

14.2 Authorship eligibility guidelines and any intended use of professional writers

For ADVOR, the Steering Committee will comprise the Publication Committee. KCE may serve as members of the committee. This committee will manage study publications with the goal of publishing findings from the data. The Publication Committee will develop the final Publication Plan as a separate document. In addition, the committee will apply and reinforce the authorship guidelines set forth in the Publication Plan. Membership in the Publication Committee does not guarantee authorship. The committee will meet at regular intervals.

Publications will adhere to authorship criteria defined by the International Committee of Medical Journal Editors (ICMJE, Uniform requirements for manuscripts submitted to biomedical journals, www.icmje.org). Individual authorship criteria defined by the target journal or conference will be followed when it differs from ICMJE criteria. Authors, including KCE representatives, must at a minimum meet all of the conditions below:

- Substantial contribution to conception and design, or acquisition of data, or analysis and interpretation of data; AND
- Drafting the article or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Decisions regarding authorship and contributorship will be made by the committee. The selected authors will be responsible for drafting the publication. All selected authors must fulfill the authorship conditions stated above to be listed as authors, and all contributors who fulfill the conditions must be listed as authors.

All investigators not listed as co-authors will be acknowledged as the "ADVOR Study Investigators" and will be individually listed according to the guidelines of the applicable scientific journal when possible. Any other contributors will be acknowledged by name with their specific contribution indicated. Based on the recruitment, site investigators might also be part of the Authorship.

A methods paper describing the ADVOR study, as well as the publication containing the primary study results will be drafted by the Chief Investigator, and submitted for publication after approval of the members

of the steering committee.

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■ APPENDICES

16 APPENDIX 1: AUTHORISATION OF PARTICIPATING SITES

Appendix 1.1. Required documentation

Prior to submitting the trial to the Ethics Committee, the Principal Investigator (PI) is required to sign a protocol signature page confirming his/her agreement to conduct the trial in accordance with these documents and all of the instructions and procedures found in this protocol.

Detailed information regarding the mandatory documentation which are required before the trial can start at the participating sites can be found in the Manual of Operations.

Appendix 1.2. Procedure for initiating/opening a new site

Once all start-up documentation (see Manual of Operations) from the participating site is available at the sponsor and the IMP/study material is available at the participating site, the sponsor will send confirmation by e-mail to the PI that the study can start. Only upon receipt of this site activation confirmation the site can screen/enrol patients.

Appendix 1.3. Principal Investigator responsibilities

The PI is the responsible leader of the investigational team of the participating site. The PI is responsible that he/she and his/her investigational team conducts the trial according the instructions and procedures documented in this protocol. Full list of PI's legal responsibilities is listed in the Clinical Trial Agreement.

The PI has the primary responsibility to protect the rights and welfare of the patient in the trial. The PI's primary responsibilities also include the following:

- Delegation of Responsibilities

  PI must personally perform or delegate to qualified sub-investigator or investigational staff all of the necessary tasks to carry out this trial. Even when specific tasks are delegated, the PI remains ultimately responsible for proper conduct of the trial and fulfilment of all associated obligations.

- Oversight of Investigational Team

  The PI must provide members of the investigational team with sufficient oversight, training and information to facilitate appropriate safety procedures and protocol adherence. In addition, the EC must be informed if a PI is no longer able to fulfil his or her duties for any reason including, but not limited to, traveling for a prolonged period of time.

- Evaluation of Adequacy of Resources

  Pis must ensure that adequate resources (facilities, equipment, supplies, and personnel) exist to conduct the research, protect subjects and ensure the integrity of the research.

- Document Retention

The PI must ensure adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial patients. Source data should be attributable, legible, contemporaneous, original, accurate and complete. PI must ensure that this source data is reported to the sponsor in the CRF and in the required reports according to the timelines defined in this protocol.

17 APPENDIX 2: EQ-5D QUESTIONNAIRE (DUTCH)

Vink onder elke titel het ENE vakje aan dat het best uw gezondheid VANDAAG beschrijft.

MOBILITEIT

Ik heb geen problemen met rondwandelen ❑
Ik heb een beetje problemen met rondwandelen ❑
Ik heb matige problemen met rondwandelen ❑
Ik heb ernstige problemen met rondwandelen ❑
Ik ben niet in staat om rond te wandelen ❑

ZELFZORG

Ik heb geen problemen met mijzelf te wassen of aan te kleden ❑
Ik heb een beetje problemen met mijzelf te wassen of aan te kleden ❑
Ik heb matige problemen met mijzelf te wassen of aan te kleden ❑
Ik heb ernstige problemen met mijzelf te wassen of aan te kleden ❑
Ik ben niet in staat mijzelf te wassen of aan te kleden ❑

DAGELIJKSE ACTIVITEITEN *(bijv. werk, studie, huishouden, gezins- en vrijetijdsactiviteiten)*

Ik heb geen problemen met mijn dagelijkse activiteiten ❑
Ik heb een beetje problemen met mijn dagelijkse activiteiten ❑
Ik heb matige problemen met mijn dagelijkse activiteiten ❑
Ik heb ernstige problemen met mijn dagelijkse activiteiten ❑
Ik ben niet in staat mijn dagelijkse activiteiten uit te voeren ❑

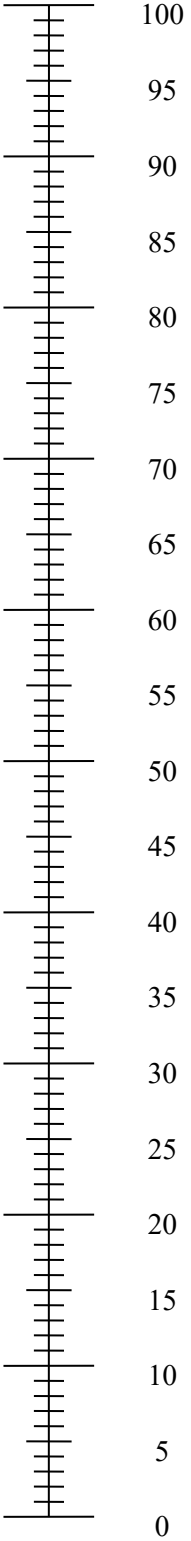
PIJN / ONGEMAK

Ik heb geen pijn of ongemak ❑
Ik heb een beetje pijn of ongemak ❑
Ik heb matige pijn of ongemak ❑
Ik heb ernstige pijn of ongemak ❑
Ik heb extreme pijn of ongemak ❑

ANGST / DEPRESSIE

Ik ben niet angstig of depressief ❑
Ik ben een beetje angstig of depressief ❑
Ik ben matig angstig of depressief ❑
Ik ben erg angstig of depressief ❑
Ik ben extreem angstig of depressief ❑

- We willen weten hoe goed of slecht uw gezondheid VANDAAG is.
- Deze meetschaal (te vergelijken met een thermometer) is genummerd van 0 tot 100.
- 100 staat voor de beste gezondheid die u zich kunt voorstellen.
  0 staat voor de slechtste gezondheid die u zich kunt voorstellen.
- Plaats een X op de meetschaal om aan te geven hoe uw gezondheid VANDAAG is.
- Noteer nu het getal dat u aangeduid hebt op de meetschaal in het onderstaande vakje.



UW GEZONDHEID VANDAAG = ☐

18 APPENDIX 3 : EQ-5D QUESTIONNAIRE (FRENCH)

Pour chaque rubrique, veuillez cocher UNE case, celle qui décrit le mieux votre santé AUJOURD'HUI.

Mobilité

Je n'ai aucun problème pour me déplacer à pied ❑
J'ai des problèmes légers pour me déplacer à pied ❑
J'ai des problèmes modérés pour me déplacer à pied ❑
J'ai des problèmes sévères pour me déplacer à pied ❑
Je suis incapable de me déplacer à pied ❑

Autonomie de la personne

Je n'ai aucun problème pour me laver ou m'habiller tout(e) seul(e) ❑
J'ai des problèmes légers pour me laver ou m'habiller tout(e) seul(e) ❑
J'ai des problèmes modérés pour me laver ou m'habiller tout(e) seul(e) ❑
J'ai des problèmes sévères pour me laver ou m'habiller tout(e) seul(e) ❑
Je suis incapable de me laver ou de m'habiller tout(e) seul(e) ❑

Activités courantes *(exemples: travail, études, travaux ménagers, activités familiales ou loisirs)*

Je n'ai aucun problème pour accomplir mes activités courantes ❑
J'ai des problèmes légers pour accomplir mes activités courantes ❑
J'ai des problèmes modérés pour accomplir mes activités courantes ❑
J'ai des problèmes sévères pour accomplir mes activités courantes ❑
Je suis incapable d'accomplir mes activités courantes ❑

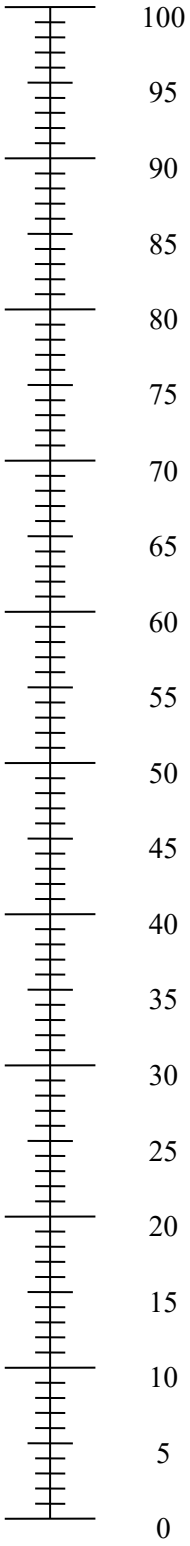
Douleurs / gêne

Je n'ai ni douleur ni gêne ❑
J'ai des douleurs ou une gêne légère(s) ❑
J'ai des douleurs ou une gêne modérée(s) ❑
J'ai des douleurs ou une gêne sévère(s) ❑
J'ai des douleurs ou une gêne extrême(s) ❑

Anxiété / Dépression

Je ne suis ni anxieux(se), ni déprimé(e) ❑
Je suis légèrement anxieux(se) ou déprimé(e) ❑
Je suis modérément anxieux(se) ou déprimé(e) ❑
Je suis sévèrement anxieux(se) ou déprimé(e) ❑
Je suis extrêmement anxieux(se) ou déprimé(e) ❑

- Nous aimerions savoir dans quelle mesure votre santé est bonne ou mauvaise AUJOURD'HUI.
- Cette échelle est numérotée de 0 à 100.
- 100 correspond à la meilleure santé que vous puissiez imaginer.
  0 correspond à la pire santé que vous puissiez imaginer.
- Veuillez faire une croix (X) sur l'échelle afin d'indiquer votre état de santé AUJOURD'HUI.
- Maintenant, veuillez noter dans la case ci-dessous le chiffre que vous avez coché sur l'échelle.



VOTRE SANTÉ AUJOURD'HUI = ☐

19 APPENDIX 4 : URINARY COLLECTION PROCEDURE

Urinary collection procedure:

Patients need to empty their bladder before the administration of the start dose of loop diuretics.

The urinary collection needs to start immediately after first bolus administration of loop diuretics and acetazolamide or placebo and the collection will stop the latest as close to but prior to the morning bolus of study medication on day 3. This collection will be defined as the Total Urinary Collection in the study. Importantly, the total volume needs to be written down in the study files as this is will be used for tertiary end-point analysis and is needed for the clinician to decide if escalation of therapy is needed.

Total Urinary Collection equals the sum of Urinary Collection period 1 + Urinary Collection period 2:

Urinary Collection period 1 = Urine collection that starts immediately after first bolus administration until the morning of day 2. This Urinary Output 1 value will be reported in the CRF together with start- and stop date/time of this first collection period.

Urinary Collection period 2 = Urine collection that starts with the end of Urinary Output 1 until the morning of day 3 prior to the morning bolus of study medication. This Urinary Output 2 value will be reported in the CRF together with start- and stop date/time of this second collection period.

How to collect the urine?

Urinary catheter insertion is strongly recommended but not mandatory to achieve an optimal urine collection. However, in case of urinary incontinence, placement of a urinary catheter is mandatory.

Alternatively, the patient needs to use (an) urinary container(s) to collect ALL urine. Prior to the stop of urinary collection period the patient need to be instructed to empty their bladder. Care should be taken to ensure ALL urine is collected.

At the end of a Urinary Collection period all urine of this period needs to be collected in the urinary container(s).

Which measurements need to be done?

For each urinary collection period the container(s) or a sample (if multiple containers are kept, they need to be mixed before taking a sample) will need to be sent to the local lab for analysis of the volume, Cr, total protein and Na (and bumetanide level, if available).

20 APPENDIX 5 : CONCOMITANT MEDICATION

During the trial the Principal Investigator or delegated team member should check if the subject is taking concomitant medication (CM).

The CM which need to be recorded for the trial can be divided in 3 different types of concomitant medication (CM):

- **Neurohumoral blockers**: renin-angiotensin system blockers, sacubitril/valsartan, beta-blockers, and mineralocorticoid receptor antagonists
- **Diuretics**
- **Other concomitant medication**: pre-defined list of medication (ivabradine, hydralazine, molsidomine, etc.) The full list of other CM can be found in the current eCRF version.

