

Novartis Institutes for BioMedical Research

CLR325

Clinical Trial Protocol CCLR325X2202

**A randomized, subject and investigator-blind,  
placebo-controlled study of CLR325 in  
chronic stable heart failure patients**

Document type:	Amended Protocol Version
EUDRACT number:	2016-001387-12
Version number:	v05 (Clean)
Development phase:	Ila
Release date:	27-Aug-2018

Property of Novartis  
Confidential  
May not be used, divulged, published, or otherwise disclosed  
without the consent of Novartis

## **Site Operations Manual (SOM)**

A Site Operations Manual (SOM) accompanies this protocol, providing the operational details for study conduct.

### **Notification of serious adverse events**

Refer to [Section 9.2](#) of the protocol for definitions and reporting requirements for Serious Adverse Events (within 24 hours after awareness of the SAE to the local Novartis Chief Medical Office and Patient Safety (CMO & PS) Department and notify the Study Lead).

Contact information is listed in the Site Operations Manual.

## Table of contents

Site Operations Manual (SOM).....	2
Notification of serious adverse events.....	2
Table of contents .....	3
List of tables .....	6
List of figures .....	6
List of abbreviations .....	7
Pharmacokinetic definitions and symbols .....	9
Amendment 5 (August 2018) .....	10
Amendment 4 (October 2017).....	12
Amendment 3 (February 2017) .....	14
Amendment 2 (Aug 2016).....	15
Amendment 1 (April 2016).....	16
Protocol synopsis.....	18
1 Introduction .....	20
1.1 Background.....	20
<b>Corporate Confidential Information</b>	
1.2 Study purpose .....	23
2 Study objectives.....	24
2.1 Primary objective(s).....	24
2.2 Secondary objective(s).....	24
<b>Corporate Confidential Information</b>	
3 Investigational plan .....	25
3.1 Study design.....	25
<b>Corporate Confidential Information</b>	
3.4 Rationale for choice of comparator .....	29
<b>Corporate Confidential Information</b>	
3.6 Risks and benefits .....	29
3.6.1 Potential risks associated with blood collection.....	30
3.6.2 Potential risks associated with immunogenicity .....	31
3.6.3 Potential risks associated with infusion-related reactions.....	31
3.6.4 Potential risks associated with systemic inflammation.....	31
3.6.5 Potential risks associated with a PA catheter .....	32
4 Population.....	32
4.1 Inclusion criteria .....	32

4.2	Exclusion criteria	33
5	Restrictions for Study Subjects	34
5.1	Contraception requirements	34
5.2	Prohibited treatment	34
5.3	Dietary restrictions and smoking	34
5.4	General restrictions	34
6	Treatment	35
6.1	Study treatment	35
6.1.1	Investigational treatment	35
6.2	Treatment arms	35
6.3	Permitted dose adjustments and interruptions of study treatment	35
6.4	Treatment assignment	35
6.5	Treatment blinding	36
6.6	Emergency breaking of assigned treatment code	36
6.7	Treatment exposure and compliance	37
6.8	Recommended treatment of adverse events	37
6.9	Rescue medication	37
6.10	Concomitant treatment	37
7	Discontinuation and study completion	37
7.1	Discontinuation of study treatment	37
7.2	Study completion and post-study treatment	39
7.2.1	Lost to follow-up	39
7.3	Withdrawal of consent	39
7.4	Study Stopping rules	40
7.5	Early study termination	40
8	Procedures and assessments	41
8.1	Informed consent procedures	45
8.2	Subject demographics/other baseline characteristics	45
8.3	Efficacy / Pharmacodynamics	46
	<b>Corporate Confidential Information</b>	
8.3.2	Invasive hemodynamic monitoring	46
8.3.3	Echocardiogram	47
8.3.4	Mixed venous blood gas (SvO <sub>2</sub> )	47
8.4	Safety	47
8.4.1	Physical examination	47
8.4.2	Vital signs	48