Depending on the visit day, specific types of CM will need to be recorded in the eCRF

Visit	Neurohumoral blocker(s)	Diuretic(s)	Other CM
Screening	X	X	X
Day 1, 2, 3		X	X
Day 4	X	X	X
Discharge, 3 month FU	X	X	x

During the screening phase, the daily maintenance dose of the neurohumoral blockers and diuretics will need to be recorded in the eCRF. In addition, the list of the other concomitant medication will need to be reported in the eCRF.

During the treatment phase, the list of other concomitant medications will need to be reviewed on a daily basis. Listed medication which the subject is treated with, will need to be reported in the eCRF on the respectively day of treatment. Additionally on day 4 also the daily dose of the neurohumoral blockers and diuretics will need to be recorded in the eCRF.

During the follow-up phase, the daily dose of neurohumoral blockers and diuretics the subject is taking at the time of discharge and 3 month FU visit will need to be recorded in the eCRF. The list of other concomitant medications will need to be reported at the time of discharge and 3 months. In addition, it needs to be reported in the eCRF if the subject received iron treatment during the treatment phase and/or during the follow-up phase.

21 APPENDIX 6 : AMENDMENT HISTORY

Amendment No.	Protocol version no.	Date issued	Details of changes made
01	Version 2.0	03 January 2019	Cfr. explanation below.

List details of all protocol amendments here whenever a new version of the protocol is produced.

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the EC committee or FAMHP.

Details of changes made for amendment No. 01:

- Clarification of maintenance dose of loop diuretics. To avoid any confusion, half of start dose given at randomization has been erased and we stated “1x orally daily maintenance dose”.
- Clarification volume assessment scoring
- If patients are being discharged earlier than day 4, parameters planned for the morning of day 4 will still be assessed but at discharge (i.e. EQ5D, NT-proBNP/BNP, blood samples for sub-study if applicable)
- Removal of an endpoint event, i.e. rehospitalisation due to any cardiac event.
- Clarification exclusion criteria:
  - **Use of nitrates *and/or* molsidomine is allowed, but at the discretion of the treating physician** instead of use of nitrates is allowed only when systolic blood pressure is above 140mmHg.
  - **Treatment with acetazolamide within one month prior to randomization is not allowed** instead of treatment with acetazolamide during the index hospitalisation before randomization.
  - **Use of any non-protocol defined diuretic agent with the exception of mineralocorticoid receptor antagonists during the treatment phase of the study is not allowed** instead of Use of any non-protocol defined diuretic agent with the exception of mineralocorticoid receptor antagonists.
- Addition of one exclusion criteria: Subjects with urinary incontinence who are not willing to receive a bladder catheter
- Collection of additional study data:
  - Use of iron per os and/or intravenously throughout the study will be recorded at discharge and at follow-up.
  - A predefined list of concomitant medication will need to be completed at discharge and at 3 months follow-up (cfr. appendix 5)
- Details concerning the collection of concomitant medication have been replaced by appendix 5.
- Follow-up appointment will be 3 months (+ 14 days) after the start of study medication, instead of after hospital discharge.
- For completeness, admission has been changed to screening.
- Throughout the protocol, minor changes (e.g. typographical errors, clarifications, etc.) have been added.

Original Statistical Analysis Plan

Version - 1.0

17th of January 2022

A multi-centre, randomized, double-blind, phase IV clinical trial on the diuretic effects of Acetazolamide (Diamox ®) in patients with Decompensated heart failure and Volume OveRload

STATISTICAL ANALYSIS PLAN

Version number: version 1.0

Version date: 17 January 2022

RESEARCH REFERENCE NUMBERS

EudraCT Number:	2018-001345-14
Clinical trials.gov Number:	NCT03505788
SPONSOR Number:	ZOLCAR17001
FUNDER Number:	KCE-17001
Protocol version:	2.0, dated 03/01/2019

1 DOCUMENT HISTORY

Version number	Version Date	Author	Reason for change
Version 1.0	17 January 2022	ADVOR Scientific Study Team [redacted] (Statistician)	First version

2 TABLE OF CONTENTS

3 LIST OF ABBREVIATIONS

The following table presents the abbreviations and acronyms used in the Statistical Analysis Plan:

CI	Confidence Interval
EAC	Endpoint Adjudication Committee
eCRF	electronic Case Report Form
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
EQ-5D	EuroQol five dimension (questionnaire)
HF	Heart Failure
HFpEF	Heart Failure with preserved Ejection Fraction
HFrEF	Heart Failure with reduced Ejection Fraction
HR	Hazard Ratio
IMP	Investigational Medicinal Product
IRT	Interactive Response Technology
ITT	Intention To Treat
ITTAS	Intention to treat analysis set
IV	Intravenous
KCl	Potassium chlorate
LVEF	Left Ventricular Ejection Fraction
$MgSo_4$	Magnesium sulfate
$NaHCO_3$	Sodium bicarbonate
OR	Odds ratio
Qol	Quality of Life
SAS	Statistical Analysis System
SD	Source Documents
SGLT2-i	Sodium-glucose Cotransport 2 Inhibitor
SOC	Standard Of Care
SUSAR	Suspected Unexpected Serious Adverse Reactions

4 STUDY SYNOPSIS

The ADVOR study is a phase IV randomized, multicentre, double-blind study, comparing monotherapy with high-dose loop diuretics (standard of care - SOC) versus combination therapy with acetazolamide and high-dose loop diuretics in patients with decompensated heart failure (HF) and clinical signs of volume overload. For more details regarding the intervention see section 4.3

The study consists of 3 phases:

- screening phase: starting from identifying a study subject prior to / during hospitalization until the first dose of study medication will be given
- treatment phase: starting from the first dose of study medication administration until the morning of day 4 or earlier in case of successful decongestion prior to day 4.
- follow-up phase: starting when the treatment phase ends until 3 months after the study start dose.

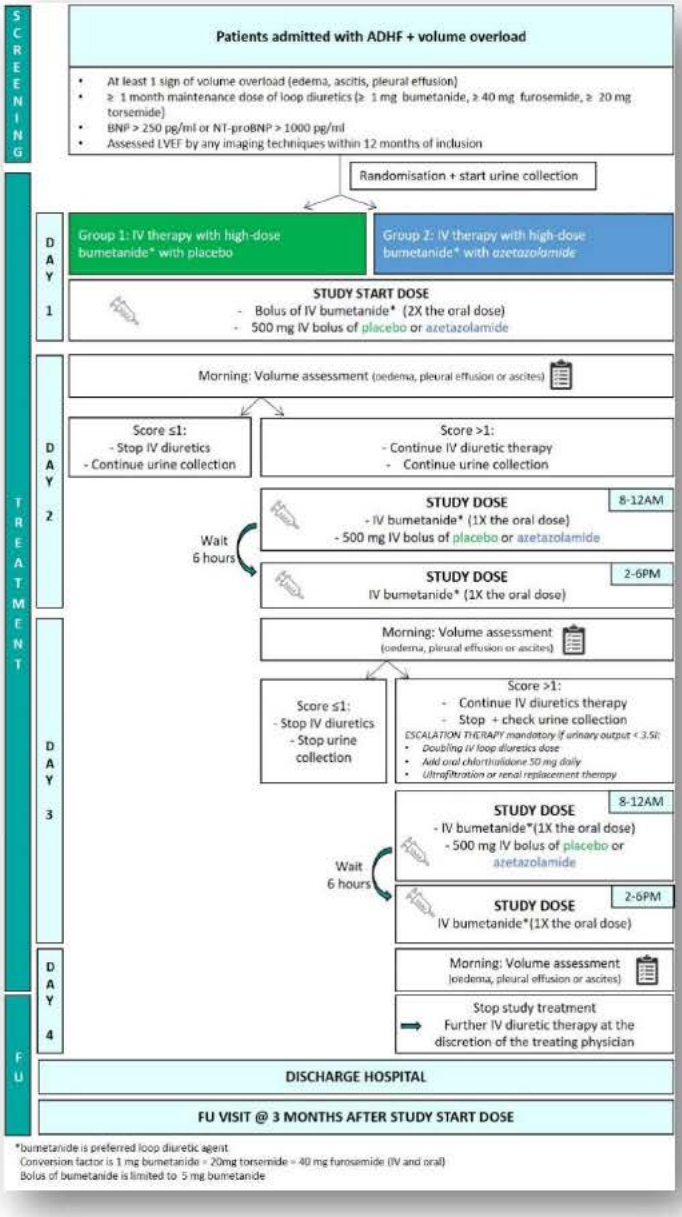


Figure 1. Trial Flow Chart

4.1 Study objectives and endpoints

4.1.1 Rationale

Guidelines from international cardiac societies lack high-quality data on the optimal dosing, timing and method of delivery of diuretic agents. In the Diuretic Optimization Strategies Evaluation (DOSE) trial[1], which is the only randomized clinical trial on diuretic therapy for decompensated HF patients, no differences in patients' global assessment of symptoms or change in renal function were observed when loop diuretics were administered by bolus as compared with continuous infusion or at high versus low dose during a hospitalization for decompensated HF. Of note, only a minority of patients (15%) were adequately decongested after 72 h in the DOSE trial, Acetazolamide is a largely forgotten diuretic, but can potentially boost diuretic response. Acetazolamide targets the proximal tubule of the kidney, where the majority of sodium is reabsorbed. The ADVOR trial will examine if an improved application of existing decongestive therapies by adding acetazolamide, will result in a better outcome for patients and society.

4.1.2 Primary Objective and outcome measure

Primary Objective	Primary Endpoint
To investigate if combination therapy with acetazolamide improves loop diuretic efficacy to increase diuresis in decompensated HF patients with volume overload, allowing for a better/faster decongestion and a lower total dose of loop diuretics.	The proportion of patients achieving decongestion on the morning of day 4 without the need for escalating therapy on the morning of day 3 (treatment success).

<u>Definition of primary endpoint</u>

Congestion is assessed using the ***volume assessment score*** (also called the congestion score see figure 2). Freedom from volume-overload will be defined as not more than trace oedema, no residual pleural effusion, and no residual ascites (Figure 2).
So a (sum) score ≤ 1 indicates that decongestion is achieved (being dry), a score > 1 is considered to be volume overloaded (decongestion not achieved).

The primary endpoint is a binary endpoint, with a success defined in case of:

- decongestion (volume assessment score ≤ 1) on the morning of day 2
- or decongestion (volume assessment score ≤ 1) on the morning of day 3
- or decongestion (volume assessment score ≤ 1) on the morning of day 4 **without** the need for escalating diuretic strategy on the morning of day 3, which is needed if total urinary output on the morning of day 3 < 3.5l (sum of urinary output from the start of IMP until the morning of day 3).

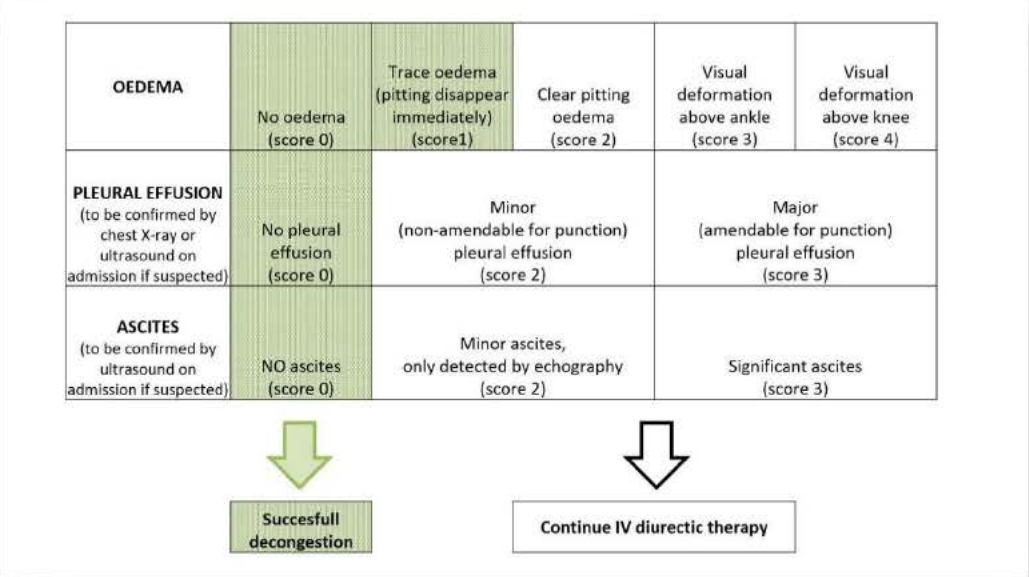


Figure 2. Clinical Congestion Assessment

The SOC therapy results in ~15% effective decongestion[1]. It is estimated that the combination therapy should have a success rate of 25%, which represents a clear meaningful absolute benefit of 10% more patients with appropriate decongestion after 72h.

4.1.3 Secondary objectives and endpoints

Secondary Objectives	Secondary Endpoints
To investigate if combination therapy with acetazolamide: - leads to improved clinical outcome in decompensated HF (less HF readmissions, lower all-cause mortality) - shortens the length of stay in patients with decompensated HF, which is expected to reduce health care expenditure - leads to improved quality of life	 - Combined endpoint of all-cause mortality and HF readmission during 3 months of follow-up - Length of index hospital admission - Change in EuroQoL

Definition of secondary endpoints

- The combined endpoint of all-cause mortality and HF readmission during 3 months of follow-up is a binary endpoint. The time to the combined endpoint will be calculated as the time between start of study medication (day 1) and the date of (first) HF readmission or mortality (whichever comes first). *HF readmission* is defined as either a hospital admission or an unscheduled contact at the emergency department **and** when

treatment with intravenous loop diuretics is initiated because of worsening HF.

- Length of index hospital admission (calculated in days) is obtained as the date of discharge from the hospital minus the date of screening +1.

- EuroQol is a five dimension patient-reported QoL questionnaire (EQ-5D-5L). The EQ-5D-5L health states, defined by the EQ-5D-5L descriptive system, are converted into a single index value (https://kce.fgov.be/nl/een-belgische-waardenset-voor-de-eq-5d-5l-%E2%80%93-hoe-gezondheidsgerelateerde-levenskwaliteit-waarderen). The VAS assessment of the EQ-5D will also be used. The EQ VAS records the patient's self-rated health on a vertical visual analogue scale.

  The index values and EQ-VAS at baseline, the morning of day 4 or discharge (whatever comes first), and at 3 months of follow-up will be used for the secondary objective.

  During the study the baseline EQ-5D-5L questionnaire has been completed as soon as possible after screening up to day 1.

4.1.4 Safety objectives and endpoints

Safety Objective	Safety Endpoints
To evaluate the safety of the combination therapy with acetazolamide in this patient population.	- Severe metabolic acidosis at any time during study treatment - Doubling or more (≥2x) of baseline serum Creatinine, or ≥ 50% decrease in baseline eGFR at any time during study treatment - Hypokalaemia at any time during study treatment - Hypotension at any time during study treatment

Definition of Safety endpoints

- Severe metabolic acidosis: a value of bicarbonate <12 mmol/L at any time during study treatment (day 1- day 4)
- Worsening of eGFR or the need for renal replacement therapy during the index hospitalization. eGFR is obtained via the CKD-EPI formula. The eGFR values will be calculated (in mL/min/1.73 m2) from the local laboratory creatinine measurements using the CKD-EPI formula (Levey at al 2009)[2].
- Hypokalaemia: a value of potassium ≤ 3,0 mmol/L at any time during study treatment (day 1- day 4)
- Hypotension: a value of systolic blood pressure < 85 mmHg at any time during study treatment (day 1- day 4)

Baseline laboratory value

For all laboratory variables, the baseline value is defined as the last value prior to the first dose of study drug.

4.1.5 Exploratory objectives and endpoints

Exploratory Objectives	Exploratory endpoints
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on body weight.	Change in body weight.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will reduce the incidence of all cause rehospitalisation's.	All cause rehospitalisation during first 3 months after study start dose.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on total urinary volume and natriuresis.	Total urinary volume and natriuresis starting from first intravenous (IV) diuretic administration at randomization until the morning of day 3.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on plasma natriuretic peptides.	Change in plasma natriuretic peptides change from screening until day 4 or at discharge (whatever comes first) and at 3 months follow up visit.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on the total dose of IV loop diuretics used during first 4 days.	Total dose of IV loop diuretics used during first 4 days.
Effect of decongestion on uptitration of neurohormonal blockers	Changes in doses of neurohumoral blockers.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will reduce the need for renal replacement therapy.	Need for renal replacement therapy or ultrafiltration during first 3 months after study start dose.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will reduce the incidence of hyponatremia during treatment phase.	Hyponatremia during treatment phase.
To evaluate if liver dysfunction at screening has an effect on the treatment effect on the primary endpoint.	Liver dysfunction at screening and primary endpoint.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on plasma volume changes during treatment phase.	Change in plasma volume during treatment phase (assessed by albumin and hematocrit)

Exploratory Objectives	Exploratory endpoints
To evaluate if iron deficiency at screening has an effect on the treatment effect on the primary endpoint	Iron deficiency at screening and primary endpoint
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on congestion score during the study.	Changes in congestion score (total and each subscore) during treatment phase and 3 months of FU.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on EQ-VAS score during the study.	Changes in EQ-VAS score during treatment phase and 3 months of FU.

4.2 Patient population

4.2.1 Inclusion Criteria

- Signed written informed consent must be obtained before any study assessment is performed
- Male or female patients of 18 years of age or older
- An elective or emergency hospital admission with clinical diagnosis of decompensated HF with at least one clinical sign of volume overload (e.g. oedema (score 2 or more), ascites confirmed by echography or pleural effusion confirmed by chest X-ray or echography)
- Maintenance therapy with oral loop diuretics at a dose of at least 1 mg bumetanide or an equivalent dose for at least 1 month before hospital admission (Conversion: 1 mg bumetanide = 40 mg furosemide = 20 mg torsemide)
- Plasma NTproBNP levels >1000 ng/L or BNP levels >250 ng/L at the time of screening
- Assessed LVEF by any imaging technique; i.e. echocardiography, catheterization, nuclear scan or magnetic resonance imaging within 12 months of inclusion

4.2.2 Exclusion Criteria

- Concurrent diagnosis of an acute coronary syndrome defined as typical chest pain in addition to a troponin rise above the 99th percentile and electrocardiographic changes suggestive of cardiac ischemia
- History of congenital heart disease requiring surgical correction
- History of a cardiac transplantation and/or ventricular assist device
- Systolic blood pressure <90 mmHg or mean arterial pressure <65 mmHg at screening
- Expected use of intravenous inotropes, vasopressors or nitroprusside during the study. The use of nitrates and/or molsidomine is allowed at the discretion of the treating physician.
- Estimated glomerular filtration rate <20 mL/min/1.73m² at screening
- Use of renal replacement therapy or ultrafiltration at any time before study inclusion
- Treatment with intravenous loop diuretics > 2 mg bumetanide or an equivalence of another loop diuretic during the index hospitalization and prior to randomization
- Treatment with acetazolamide within 1 month prior to randomization
- Exposure to nephrotoxic agents (i.e. contrast dye) anticipated within the next 3 days
- Use of any non-protocol defined diuretic agent with the exception of mineralocorticoid

receptor antagonists during the treatment phase of the study. Thiazides, metolazone, indapamide and amiloride should be stopped upon study inclusion. If patient is taking a combination drug including a thiazide-type diuretic, the thiazide-type diuretic should be stopped.
- Current use of sodium-glucose transporter-2 inhibitors
- Subjects who are pregnant or breastfeeding
- Subjects with urinary incontinence who are not willing to receive a bladder catheter.

4.3 Intervention

At the moment of randomization, oral loop diuretics are stopped and the patient receives an IV bolus of loop diuretics at a dose equal to the double of his oral daily maintenance dose with a maximal dose of 5 mg bumetanide (=200 mg furosemide). Bumetanide is the preferred loop diuretic agent to be used in this trial. Between administering the start dose and next treatment dose a minimum of 6 hours is required. During the remaining part of the treatment phase, the patient will continue to receive 2 IV treatment doses every day provided that the treating physician has concluded during the morning rounds that the patient is still volume overloaded (see Figures 2, volume assessment score > 1). The IV loop diuretic dose will be the orally daily maintenance dose, administered between 8:00 and 12:00 am, together with an intravenous bolus of 500 mg of acetazolamide or placebo. The second dose of IV loop diuretics, again dosing equal to the orally daily maintenance dose, will be given 6 hours after the morning dose. Any patient with more than trace oedema, residual pleural effusion or residual ascites would be considered to be still volume overloaded (see Figures 2). Residual pleural effusion and/or ascites should always be confirmed by chest X-ray or echography. If pleural effusion/ascites is used as an inclusion criteria, chest X-ray and/or echography should be repeated until decongestion has been achieved (volume assessment score ≤ 1). If not present at inclusion, new evidence of pleural effusion and/or ascites may arise during the treatment phase, but if scored it should be confirmed by a chest X-ray or echography. If the patient is not volume overloaded anymore, the intravenous administration of study medication should be stopped. Once decongestion is achieved (volume assessment score ≤ 1) during the treatment phase, no volume assessment should be performed anymore the following morning.

4.3.1 STOP TREATMENT

The treating physician is allowed (but not obliged) to stop the study treatment, in case of persistent volume-overload in following cases:
- symptomatic hypotension with a systolic blood pressure <100 mmHg
- asymptomatic hypotension with a systolic blood pressure <90 mmHg
- an increase of serum Creatinine levels x 1.5 of the serum Creatinine level compared to admission value
- occurrence of metabolic acidosis (pH < 7.2)

The discontinuation of the study treatment will be seen as a failure for the primary endpoint (decongestion not achieved). However, if any of these events occur when the patient is judged to be euvolemic, the study treatment is stopped and the primary endpoint is considered to be successful (decongestion achieved).

Freedom from volume-overload (i.e. successful decongestion) on the morning of day 4 will be defined as not more than trace oedema, no residual pleural effusion, and no residual ascites (Figure 2).

4.3.2 Treatment DOSE ADJUSTMENTS in case of an inappropriate diuretic response

If the total urinary output on the morning of day 3 is < 3500 mL and the patient is still volume overloaded, an escalation of decongestive treatment is mandatory. One of the outlined three options can be chosen at the discretion of the treating physician.
Escalation therapy options:

- doubling of the IV dose of the loop diuretics
- add oral chlorthalidone 50 mg once daily
- ultrafiltration or renal replacement therapy might be considered

The decision to proceed with escalation therapy will be collected in the case report as the need for escalation implies a failure for the primary endpoint.

4.3.3 Background therapy

24h oral **intake of fluid and sodium will be restricted to 1500 mL and 1.5 g,** respectively. It is recommended that all patients receive the same maintenance infusion with 500 mL glucose 5% and 3g $MgSO_4$ administered over 24h time interval, until complete decongestion or end of the study treatment phase. All non-protocol fluids administered (including those for administration of intravenous medication) should be limited.

In case of **serum potassium levels <4 mmol/L**, 40 mmol of KCl is added to the maintenance infusion. Oral potassium supplements may be used at the discretion of the treating physician, but their use will be prospectively registered.

In case of **metabolic acidosis with serum bicarbonate levels <20 mmol/L**, it is recommended to administer intravenously 100 ml of $NaHCO_3$ 8.4%.

Treatment with **neurohumoral blockers** (e.g. renin-angiotensin system blockers, sacubitril/valsartan, beta-blockers, and mineralocorticoid receptor antagonists) may be continued at the same or lower dosage at the discretion of the treating physician, until the end of the treatment phase (max 4 days) or until complete decongestion is achieved, whatever comes first. Dose increases for any of these medications are not allowed during the screening and treatment phase with the exception of mineralocorticoid receptor antagonists in case of hypokalemia despite intravenous potassium supplement. In addition, starting an SGLT2 inhibitor and a switch from renin-angiotensin system blockers to sacubitril/valsartan is not allowed during the screening and treatment phase, but might be pursued after decongestion is achieved. After decongestion, it is strongly recommended to up-titrate doses of neurohumoral blockers according to the guidelines in the HFrEF patients. Dosages of neurohumoral blockers are collected at screening, at discharge and at three months follow-up.

4.3.4 Randomisation and blinding

The ADVOR study is a randomized double blind clinical trial with 2 treatment arms; therapy with high-dose loop diuretics and placebo vs therapy with high-dose loop diuretics and acetazolamide. An automated web-based system is used to randomly assign patients in a 1:1 ratio with variable blocks sizes, stratified for LVEF according to study centre. To ensure an equal proportion of HFpEF

versus HFrEF patients in both study arms, the population will be stratified at inclusion according to LVEF ≤40% versus >40%. Permuted block randomization according to centre and LVEF stratum will be used to achieve this.

Patient, site personnel, sponsor personnel and data analysts will remain blinded to the identity of the treatment from the time of randomization until database lock. Though we do not foresee serious adverse events related to the study drug, the study code should only be broken for valid medical or safety reasons e.g. in the case of a severe adverse event where it is necessary for the investigator or treating health care professional to know which treatment the patient is receiving before the participant can be treated.
Following rules apply for unblinding;

- Rapid unblinding of a patient can be performed by a physician of the study team. Detailed information concerning the unblinding procedures is provided in the Manual of Operations.
- On receipt of the treatment allocation details, the PI or treating health care professional will continue to deal with the participant's medical emergency as appropriate.
- The PI/Investigation team documents the breaking of the code and the reasons for doing so on the medical notes and eCRF. It will also be documented at the end of the trial in any final study report and/or statistical report.

Unblinded data are to be kept strictly confidential until the time of unblinding of the trial and will not be accessible by anyone else involved in the trial with the following exceptions: (1) the Project Manager of the company responsible for the labelling and packaging of the IMP, (2) the IRT system programmers who work on the randomization and drug management system; and (3) the data manager who prepares reports required for regulatory reporting (suspected unexpected serious adverse reactions [SUSAR] reporting). These individuals will not be involved in the day-to-day running of the study.

4.4 Endpoint adjudication

Because the primary endpoint requires physical examination of the blinded study physician, adjudication of the primary event is not possible. However, all admission labelled as HF readmission, all-cause mortality and the need for renal replacement therapy will be adjudicated by the Endpoint Adjudication Committee (EAC)

For the endpoints HF readmission, all-cause mortality and the need for renal replacement therapy a complete endpoint package will need to be send to the sponsor. This package should include anonymized source documents (SDs) relevant to the endpoint reported (e.g. discharge letter, emergency room notes, etc). The SD should include at least the reason for admission/death and the received treatment for the event (if applicable). This package will be assessed by the Endpoint adjudication members of the Endpoint adjudication committee.

In case of discrepancy between the evaluation of the EAC members regarding the same event, the Trial Data Manager will contact the EAC members to inform them on the discrepancies found so the both of them can come to a final decision. The discrepancies and the final decision will be documented.

4.5 Sample Size

The ADVOR study is powered for primary endpoint which is the most relevant endpoint with respect to the study hypothesis and reliable data from large randomized clinical trials are available to make a formal power calculation.
In the DOSE trial, which recruited a similar study population as targeted in the ADVOR study, successful decongestion with a similar definition was approximately 11% vs 18% after 72 h in the low vs high-dose loop diuretics arm. The high-dose loop diuretics arm of the DOSE trial is quite comparable to the standard of care group in the ADVOR study as the loop diuretic dose used in the latter is only slightly lower (2x instead of 2.5x the oral maintenance outpatient dose) and non-loop diuretics, which were infrequently used in the DOSE trial, are not allowed. Because of these slight differences, 15% is chosen as an estimate for occurrence of the primary endpoint in the monotherapy with high-dose loop diuretics (SOC) group.

No reliable data are available from large clinical trials to estimate occurrence of the primary endpoint in the acetazolamide arm of the ADVOR study. Therefore, after thorough discussion with the advisory board a success rate of 25% was chosen, which represents a clear meaningful benefit of 10% more patients with appropriate decongestion after 72 h. Using both estimates, considering a type I error rate α=0.05 and type II error rate β=0.20 (yielding a statistical power of 80%), the targeted sample size for the ADVOR study is calculated at n = 494. A 5% drop out has been calculated in order to estimate the total number of 519 patients to be enrolled in the study.

5 STATISTICAL ANALYSIS

5.1 General principles

This statistical analysis plan dated 17 January 2022 is based on the statistical information documented in the study protocol version 2.0 dated 03 January 2019 (see section 10), the methods paper published in the European Journal of Heart Failure (2018)[3] and guidance by the European Medicines Agency (EMA)[4] and the Food and Drug Administration[5] regarding the management of clinical trials during COVID-19 pandemic (2021).

The scope of this statistical analysis plan is to outline the statistical tools used for the primary and secondary objectives of the ADVOR study following the procedures documented in the study protocol version 2.0 dated 03 January 2019 in all participating centres.

Prior to the statistical analysis the collected study data will be cleaned by the data management team of the Clinical Trial Unit at the Ziekenhuis Oost-Limburg (ZOL-CTU). The cleaning will be performed according to the Data Management Plan agreed for the ADVOR trial.

All statistical hypothesis are 2-sided and a 5% significance level will be used. A correction for multiple testing will not be implemented.

Trial results will be reported according to the CONSORT statement on reporting randomized controlled trials.
For the analysis of the primary outcome, each secondary and exploratory outcome the following information will be presented:

- the number of patients included in each analysis, by treatment arm
- a summary statistic of the outcome (e.g. number (%), mean (SD)), by treatment arm

- the estimated treatment effect
- a 95% confidence interval for the estimated treatment effect
- A two-sided p-value for all analyses, a significance level of 5% will be used.

All statistical analysis will be done with Statistical Analysis System (SAS) for Windows, SAS 9.4.

5.2 Interim analyses

No interim analysis is planned regarding the ADVOR study.

5.3 Multiplicity adjustment

There will be no correction for multiplicity of testing as there is only one primary endpoint. All other endpoints and subgroup analyses will be considered as exploratory.

5.4 Blind review

Once the database is cleaned the dataset will be locked and transferred to an independent academic statistical center (DSI/CenStat - University Hasselt) for efficacy and safety analysis according to the statistical analysis plan

5.5 Data sets to be analysed

For the *primary endpoint* the statistical analysis will be based on an intention-to-treat analysis set (ITTAS) including all randomized patients. Patients will be analysed according to the treatment group they were allocated to according to the electronic randomization system (irrespective of the actual treatment received).

For the *secondary endpoints* the statistical analyses will be based on the same data set as will be used for the primary endpoint analysis.

For the *safety endpoints*, all patients that were randomized and received at least 1 dose of the investigated drug or placebo will be included (safety population). For the safety analysis, patients will be grouped based on the actual study treatment received.

5.6 Subject disposition

After closing of the database, a CONSORT diagram will be produced for transparent status of the subject reporting.

5.7 Violations and deviations

The important protocol deviations listed below will be summarised by randomized treatment group

- Patients who were randomized but did not meet inclusion and exclusion criteria
- Patients who received study treatment with a wrong treatment vial number at any time during the study
- Patients who received only acetazolamide/placebo but no loop diuretics on day 1, 2 or 3

As the primary analysis is an intention-to-treat analysis, protocol deviation will not imply exclusion from the primary analysis.

5.8 Concomitant therapies

The frequency of baseline medication will be shown for the intention-to-treat analysis set (ITTAS) per treatment group, counts and % will be presented grouping the baseline medication per drug classes.

5.9 Baseline characteristics

To describe the study population, characteristics of all the patients in the ITTAS population will be presented. Numbers (%) or means (SD) and medians (IQR) will be given for each treatment group as appropriate.

5.10 Analysis of the primary outcome

5.10.1 Main analysis

The primary statistical analysis in ADVOR will be an intention-to-treat (see section 5.5).

The primary endpoint is a binary outcome with a success defined as decongestion achieved on the morning of day 4 (or earlier) without the need for escalating diuretic strategy (doubling loop diuretic dose, addition of chlorthalidone, or ultrafiltration) during IV diuretic therapy on morning of day 3.

For descriptive purposes, the number (%) of failure and success for the primary outcome will be given per treatment arm.

The treatment effect for this primary outcome will be evaluated by means of a generalized linear mixed model (logistic model[67]). The statistical model will include a fixed treatment effect, and a random centre effect. The results of this model will be presented as an odds-ratio (OR), 95% confidence interval and the associated p value.

5.10.2 Subgroup analysis

For exploratory purposes subgroup analysis for the primary and secondary endpoints will be conducted, to assess whether the treatment effect differs according subgroups. The table below gives an overview of the subgroups that will be investigated and how they will be created:

Characteristic	Sub group
Age (years)	≤ median, > median
Sex	Male, Female
LVEF	≤ 40%, > 40%
Baseline NTproBNP (ng/L)	≤ median, > median

Characteristic	Sub group
Baseline eGFR (mL/min/1,73m2)	≤ median, > median
Etiology of HF	Ischemic, non-ischemic
Home dose loop diuretic (mg)	≤ median, > median
Baseline Congestion score	≤ median, > median
Hyponatremia (mmol/L)	≤ 130, > 130
Thiazide	Yes, No
Atrial Fibrillation	Yes, No

The statistical model formulated for the analysis of the primary endpoints will be extended to include a fixed effect for the subgroup and an interaction term between the study arm and the subgroup. The interaction term allows for a subgroup-specific treatment effect. The interaction term will be considered significant at the 5% level.
Within each subgroup, summary statistics of the primary outcome by treatment arm will be presented; the OR for the treatment effect and 95% confidence interval. A p-value for the interaction test will also be reported. A forest plot, of the ORs (and 95%CI) per subgroup, will be used to summarize the results visually.

5.10.3 Treatment of missing data

The proposed statistical model assumes the missing data mechanism to be missing at random. If the level of missing data for the primary outcome in the ITTAS populations exceeds 5%, a sensitivity analysis to assess the robustness of the analysis of the primary outcome by means of multiple imputation technique will be performed for the primary endpoint.

5.10.4 Other sensitivity analyses

The COVID-19 pandemic has impacted clinical development and ongoing clinical trials. Public Health measures to control the virus may impact the ability to collect data. Additionally, the number of HF admissions were reduced by 40% in Europe between March and June 2020. The change in patient behaviours and characteristics might lead to for example less hospitalizations, more advanced HF presentations, less hospital admission duration, and less protocol adherence which all might impact study results.

Several mitigation strategies were discussed in the steering committee meeting. (a) Extra efforts to capture potential reasons for missing data will be employed. (b) Due to slower enrolment extension of the trial was considered until complete enrolment of the predefined sample size. The trial completion was achieved before the expiration date of the Investigational Medicinal Product (IMP).

Because of the aforementioned potential differences in patient characteristics, a sensitivity analysis for the primary endpoint will be performed.

The treatment effect for the primary endpoint is evaluated by means of a generalized linear mixed model, including a fixed treatment effect and random centre effect. As a sensitivity analysis, the model will be extended by including a fixed effect (binary) indicating if the endpoint was assessed before or after the first identified COVID-19 case in Belgium dated 03 February 2020 and the interaction term between this variable and treatment.

5.11 Analysis of secondary outcomes

All statistical models implemented for the analysis of the secondary outcomes are mixed-effects models, including a fixed treatment effect and random centre effect. For the outcomes measured at multiple time points also random patient effects (intercept, slope) are included.

5.11.1 Secondary outcome 1

The combined endpoint of all-cause mortality and HF readmission during 3 months of follow-up, will be analysed as a binary and as a time-to-event endpoint.

- For descriptive purposes, the number (%) of failure and success for the combined outcome of all-cause mortality and HF readmission during 3 months of follow-up will be given per treatment arm. To investigate the treatment effect on the occurrence of the combined endpoint, a generalized linear mixed model for a binary outcome will be employed. The model will incorporate a fixed treatment effect and random centre effect. The results of this model will be presented as odds-ratio, 95% confidence interval and the associated p value.

  If the treatment effect on the composite endpoint of 'all-cause mortality and HF readmission' turns out to be statistically significant, both components will be evaluated separately in a hierarchical fashion with HF readmissions first and all-cause mortality second. For this analysis, HF readmission will include patients dying from any cause during the 3 months of FU. The treatment effect, on the components, will be investigated by the same statistical model as used for the combined endpoint.

- To investigate the treatment effect on the time until combined endpoint of all-cause mortality and HF readmission during 3 months of follow-up a survival analysis will be performed. Time to event is defined as the time between day 1 and the date of (first) HF readmission or death if there was no HF readmission. The analysis will be censored at 3 months of follow-up or at the time when the patient was last known to be alive (for patients that withdraw or are lost to follow up), whichever occurs earlier. Survival times will be summarised descriptively using Kaplan-Meier survival curves. Formal testing for a difference in time to event will be done using a mixed-effect Cox proportional hazard model including the treatment arm and a random centre effect (ie, using a shared frailty model). The proportional hazards assumption will be verified by plotting log cumulative hazard versus log-time for the intervention and SOC group. Model validity will be further explored using plots of Cox-Snell and Martingale residuals. The treatment effect will be summarised as the hazard ratio (HR), 95% CI and associated p value.

To assess whether the treatment effect differs according subgroups defined on the basis of LVEF (see table in section 5.10.2) a subgroup analysis will be performed. The statistical model formulated for the analysis of this endpoints (binary and time to event version) will be extended to include a fixed effect for the subgroup and an interaction term between the study arm and the subgroup. The interaction term will be considered significant at the 5% level. Within each subgroup, summary statistics of the outcome by treatment arm will be presented; the OR/HR for the treatment effect and 95% confidence interval. A p-value for the interaction test will also be reported.

5.11.2 Secondary outcome 2

For each treatment arm mean, median and interquartile range for length of index hospitalization will be presented for patients who survived to hospital discharge. The effect of treatment on length of index hospitalization, for patients who survived to hospital discharge, is compared among treatment arms with a linear mixed model[8] (fixed treatment effect and random centre effect). A log transformation will be employed as the model assumptions (such normality) will likely be violated. Model assumptions will be investigated by means of diagnostic plots. The results of this model will be presented as (geometric) mean length of index hospitalization (and 95% CI) per treatment group, the geometric mean ratio (GMR), 95% CI and associated p value.

To assess whether the treatment effect differs according subgroups defined on the basis of LVEF (see table in section 5.10.2) a subgroup analysis will be performed. The statistical model formulated for the analysis of this endpoint will be extended to include a fixed effect for the subgroup and an interaction term between the study arm and the subgroup. The interaction term will be considered significant at the 5% level. Within each subgroup, summary statistics of the outcome by treatment arm will be presented; the GMR for the treatment effect and 95% confidence interval. A p-value for the interaction test will also be reported.

5.11.3 Secondary outcome 3

The QoL scores (EQ-5D total score and EQ-VAS) will be presented using line plots for each study arm to illustrate trends over time. Depending on the distribution of the data, the means and 95% CI of means or medians and inter-quartile ranges at baseline, the morning of day 4 or discharge (whatever comes first), and at 3 months of follow up will be reported. Evolution in the QoL will be investigated via a linear mixed model, with the QoI score of morning of day 4 or discharge (whatever comes first), and 3 months follow up as the longitudinal dependent variable, with a fixed treatment effect, day, the interaction of treatment by day, and baseline QoL, random centre effect and random patient effect (intercept, slope) will be used. Model assumptions will be investigated by means of diagnostic plots. Transformation will be employed when the model assumptions (such normality) are violated.
The analysis will be performed following the recommendations of the Belgian guidelines for health economic evaluation to value health care resource use will be used:
https://kce.fgov.be/nl/een-belgische-waardenset-voor-de-eq-5d-5l-%E2%80%93-hoe-gezondheidsgerelateerde-levenskwaliteit-waarderen

A subgroup analysis with subgroups defined on the basis of LVEF, will also be performed.

5.12 Analysis of safety outcomes

For the safety endpoints, all patients that were randomized and received at least 1 dose of the investigated drug or placebo will be included. For the safety analysis, patients will be grouped based on the actual study treatment received.

The safety outcomes are all binary outcomes. For descriptive purposes, the number (%) of failure and success for each safety outcome will be given per treatment arm.
For each safety outcome, the treatment effect will be evaluated by means of a generalized linear mixed model (logistic model). The statistical model will include a fixed treatment effect, and a random centre effect. The results of this model will be presented as an odds-ratio (OR), 95% confidence interval and the associated p value.

6 SIGNATURES

The undersigned have reviewed this plan and approve it as final. They find it to be consistent with the requirements of the protocol as it applies to their respective areas. They also find it to be compliant with ICH-E9 principles and, in particular, confirm that this analysis plan was developed in a completely blinded manner (i.e. without knowledge of the effect of the intervention(s) being assessed).

	Name	Date	Signature
Project Manager	[redacted]	18 JAN 2022	[redacted]
Data Manager	[redacted]	18 JAN 2022	[redacted]
Statistician	[redacted]	nd door (Authentication) (Authentication) Datum: 2022.01.19 09:45:46 +01'00'	
Chief Investigator	[redacted]	18 Jan 2022	[redacted]

7 APPENDIX A – BASELINE CHARACTERISTICS

7.1 Patient characteristics and baseline comparisons

This includes following variables:

Baseline Characteristic	Variable	Type of variable	Overall population (n= x)	High-dose loop diuretics + 500 mg acetazolamide (IV) (n=x)	High-dose loop diuretics + placebo (IV) (n=x)
Age (years)	DEM_AGE	Continue			
Female sex (N,%)	DEM_SEX	Binary			
Caucasian (N,%)	DEM_RACE	Binary			
LVEF (N,%) LVEF ≤ 40% (N,%) LVEF > 40%	MH_LVEFRES	Continue Maar in tabel nadien binair			
Etiology of HF Ischemic (N,%) CABG (N, %) PTCA (N, %) Thrombolysis (N, %) **Non-ichemic (N,%)** **Valvular (N, %)** Idiopathic (N,%) Hypertensive (N,%) Toxic (N,%)	MH_HFOPT, MH_HF_ISOPT, MH_HF_NISOPT	Nominal			
NYHA class at enrolment Class II (N,%) Class III (N,%) Class IV (N,%)	MH_NYHAOPT	Ordinal			
Comorbid conditions Atrial fibrillation or atrial flutter (N,%) Stroke (N,%) Diabetes (N,%) Hypertension (N,%) Valve surgery (N,%) PAD (N,%) COPD (N,%)	MH_AFYN, MH_STROKEYN, MH_DIABYN, MH_HYPERTOPT, MH_VALVESURYN, MH_PADYN, MH_COPDYN	Nominal			
CRT/ICD at enrolment None (N,%) pacemaker – single chamber (N,%) pacemaker-dual chamber (N,%) ICD-primary prevention (N,%) ICD-secondary prevention (N,%) CRT-D (N,%) CRT-P (N,%)	MH_CADOPT,	Nominal			
Smoking status at enrolment Never (N,%)	MH_SMOKEOPT	Nominal			

Baseline Characteristic	Variable	Type of variable	Overall population (n= x)	High-dose loop diuretics + 500 mg acetazolamide (IV) (n=x)	High-dose loop diuretics + placebo (IV) (n=x)
Former (N,%) Current (N,%)					
Malignancy requiring chemo/radiotherapy No (N,%) Yes-past (N,%) Yes-current (N,%) Yes-past and current (N,%)	MH_TUMORYN	Nominal			
Weight (kg)	VS_WGHT_D0	Continue			
Pulse (beats/min)	VS_PULSE_D0	Continue			
Blood pressure (systolic) (mmHg)	VS_BPS_D0	Continue			
Blood pressure (diastolic) (mmHg)	VS_BPD_D0	Continue			
Mean Arterial Pressure (MAP)	VS_MAP_D0	Continue			
Congestion score	VOA_SCORE_D0	Ordinal			
Laboratory values Hematology (g/dL) Hematocrit (%) Sodium (mmol/L) Potassium (mmol/L) Chloride (mmol/L) Serum osmolality (mOsm/kg) **Serum urea (mg/dL)** **eGFR (mL/min/1,73m2)** Total protein (g/L) Albumin (g/L) Fe (µg/dL) Ferritin (µg/dL) Transferrin saturation (TSAT) (%) LDH (U/L) Troponin (ng/L) NTproBNP/BNP	LABB_HGB_D0, LABB_HT_D0, LABB_NA_D0, LABB_K_D0, LABB_CL_D0, LABB_OSM_D0, LABB_UREA_D0, LABB_GFR_D0, LABB_PROT_D0, LABB_ALB_D0, LABB_FE_D0, LABB_FERR_D0, LABB_TSAT_D0, LABB_LDH_D0, LABB_TROP_D0, LABB_BNPOPT_D0, LABB_BNP_D0	Continue			
Diuretics Furosemide (mg) Thiazide (N,%) MRA (N,%)	Report parent 'Screening': DIU_TYPE + DIU DOSIS Report parent 'Screening': DIU_TYPE	Continue Nominal			
Neurohumoral blockers ACE (N,%) Beta-blocker (N,%) ARB (N,%) Entresto (N,%)	Report parent 'Screening': NH_TYPE	Nominal			
Other concomitant medication Ivabradine (N,%) **Hydralazine (N,%)**	CONMED_OTHER_D0	Nominal			

Baseline Characteristic	Variable	Type of variable	Overall population (n= x)	High-dose loop diuretics + 500 mg acetazolamide (IV) (n=x)	High-dose loop diuretics + placebo (IV) (n=x)
Molsidomine (N,%) **Isosorbide dinitrate, PO (N,%)** Digoxin (N,%) Aspirin(N,%) NOAC/DOAC VKA (N,%) P2Y12 (N,%) Statin (N,%) XO-inhibitor (N,%) Amiodarone (N,%) Flecainide (N,%) IV inotropes (N,%) IV vasodilators (N,%) Other anti-hypertensive drugs (N,%) **Iron, PO (N,%)** **Iron, IV (N,%)** SGLT2-i (N,%) None (N,%)					
EQ-5D-5L (N, %) EQ-5D-5L score	EQ5D_D0 Report parent 'Screening': EQ5D_MOBOPT, EQ5D_SCOPT, EQ5D_ACTOPT, EQ5D_PAINOPT, EQ5D_ANXOPT	Nominal Continue			
EQ-5D-5L (VAS) (N,%) (0-100)	EQ5D_SCORESL	Continue			

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Final Statistical Analysis Plan

Version - 2.0

17th of March 2022

A multi-centre, randomized, double-blind, phase IV clinical trial on the diuretic effects of Acetazolamide (Diamox ®) in patients with Decompensated heart failure and Volume OveRload

STATISTICAL ANALYSIS PLAN

Version number: version 2.0

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1 DOCUMENT HISTORY

Version number	Version Date	Author	Reason for change
Version 1.0	17 January 2022	ADVOR Scientific Study Team [redacted] (Statistician)	First version
Version 2.0	17 March 2022	ADVOR Scientific Study Team [redacted] (Statistician)	Appendix A Baseline Characteristics: The Baseline Characteristics Iron PO and Iron IV mentioned in the Appendix A have been removed because these data will not be collected during the study.

2 TABLE OF CONTENTS

3 LIST OF ABBREVIATIONS

The following table presents the abbreviations and acronyms used in the Statistical Analysis Plan:

CI	Confidence Interval
EAC	Endpoint Adjudication Committee
eCRF	electronic Case Report Form
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
EQ-5D	EuroQol five dimension (questionnaire)
HF	Heart Failure
HFpEF	Heart Failure with preserved Ejection Fraction
HFrEF	Heart Failure with reduced Ejection Fraction
HR	Hazard Ratio
IMP	Investigational Medicinal Product
IRT	Interactive Response Technology
ITT	Intention To Treat
ITTAS	Intention to treat analysis set
IV	Intravenous
KCl	Potassium chlorate
LVEF	Left Ventricular Ejection Fraction
$MgSo_4$	Magnesium sulfate
$NaHCO_3$	Sodium bicarbonate
OR	Odds ratio
Qol	Quality of Life
SAS	Statistical Analysis System
SD	Source Documents
SGLT2-i	Sodium-glucose Cotransport 2 Inhibitor
SOC	Standard Of Care
SUSAR	Suspected Unexpected Serious Adverse Reactions

4 STUDY SYNOPSIS

The ADVOR study is a phase IV randomized, multicentre, double-blind study, comparing monotherapy with high-dose loop diuretics (standard of care - SOC) versus combination therapy with acetazolamide and high-dose loop diuretics in patients with decompensated heart failure (HF) and clinical signs of volume overload. For more details regarding the intervention see section 4.3

The study consists of 3 phases:

- screening phase: starting from identifying a study subject prior to / during hospitalization until the first dose of study medication will be given
- treatment phase: starting from the first dose of study medication administration until the morning of day 4 or earlier in case of successful decongestion prior to day 4.
- follow-up phase: starting when the treatment phase ends until 3 months after the study start dose.

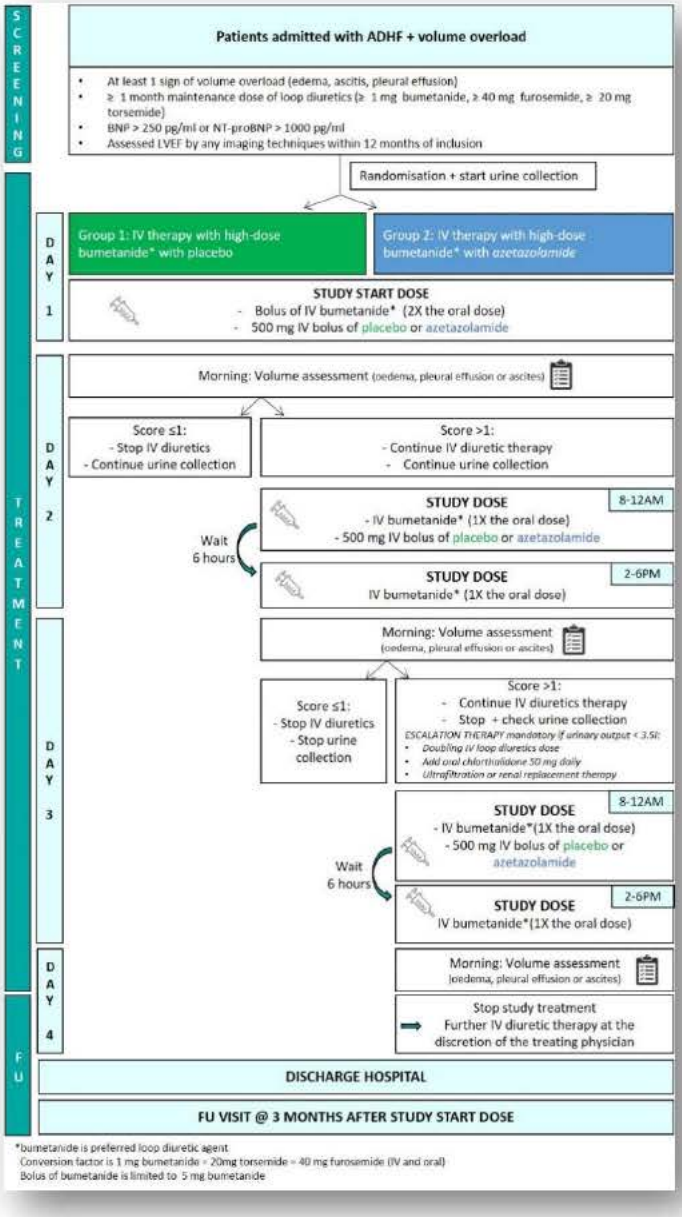

Figure 1. Trial Flow Chart

4.1 Study objectives and endpoints

4.1.1 Rationale

Guidelines from international cardiac societies lack high-quality data on the optimal dosing, timing and method of delivery of diuretic agents. In the Diuretic Optimization Strategies Evaluation (DOSE) trial[1], which is the only randomized clinical trial on diuretic therapy for decompensated HF patients, no differences in patients' global assessment of symptoms or change in renal function were observed when loop diuretics were administered by bolus as compared with continuous infusion or at high versus low dose during a hospitalization for decompensated HF. Of note, only a minority of patients (15%) were adequately decongested after 72 h in the DOSE trial, Acetazolamide is a largely forgotten diuretic, but can potentially boost diuretic response. Acetazolamide targets the proximal tubule of the kidney, where the majority of sodium is reabsorbed. The ADVOR trial will examine if an improved application of existing decongestive therapies by adding acetazolamide, will result in a better outcome for patients and society.

4.1.2 Primary Objective and outcome measure

Primary Objective	Primary Endpoint
To investigate if combination therapy with acetazolamide improves loop diuretic efficacy to increase diuresis in decompensated HF patients with volume overload, allowing for a better/faster decongestion and a lower total dose of loop diuretics.	The proportion of patients achieving decongestion on the morning of day 4 without the need for escalating therapy on the morning of day 3 (treatment success).

<u>Definition of primary endpoint</u>

Congestion is assessed using the ***volume assessment score*** (also called the congestion score see figure 2). Freedom from volume-overload will be defined as not more than trace oedema, no residual pleural effusion, and no residual ascites (Figure 2).
So a (sum) score ≤ 1 indicates that decongestion is achieved (being dry), a score > 1 is considered to be volume overloaded (decongestion not achieved).

The primary endpoint is a binary endpoint, with a success defined in case of:

- decongestion (volume assessment score ≤ 1) on the morning of day 2
- or decongestion (volume assessment score ≤ 1) on the morning of day 3
- or decongestion (volume assessment score ≤ 1) on the morning of day 4 **without** the need for escalating diuretic strategy on the morning of day 3, which is needed if total urinary output on the morning of day 3 < 3.5l (sum of urinary output from the start of IMP until the morning of day 3).

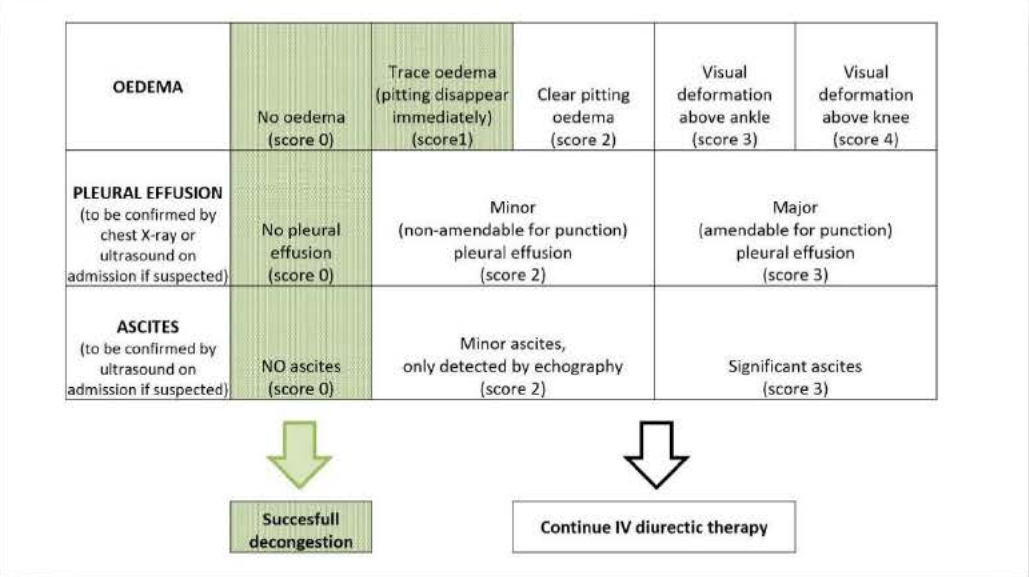

Figure 2. Clinical Congestion Assessment

The SOC therapy results in ~15% effective decongestion[1]. It is estimated that the combination therapy should have a success rate of 25%, which represents a clear meaningful absolute benefit of 10% more patients with appropriate decongestion after 72h.

4.1.3 Secondary objectives and endpoints

Secondary Objectives	Secondary Endpoints
To investigate if combination therapy with acetazolamide: - leads to improved clinical outcome in decompensated HF (less HF readmissions, lower all-cause mortality) - shortens the length of stay in patients with decompensated HF, which is expected to reduce health care expenditure - leads to improved quality of life	 - Combined endpoint of all-cause mortality and HF readmission during 3 months of follow-up - Length of index hospital admission - Change in EuroQoL

Definition of secondary endpoints

- The combined endpoint of all-cause mortality and HF readmission during 3 months of follow-up is a binary endpoint. The time to the combined endpoint will be calculated as the time between start of study medication (day 1) and the date of (first) HF readmission or mortality (whichever comes first). *HF readmission* is defined as either a hospital admission or an unscheduled contact at the emergency department **and** when

treatment with intravenous loop diuretics is initiated because of worsening HF.

- Length of index hospital admission (calculated in days) is obtained as the date of discharge from the hospital minus the date of screening +1.

- EuroQol is a five dimension patient-reported QoL questionnaire (EQ-5D-5L). The EQ-5D-5L health states, defined by the EQ-5D-5L descriptive system, are converted into a single index value (https://kce.fgov.be/nl/een-belgische-waardenset-voor-de-eq-5d-5l-%E2%80%93-hoe-gezondheidsgerelateerde-levenskwaliteit-waarderen). The VAS assessment of the EQ-5D will also be used. The EQ VAS records the patient's self-rated health on a vertical visual analogue scale.

  The index values and EQ-VAS at baseline, the morning of day 4 or discharge (whatever comes first), and at 3 months of follow-up will be used for the secondary objective.

  During the study the baseline EQ-5D-5L questionnaire has been completed as soon as possible after screening up to day 1.

4.1.4 Safety objectives and endpoints

Safety Objective	Safety Endpoints
To evaluate the safety of the combination therapy with acetazolamide in this patient population.	- Severe metabolic acidosis at any time during study treatment - Doubling or more (≥2x) of baseline serum Creatinine, or ≥ 50% decrease in baseline eGFR at any time during study treatment - Hypokalaemia at any time during study treatment - Hypotension at any time during study treatment

Definition of Safety endpoints

- Severe metabolic acidosis: a value of bicarbonate <12 mmol/L at any time during study treatment (day 1- day 4)
- Worsening of eGFR or the need for renal replacement therapy during the index hospitalization. eGFR is obtained via the CKD-EPI formula. The eGFR values will be calculated (in mL/min/1.73 m2) from the local laboratory creatinine measurements using the CKD-EPI formula (Levey at al 2009)[2].
- Hypokalaemia: a value of potassium ≤ 3,0 mmol/L at any time during study treatment (day 1- day 4)
- Hypotension: a value of systolic blood pressure < 85 mmHg at any time during study treatment (day 1- day 4)

Baseline laboratory value

For all laboratory variables, the baseline value is defined as the last value prior to the first dose of study drug.

4.1.5 Exploratory objectives and endpoints

Exploratory Objectives	Exploratory endpoints
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on body weight.	Change in body weight.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will reduce the incidence of all cause rehospitalisation's.	All cause rehospitalisation during first 3 months after study start dose.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on total urinary volume and natriuresis.	Total urinary volume and natriuresis starting from first intravenous (IV) diuretic administration at randomization until the morning of day 3.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on plasma natriuretic peptides.	Change in plasma natriuretic peptides change from screening until day 4 or at discharge (whatever comes first) and at 3 months follow up visit.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on the total dose of IV loop diuretics used during first 4 days.	Total dose of IV loop diuretics used during first 4 days.
Effect of decongestion on uptitration of neurohormonal blockers	Changes in doses of neurohumoral blockers.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will reduce the need for renal replacement therapy.	Need for renal replacement therapy or ultrafiltration during first 3 months after study start dose.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will reduce the incidence of hyponatremia during treatment phase.	Hyponatremia during treatment phase.
To evaluate if liver dysfunction at screening has an effect on the treatment effect on the primary endpoint.	Liver dysfunction at screening and primary endpoint.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on plasma volume changes during treatment phase.	Change in plasma volume during treatment phase (assessed by albumin and hematocrit)

Exploratory Objectives	Exploratory endpoints
To evaluate if iron deficiency at screening has an effect on the treatment effect on the primary endpoint	Iron deficiency at screening and primary endpoint
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on congestion score during the study.	Changes in congestion score (total and each subscore) during treatment phase and 3 months of FU.
To determine whether acetazolamide compared with placebo in combination with loop diuretics will have an effect on EQ-VAS score during the study.	Changes in EQ-VAS score during treatment phase and 3 months of FU.

4.2 Patient population

4.2.1 Inclusion Criteria

- Signed written informed consent must be obtained before any study assessment is performed
- Male or female patients of 18 years of age or older
- An elective or emergency hospital admission with clinical diagnosis of decompensated HF with at least one clinical sign of volume overload (e.g. oedema (score 2 or more), ascites confirmed by echography or pleural effusion confirmed by chest X-ray or echography)
- Maintenance therapy with oral loop diuretics at a dose of at least 1 mg bumetanide or an equivalent dose for at least 1 month before hospital admission (Conversion: 1 mg bumetanide = 40 mg furosemide = 20 mg torsemide)
- Plasma NTproBNP levels >1000 ng/L or BNP levels >250 ng/L at the time of screening
- Assessed LVEF by any imaging technique; i.e. echocardiography, catheterization, nuclear scan or magnetic resonance imaging within 12 months of inclusion

4.2.2 Exclusion Criteria

- Concurrent diagnosis of an acute coronary syndrome defined as typical chest pain in addition to a troponin rise above the 99th percentile and electrocardiographic changes suggestive of cardiac ischemia
- History of congenital heart disease requiring surgical correction
- History of a cardiac transplantation and/or ventricular assist device
- Systolic blood pressure <90 mmHg or mean arterial pressure <65 mmHg at screening
- Expected use of intravenous inotropes, vasopressors or nitroprusside during the study. The use of nitrates and/or molsidomine is allowed at the discretion of the treating physician.
- Estimated glomerular filtration rate <20 mL/min/1.73m² at screening
- Use of renal replacement therapy or ultrafiltration at any time before study inclusion
- Treatment with intravenous loop diuretics > 2 mg bumetanide or an equivalence of another loop diuretic during the index hospitalization and prior to randomization
- Treatment with acetazolamide within 1 month prior to randomization
- Exposure to nephrotoxic agents (i.e. contrast dye) anticipated within the next 3 days
- Use of any non-protocol defined diuretic agent with the exception of mineralocorticoid

receptor antagonists during the treatment phase of the study. Thiazides, metolazone, indapamide and amiloride should be stopped upon study inclusion. If patient is taking a combination drug including a thiazide-type diuretic, the thiazide-type diuretic should be stopped.
- Current use of sodium-glucose transporter-2 inhibitors
- Subjects who are pregnant or breastfeeding
- Subjects with urinary incontinence who are not willing to receive a bladder catheter.

4.3 Intervention

At the moment of randomization, oral loop diuretics are stopped and the patient receives an IV bolus of loop diuretics at a dose equal to the double of his oral daily maintenance dose with a maximal dose of 5 mg bumetanide (=200 mg furosemide). Bumetanide is the preferred loop diuretic agent to be used in this trial. Between administering the start dose and next treatment dose a minimum of 6 hours is required. During the remaining part of the treatment phase, the patient will continue to receive 2 IV treatment doses every day provided that the treating physician has concluded during the morning rounds that the patient is still volume overloaded (see Figures 2, volume assessment score > 1). The IV loop diuretic dose will be the orally daily maintenance dose, administered between 8:00 and 12:00 am, together with an intravenous bolus of 500 mg of acetazolamide or placebo. The second dose of IV loop diuretics, again dosing equal to the orally daily maintenance dose, will be given 6 hours after the morning dose. Any patient with more than trace oedema, residual pleural effusion or residual ascites would be considered to be still volume overloaded (see Figures 2). Residual pleural effusion and/or ascites should always be confirmed by chest X-ray or echography. If pleural effusion/ascites is used as an inclusion criteria, chest X-ray and/or echography should be repeated until decongestion has been achieved (volume assessment score ≤ 1). If not present at inclusion, new evidence of pleural effusion and/or ascites may arise during the treatment phase, but if scored it should be confirmed by a chest X-ray or echography. If the patient is not volume overloaded anymore, the intravenous administration of study medication should be stopped. Once decongestion is achieved (volume assessment score ≤ 1) during the treatment phase, no volume assessment should be performed anymore the following morning.

4.3.1 STOP TREATMENT

The treating physician is allowed (but not obliged) to stop the study treatment, in case of persistent volume-overload in following cases:
- symptomatic hypotension with a systolic blood pressure <100 mmHg
- asymptomatic hypotension with a systolic blood pressure <90 mmHg
- an increase of serum Creatinine levels x 1.5 of the serum Creatinine level compared to admission value
- occurrence of metabolic acidosis (pH < 7.2)

The discontinuation of the study treatment will be seen as a failure for the primary endpoint (decongestion not achieved). However, if any of these events occur when the patient is judged to be euvolemic, the study treatment is stopped and the primary endpoint is considered to be successful (decongestion achieved).

Freedom from volume-overload (i.e. successful decongestion) on the morning of day 4 will be defined as not more than trace oedema, no residual pleural effusion, and no residual ascites (Figure 2).

4.3.2 Treatment DOSE ADJUSTMENTS in case of an inappropriate diuretic response

If the total urinary output on the morning of day 3 is < 3500 mL and the patient is still volume overloaded, an escalation of decongestive treatment is mandatory. One of the outlined three options can be chosen at the discretion of the treating physician.
Escalation therapy options:

- doubling of the IV dose of the loop diuretics
- add oral chlorthalidone 50 mg once daily
- ultrafiltration or renal replacement therapy might be considered

The decision to proceed with escalation therapy will be collected in the case report as the need for escalation implies a failure for the primary endpoint.

4.3.3 Background therapy

24h oral **intake of fluid and sodium will be restricted to 1500 mL and 1.5 g,** respectively. It is recommended that all patients receive the same maintenance infusion with 500 mL glucose 5% and 3g $MgSO_4$ administered over 24h time interval, until complete decongestion or end of the study treatment phase. All non-protocol fluids administered (including those for administration of intravenous medication) should be limited.

In case of **serum potassium levels <4 mmol/L**, 40 mmol of KCl is added to the maintenance infusion. Oral potassium supplements may be used at the discretion of the treating physician, but their use will be prospectively registered.

In case of **metabolic acidosis with serum bicarbonate levels <20 mmol/L**, it is recommended to administer intravenously 100 ml of $NaHCO_3$ 8.4%.

Treatment with **neurohumoral blockers** (e.g. renin-angiotensin system blockers, sacubitril/valsartan, beta-blockers, and mineralocorticoid receptor antagonists) may be continued at the same or lower dosage at the discretion of the treating physician, until the end of the treatment phase (max 4 days) or until complete decongestion is achieved, whatever comes first. Dose increases for any of these medications are not allowed during the screening and treatment phase with the exception of mineralocorticoid receptor antagonists in case of hypokalemia despite intravenous potassium supplement. In addition, starting an SGLT2 inhibitor and a switch from renin-angiotensin system blockers to sacubitril/valsartan is not allowed during the screening and treatment phase, but might be pursued after decongestion is achieved. After decongestion, it is strongly recommended to up-titrate doses of neurohumoral blockers according to the guidelines in the HFrEF patients. Dosages of neurohumoral blockers are collected at screening, at discharge and at three months follow-up.

4.3.4 Randomisation and blinding

The ADVOR study is a randomized double blind clinical trial with 2 treatment arms; therapy with high-dose loop diuretics and placebo vs therapy with high-dose loop diuretics and acetazolamide. An automated web-based system is used to randomly assign patients in a 1:1 ratio with variable blocks sizes, stratified for LVEF according to study centre. To ensure an equal proportion of HFpEF

versus HFrEF patients in both study arms, the population will be stratified at inclusion according to LVEF ≤40% versus >40%. Permuted block randomization according to centre and LVEF stratum will be used to achieve this.

Patient, site personnel, sponsor personnel and data analysts will remain blinded to the identity of the treatment from the time of randomization until database lock. Though we do not foresee serious adverse events related to the study drug, the study code should only be broken for valid medical or safety reasons e.g. in the case of a severe adverse event where it is necessary for the investigator or treating health care professional to know which treatment the patient is receiving before the participant can be treated.
Following rules apply for unblinding;

- Rapid unblinding of a patient can be performed by a physician of the study team. Detailed information concerning the unblinding procedures is provided in the Manual of Operations.
- On receipt of the treatment allocation details, the PI or treating health care professional will continue to deal with the participant's medical emergency as appropriate.
- The PI/Investigation team documents the breaking of the code and the reasons for doing so on the medical notes and eCRF. It will also be documented at the end of the trial in any final study report and/or statistical report.

Unblinded data are to be kept strictly confidential until the time of unblinding of the trial and will not be accessible by anyone else involved in the trial with the following exceptions: (1) the Project Manager of the company responsible for the labelling and packaging of the IMP, (2) the IRT system programmers who work on the randomization and drug management system; and (3) the data manager who prepares reports required for regulatory reporting (suspected unexpected serious adverse reactions [SUSAR] reporting). These individuals will not be involved in the day-to-day running of the study.

4.4 Endpoint adjudication

Because the primary endpoint requires physical examination of the blinded study physician, adjudication of the primary event is not possible. However, all admission labelled as HF readmission, all-cause mortality and the need for renal replacement therapy will be adjudicated by the Endpoint Adjudication Committee (EAC)

For the endpoints HF readmission, all-cause mortality and the need for renal replacement therapy a complete endpoint package will need to be send to the sponsor. This package should include anonymized source documents (SDs) relevant to the endpoint reported (e.g. discharge letter, emergency room notes, etc). The SD should include at least the reason for admission/death and the received treatment for the event (if applicable). This package will be assessed by the Endpoint adjudication members of the Endpoint adjudication committee.

In case of discrepancy between the evaluation of the EAC members regarding the same event, the Trial Data Manager will contact the EAC members to inform them on the discrepancies found so the both of them can come to a final decision. The discrepancies and the final decision will be documented.

4.5 Sample Size

The ADVOR study is powered for primary endpoint which is the most relevant endpoint with respect to the study hypothesis and reliable data from large randomized clinical trials are available to make a formal power calculation.
In the DOSE trial, which recruited a similar study population as targeted in the ADVOR study, successful decongestion with a similar definition was approximately 11% vs 18% after 72 h in the low vs high-dose loop diuretics arm. The high-dose loop diuretics arm of the DOSE trial is quite comparable to the standard of care group in the ADVOR study as the loop diuretic dose used in the latter is only slightly lower (2x instead of 2.5x the oral maintenance outpatient dose) and non-loop diuretics, which were infrequently used in the DOSE trial, are not allowed. Because of these slight differences, 15% is chosen as an estimate for occurrence of the primary endpoint in the monotherapy with high-dose loop diuretics (SOC) group.

No reliable data are available from large clinical trials to estimate occurrence of the primary endpoint in the acetazolamide arm of the ADVOR study. Therefore, after thorough discussion with the advisory board a success rate of 25% was chosen, which represents a clear meaningful benefit of 10% more patients with appropriate decongestion after 72 h. Using both estimates, considering a type I error rate α=0.05 and type II error rate β=0.20 (yielding a statistical power of 80%), the targeted sample size for the ADVOR study is calculated at n = 494. A 5% drop out has been calculated in order to estimate the total number of 519 patients to be enrolled in the study.

5 STATISTICAL ANALYSIS

5.1 General principles

This statistical analysis plan dated 17 January 2022 is based on the statistical information documented in the study protocol version 2.0 dated 03 January 2019 (see section 10), the methods paper published in the European Journal of Heart Failure (2018)[3] and guidance by the European Medicines Agency (EMA)[4] and the Food and Drug Administration[5] regarding the management of clinical trials during COVID-19 pandemic (2021).

The scope of this statistical analysis plan is to outline the statistical tools used for the primary and secondary objectives of the ADVOR study following the procedures documented in the study protocol version 2.0 dated 03 January 2019 in all participating centres.

Prior to the statistical analysis the collected study data will be cleaned by the data management team of the Clinical Trial Unit at the Ziekenhuis Oost-Limburg (ZOL-CTU). The cleaning will be performed according to the Data Management Plan agreed for the ADVOR trial.

All statistical hypothesis are 2-sided and a 5% significance level will be used. A correction for multiple testing will not be implemented.

Trial results will be reported according to the CONSORT statement on reporting randomized controlled trials.
For the analysis of the primary outcome, each secondary and exploratory outcome the following information will be presented:

- the number of patients included in each analysis, by treatment arm
- a summary statistic of the outcome (e.g. number (%), mean (SD)), by treatment arm

- the estimated treatment effect
- a 95% confidence interval for the estimated treatment effect
- A two-sided p-value for all analyses, a significance level of 5% will be used.

All statistical analysis will be done with Statistical Analysis System (SAS) for Windows, SAS 9.4.

5.2 Interim analyses

No interim analysis is planned regarding the ADVOR study.

5.3 Multiplicity adjustment

There will be no correction for multiplicity of testing as there is only one primary endpoint. All other endpoints and subgroup analyses will be considered as exploratory.

5.4 Blind review

Once the database is cleaned the dataset will be locked and transferred to an independent academic statistical center (DSI/CenStat - University Hasselt) for efficacy and safety analysis according to the statistical analysis plan

5.5 Data sets to be analysed

For the *primary endpoint* the statistical analysis will be based on an intention-to-treat analysis set (ITTAS) including all randomized patients. Patients will be analysed according to the treatment group they were allocated to according to the electronic randomization system (irrespective of the actual treatment received).

For the *secondary endpoints* the statistical analyses will be based on the same data set as will be used for the primary endpoint analysis.

For the *safety endpoints*, all patients that were randomized and received at least 1 dose of the investigated drug or placebo will be included (safety population). For the safety analysis, patients will be grouped based on the actual study treatment received.

5.6 Subject disposition

After closing of the database, a CONSORT diagram will be produced for transparent status of the subject reporting.

5.7 Violations and deviations

The important protocol deviations listed below will be summarised by randomized treatment group

- Patients who were randomized but did not meet inclusion and exclusion criteria
- Patients who received study treatment with a wrong treatment vial number at any time during the study
- Patients who received only acetazolamide/placebo but no loop diuretics on day 1, 2 or 3

As the primary analysis is an intention-to-treat analysis, protocol deviation will not imply exclusion from the primary analysis.

5.8 Concomitant therapies

The frequency of baseline medication will be shown for the intention-to-treat analysis set (ITTAS) per treatment group, counts and % will be presented grouping the baseline medication per drug classes.

5.9 Baseline characteristics

To describe the study population, characteristics of all the patients in the ITTAS population will be presented. Numbers (%) or means (SD) and medians (IQR) will be given for each treatment group as appropriate.

5.10 Analysis of the primary outcome

5.10.1 Main analysis

The primary statistical analysis in ADVOR will be an intention-to-treat (see section 5.5).

The primary endpoint is a binary outcome with a success defined as decongestion achieved on the morning of day 4 (or earlier) without the need for escalating diuretic strategy (doubling loop diuretic dose, addition of chlorthalidone, or ultrafiltration) during IV diuretic therapy on morning of day 3.

For descriptive purposes, the number (%) of failure and success for the primary outcome will be given per treatment arm.

The treatment effect for this primary outcome will be evaluated by means of a generalized linear mixed model (logistic model[67]). The statistical model will include a fixed treatment effect, and a random centre effect. The results of this model will be presented as an odds-ratio (OR), 95% confidence interval and the associated p value.

5.10.2 Subgroup analysis

For exploratory purposes subgroup analysis for the primary and secondary endpoints will be conducted, to assess whether the treatment effect differs according subgroups. The table below gives an overview of the subgroups that will be investigated and how they will be created:

Characteristic	Sub group
Age (years)	≤ median, > median
Sex	Male, Female
LVEF	≤ 40%, > 40%
Baseline NTproBNP (ng/L)	≤ median, > median

Characteristic	Sub group
Baseline eGFR (mL/min/1,73m2)	≤ median, > median
Etiology of HF	Ischemic, non-ischemic
Home dose loop diuretic (mg)	≤ median, > median
Baseline Congestion score	≤ median, > median
Hyponatremia (mmol/L)	≤ 130, > 130
Thiazide	Yes, No
Atrial Fibrillation	Yes, No

The statistical model formulated for the analysis of the primary endpoints will be extended to include a fixed effect for the subgroup and an interaction term between the study arm and the subgroup. The interaction term allows for a subgroup-specific treatment effect. The interaction term will be considered significant at the 5% level.
Within each subgroup, summary statistics of the primary outcome by treatment arm will be presented; the OR for the treatment effect and 95% confidence interval. A p-value for the interaction test will also be reported. A forest plot, of the ORs (and 95%CI) per subgroup, will be used to summarize the results visually.

5.10.3 Treatment of missing data

The proposed statistical model assumes the missing data mechanism to be missing at random. If the level of missing data for the primary outcome in the ITTAS populations exceeds 5%, a sensitivity analysis to assess the robustness of the analysis of the primary outcome by means of multiple imputation technique will be performed for the primary endpoint.

5.10.4 Other sensitivity analyses

The COVID-19 pandemic has impacted clinical development and ongoing clinical trials. Public Health measures to control the virus may impact the ability to collect data. Additionally, the number of HF admissions were reduced by 40% in Europe between March and June 2020. The change in patient behaviours and characteristics might lead to for example less hospitalizations, more advanced HF presentations, less hospital admission duration, and less protocol adherence which all might impact study results.

Several mitigation strategies were discussed in the steering committee meeting. (a) Extra efforts to capture potential reasons for missing data will be employed. (b) Due to slower enrolment extension of the trial was considered until complete enrolment of the predefined sample size. The trial completion was achieved before the expiration date of the Investigational Medicinal Product (IMP).

Because of the aforementioned potential differences in patient characteristics, a sensitivity analysis for the primary endpoint will be performed.

The treatment effect for the primary endpoint is evaluated by means of a generalized linear mixed model, including a fixed treatment effect and random centre effect. As a sensitivity analysis, the model will be extended by including a fixed effect (binary) indicating if the endpoint was assessed before or after the first identified COVID-19 case in Belgium dated 03 February 2020 and the interaction term between this variable and treatment.

5.11 Analysis of secondary outcomes

All statistical models implemented for the analysis of the secondary outcomes are mixed-effects models, including a fixed treatment effect and random centre effect. For the outcomes measured at multiple time points also random patient effects (intercept, slope) are included.

5.11.1 Secondary outcome 1

The combined endpoint of all-cause mortality and HF readmission during 3 months of follow-up, will be analysed as a binary and as a time-to-event endpoint.

- For descriptive purposes, the number (%) of failure and success for the combined outcome of all-cause mortality and HF readmission during 3 months of follow-up will be given per treatment arm. To investigate the treatment effect on the occurrence of the combined endpoint, a generalized linear mixed model for a binary outcome will be employed. The model will incorporate a fixed treatment effect and random centre effect. The results of this model will be presented as odds-ratio, 95% confidence interval and the associated p value.

  If the treatment effect on the composite endpoint of 'all-cause mortality and HF readmission' turns out to be statistically significant, both components will be evaluated separately in a hierarchical fashion with HF readmissions first and all-cause mortality second. For this analysis, HF readmission will include patients dying from any cause during the 3 months of FU. The treatment effect, on the components, will be investigated by the same statistical model as used for the combined endpoint.

- To investigate the treatment effect on the time until combined endpoint of all-cause mortality and HF readmission during 3 months of follow-up a survival analysis will be performed. Time to event is defined as the time between day 1 and the date of (first) HF readmission or death if there was no HF readmission. The analysis will be censored at 3 months of follow-up or at the time when the patient was last known to be alive (for patients that withdraw or are lost to follow up), whichever occurs earlier. Survival times will be summarised descriptively using Kaplan-Meier survival curves. Formal testing for a difference in time to event will be done using a mixed-effect Cox proportional hazard model including the treatment arm and a random centre effect (ie, using a shared frailty model). The proportional hazards assumption will be verified by plotting log cumulative hazard versus log-time for the intervention and SOC group. Model validity will be further explored using plots of Cox-Snell and Martingale residuals. The treatment effect will be summarised as the hazard ratio (HR), 95% CI and associated p value.

To assess whether the treatment effect differs according subgroups defined on the basis of LVEF (see table in section 5.10.2) a subgroup analysis will be performed. The statistical model formulated for the analysis of this endpoints (binary and time to event version) will be extended to include a fixed effect for the subgroup and an interaction term between the study arm and the subgroup. The interaction term will be considered significant at the 5% level. Within each subgroup, summary statistics of the outcome by treatment arm will be presented; the OR/HR for the treatment effect and 95% confidence interval. A p-value for the interaction test will also be reported.

5.11.2 Secondary outcome 2

For each treatment arm mean, median and interquartile range for length of index hospitalization will be presented for patients who survived to hospital discharge. The effect of treatment on length of index hospitalization, for patients who survived to hospital discharge, is compared among treatment arms with a linear mixed model[8] (fixed treatment effect and random centre effect). A log transformation will be employed as the model assumptions (such normality) will likely be violated. Model assumptions will be investigated by means of diagnostic plots. The results of this model will be presented as (geometric) mean length of index hospitalization (and 95% CI) per treatment group, the geometric mean ratio (GMR), 95% CI and associated p value.

To assess whether the treatment effect differs according subgroups defined on the basis of LVEF (see table in section 5.10.2) a subgroup analysis will be performed. The statistical model formulated for the analysis of this endpoint will be extended to include a fixed effect for the subgroup and an interaction term between the study arm and the subgroup. The interaction term will be considered significant at the 5% level. Within each subgroup, summary statistics of the outcome by treatment arm will be presented; the GMR for the treatment effect and 95% confidence interval. A p-value for the interaction test will also be reported.

5.11.3 Secondary outcome 3

The QoL scores (EQ-5D total score and EQ-VAS) will be presented using line plots for each study arm to illustrate trends over time. Depending on the distribution of the data, the means and 95% CI of means or medians and inter-quartile ranges at baseline, the morning of day 4 or discharge (whatever comes first), and at 3 months of follow up will be reported. Evolution in the QoL will be investigated via a linear mixed model, with the QoI score of morning of day 4 or discharge (whatever comes first), and 3 months follow up as the longitudinal dependent variable, with a fixed treatment effect, day, the interaction of treatment by day, and baseline QoL, random centre effect and random patient effect (intercept, slope) will be used. Model assumptions will be investigated by means of diagnostic plots. Transformation will be employed when the model assumptions (such normality) are violated.
The analysis will be performed following the recommendations of the Belgian guidelines for health economic evaluation to value health care resource use will be used:
https://kce.fgov.be/nl/een-belgische-waardenset-voor-de-eq-5d-5l-%E2%80%93-hoe-gezondheidsgerelateerde-levenskwaliteit-waarderen

A subgroup analysis with subgroups defined on the basis of LVEF, will also be performed.

5.12 Analysis of safety outcomes

For the safety endpoints, all patients that were randomized and received at least 1 dose of the investigated drug or placebo will be included. For the safety analysis, patients will be grouped based on the actual study treatment received.

The safety outcomes are all binary outcomes. For descriptive purposes, the number (%) of failure and success for each safety outcome will be given per treatment arm.
For each safety outcome, the treatment effect will be evaluated by means of a generalized linear mixed model (logistic model). The statistical model will include a fixed treatment effect, and a random centre effect. The results of this model will be presented as an odds-ratio (OR), 95% confidence interval and the associated p value.

6 SIGNATURES

The undersigned have reviewed this plan and approve it as final. They find it to be consistent with the requirements of the protocol as it applies to their respective areas. They also find it to be compliant with ICH-E9 principles and, in particular, confirm that this analysis plan was developed in a completely blinded manner (i.e. without knowledge of the effect of the intervention(s) being assessed).

	Name	Date	Signature
Project Manager	[redacted]	21 MAR 2022	[redacted]
Data Manager	[redacted]	22 MAR 2022	[redacted]
Statistician			[redacted] (Authentication) Datum: 2022.03.21 08:54:40 +01'00'
Chief Investigator	[redacted]	22 Mar 2022	[redacted]

7 APPENDIX A – BASELINE CHARACTERISTICS

7.1 Patient characteristics and baseline comparisons

This includes following variables:

Baseline Characteristic	Variable	Type of variable	Overall population (n= x)	High-dose loop diuretics + 500 mg acetazolamide (IV) (n=x)	High-dose loop diuretics + placebo (IV) (n=x)
Age (years)	DEM_AGE	Continue			
Female sex (N,%)	DEM_SEX	Binary			
Caucasian (N,%)	DEM_RACE	Binary			
LVEF (N,%) LVEF ≤ 40% (N,%) LVEF > 40%	MH_LVEFRES	Continue			
Etiology of HF Ischemic (N,%) CABG (N, %) PTCA (N, %) Thrombolysis (N, %) Non-ichemic (N,%) Valvular (N, %) Idiopathic (N,%) Hypertensive (N,%) Toxic (N,%)	MH_HFOPT, MH_HF_ISOPT, MH_HF_NISOPT	Nominal			
NYHA class at enrolment Class II (N,%) Class III (N,%) Class IV (N,%)	MH_NYHAOPT	Ordinal			
Comorbid conditions Atrial fibrillation or atrial flutter (N,%) Stroke (N,%) Diabetes (N,%) Hypertension (N,%) Valve surgery (N,%) PAD (N,%) COPD (N,%)	MH_AFYN, MH_STROKEYN, MH_DIABYN, MH_HYPERTOPT, MH_VALVESURYN, MH_PADYN, MH_COPDYN	Nominal			
CRT/ICD at enrolment None (N,%) pacemaker – single chamber (N,%) pacemaker-dual chamber (N,%) ICD-primary prevention (N,%) ICD-secondary prevention (N,%) CRT-D (N,%) CRT-P (N,%)	MH_CADOPT,	Nominal			
Smoking status at enrolment Never (N,%)	MH_SMOKEOPT	Nominal			

Former (N,%) Current (N,%)					
Malignancy requiring chemo/radiotherapy No (N,%) Yes-past (N,%) Yes-current (N,%) Yes-past and current (N,%)	MH_TUMORYN	Nominal			
Weight (kg)	VS_WGHT_D0	Continue			
Pulse (beats/min)	VS_PULSE_D0	Continue			
Blood pressure (systolic) (mmHg)	VS_BPS_D0	Continue			
Blood pressure (diastolic) (mmHg)	VS_BPD_D0	Continue			
Mean Arterial Pressure (MAP)	VS_MAP_D0	Continue			
Congestion score	VOA_SCORE_D0	Ordinal			
Laboratory values Hematology (g/dL) Hematocrit (%) Sodium (mmol/L) Potassium (mmol/L) Chloride (mmol/L) Serum osmolality (mOsm/kg) Serum urea (mg/dL) eGFR (mL/min/1,73m2) Total protein (g/L) Albumin (g/L) Fe (µg/dL) Ferritin (µg/dL) Transferrin saturation (TSAT) (%) LDH (U/L) Troponin (ng/L) NTproBNP/BNP	LABB_HGB_D0, LABB_HT_D0, LABB_NA_D0, LABB_K_D0, LABB_CL_D0, LABB_OSM_D0, LABB_UREA_D0, LABB_GFR_D0, LABB_PROT_D0, LABB_ALB_D0, LABB_FE_D0, LABB_FERR_D0, LABB_TSAT_D0, LABB_LDH_D0, LABB_TROP_D0, LABB_BNPOPT_D0, LABB_BNP_D0	Continue			
Diuretics Furosemide (mg) Thiazide (N,%) MRA (N,%)	Report parent 'Screening': DIU_TYPE + DIU DOSIS Report parent 'Screening': DIU_TYPE	Continue Nominal			
Neurohumoral blockers ACE (N,%) Beta-blocker (N,%) ARB (N,%) Entresto (N,%)	Report parent 'Screening': NH_TYPE	Nominal			
Other concomitant medication Ivabradine (N,%) Hydralazine (N,%) Molsidomine (N,%) Isosorbide dinitrate, PO (N,%) Digoxin (N,%) Aspirin(N,%) NOAC/DOAC	CONMED_OTHER_D0	Nominal			

VKA (N,%) P2Y12 (N,%) Statin (N,%) XO-inhibitor (N,%) Amiodarone (N,%) Flecainide (N,%) IV inotropes (N,%) IV vasodilators (N,%) Other anti-hypertensive drugs (N,%) SGLT2-i (N,%) None (N,%)					
EQ-5D-5L (N, %) EQ-5D-5L score	EQ5D_D0 Report parent 'Screening': EQ5D_MOBOPT, EQ5D_SCOPT, EQ5D_ACTOPT, EQ5D_PAINOPT, EQ5D_ANXOPT	Nominal Continue			
EQ-5D-5L (VAS) (N,%) (0-100)	EQ5D_SCORESL	Continue			

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Summary of Changes

Changes between original (V1.0) and final (V2.0) protocol:

Amendment No.	Protocol version no.	Date issued	Details of changes made
01	Version 2.0	03 January 2019	Cfr. explanation below.

Details of changes made for amendment No. 01:

- Clarification of maintenance dose of loop diuretics. To avoid any confusion, half of start dose given at randomization has been erased and we stated "1x orally daily maintenance dose".

- Clarification volume assessment scoring

- If patients are being discharged earlier than day 4, parameters planned for the morning of day 4 will still be assessed but at discharge (i.e. EQ5D, NT-proBNP/BNP, blood samples for sub-study if applicable)

- Removal of an endpoint event, i.e. rehospitalisation due to any cardiac event.

- Clarification exclusion criteria:

  - **Use of nitrates *and/or* molsidomine is allowed, but at the discretion of the treating physician** instead of use of nitrates is allowed only when systolic blood pressure is above 140mmHg.
  - **Treatment with acetazolamide within one month prior to randomization is not allowed** instead of treatment with acetazolamide during the index hospitalisation before randomization.
  - **Use of any non-protocol defined diuretic agent with the exception of mineralocorticoid receptor antagonists during the treatment phase of the study is not allowed** instead of Use of any non-protocol defined diuretic agent with the exception of mineralocorticoid receptor antagonists.

- Addition of one exclusion criteria: Subjects with urinary incontinence who are not willing to receive a bladder catheter

- Collection of additional study data:

  - Use of iron per os and/or intravenously throughout the study will be recorded at discharge and at follow-up.
  - A predefined list of concomitant medication will need to be completed at discharge and at 3 months follow-up (cfr. appendix 5)

- Details concerning the collection of concomitant medication have been replaced by appendix 5.

- Follow-up appointment will be 3 months (+ 14 days) after the start of study medication, instead of after hospital discharge.

- For completeness, admission has been changed to screening.

- Throughout the protocol, minor changes (e.g. typographical errors, clarifications, etc.) have been added.

Changes between original (V1.0) and final (V2.0) Statistical Analysis Plan:

Version number	**Version Date**	**Author**	**Reason for change**
Version 1.0	17 January 2022	ADVOR Scientific Study Team Liesbeth Bruckers (Statistician)	First version
Version 2.0	17 March 2022	ADVOR Scientific Study Team Liesbeth Bruckers (Statistician)	Appendix A Baseline Characteristics: The Baseline Characteristics Iron PO and Iron IV mentioned in the Appendix A have been removed because these data will not be collected during the study.