8.4.3	Height and weight .....	48
8.4.4	Laboratory evaluations .....	48
8.4.5	Electrocardiogram (ECG) .....	49
8.4.6	Immunogenicity .....	49
8.4.7	Alcohol test and drug screen .....	50
8.4.8	AICD interrogation .....	50
8.4.9	Cardiac Holter and telemetry monitoring .....	50
8.4.10	Fluid input and output .....	50
8.5	Pharmacokinetics .....	50
8.5.1	PK blood collection .....	51
8.5.2	PK assessments .....	51
8.5.3	PK urine collection (28-hour urine collection) .....	51
8.6	Other assessments .....	52
<b>Corporate Confidential Information</b>		
9	Safety monitoring .....	53
9.1	Adverse events .....	53
9.2	Serious adverse event reporting .....	54
9.2.1	Definition of SAE .....	54
9.2.2	SAE reporting .....	55
9.3	Liver safety monitoring .....	56
9.4	Renal safety monitoring .....	60
9.5	Pregnancy reporting .....	61
9.6	Prospective suicidality assessment .....	61
9.7	Early phase safety monitoring .....	61
10	Data review and database management .....	62
10.1	Site monitoring .....	62
10.2	Data collection .....	62
10.3	Database management and quality control .....	62
10.4	Data Monitoring Committee .....	63
10.5	Adjudication Committee .....	63
11	Data analysis .....	63
<b>Corporate Confidential Information</b>		
11.2	Subject demographics and other baseline characteristics .....	64
11.3	Treatments (study drug, rescue medication, other concomitant therapies, compliance) .....	64
11.4	Analysis of the primary variable(s) .....	64

11.4.1	Variable(s).....	64
11.4.2	Statistical model, hypothesis, and method of analysis.....	64
11.4.3	Handling of missing values/censoring/discontinuations.....	65
11.4.4	Supportive analyses.....	65
11.5	Analysis of secondary and exploratory variables.....	65
	<b>Corporate Confidential Information</b>	
11.5.2	Safety.....	65
11.5.3	Pharmacokinetics.....	66
11.5.4	Pharmacokinetic / pharmacodynamic interactions.....	66
11.5.5	Other assessments.....	67
11.6	Sample size calculation.....	68
11.7	Power for analysis of key secondary variables.....	68
	<b>Corporate Confidential Information</b>	
12	Ethical considerations.....	68
12.1	Regulatory and ethical compliance.....	68
12.2	Responsibilities of the investigator and IRB/IEC.....	69
12.3	Publication of study protocol and results.....	69
13	Protocol adherence.....	69
13.1	Protocol Amendments.....	69
14	References.....	71

### List of tables

Table 8-1	Assessment Schedule.....	41
Table 8-2	Permitted assessment windows.....	51
Table 9-1	Liver Event and Laboratory Trigger Definitions.....	57
Table 9-2	Follow Up Requirements for Liver Events and Laboratory Triggers...	58
Table 9-3	Specific Renal Alert Criteria and Actions.....	60

### List of figures

Figure 3-1	Study Design.....	27
------------	-------------------	----

## List of abbreviations

---

ACE	Angiotensin-converting enzyme
AICD	Automatic implantable cardioverter defibrillator
AE	adverse event
AEB	Additional Exploratory Biomarker
ALT	alanine aminotransferase
ARB	Angiotensin receptor blocker
AST	aspartate aminotransferase
BfArM	German Health Authority
BMI	Body Mass Index
BUN	blood urea nitrogen
CD-ROM	compact disc – read only memory
CFR	Code of Federal Regulation
CK	creatinine kinase
CRF	Case Report/Record Form (paper or electronic)
CV	coefficient of variation
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
GCP	Good Clinical Practice
h	hour
i.v.	intravenous
IA	Interim Analysis
IC50	Half maximal inhibitory concentration
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IRB	Institutional Review Board
kg	Kilogram
LDH	lactate dehydrogenase
mg	milligram(s)
min	Minute
ml	milliliter(s)
PA Catheter	Pulmonary Artery Catheter
PD	pharmacodynamic(s)

PK	pharmacokinetic(s)
RBC	red blood cell(s)
SAE	serious adverse event
SD	standard deviation
SOM	Site Operational Manual
SvO2	Mixed Venous Oxygen Saturation
TBL	total bilirubin
µg	Microgram
ULN	upper limit of normal
WBC	white blood cell(s)

---



## Pharmacokinetic definitions and symbols

Ae0-t	Amount of drug (or defined metabolite) excreted into the urine from time zero to time 't' where t is a defined time point after administration [mass units or % of dose]
AUC0-t	The area under the plasma (or serum or blood) concentration-time curve from time zero to time 't' where t is a defined time point after administration [mass x time / volume]
AUCinf	The area under the plasma (or serum or blood) concentration-time curve from time zero to infinity [mass x time / volume]
AUClast	The area under the plasma (or serum or blood) concentration-time curve from time zero to the time of the last quantifiable concentration [mass x time / volume]
CL	The systemic (or total body) clearance from plasma (or serum or blood) following intravenous administration [volume / time]
CLr	The renal clearance from plasma (or serum or blood) [volume / time]
Cmax,ss	The observed maximum plasma (or serum or blood) concentration following drug administration at steady state [mass / volume]
T1/2	The terminal elimination half-life [time]
Tmax	The time to reach the maximum concentration after drug administration [time]
Vss	The volume of distribution at steady state following intravenous administration [volume]

**Corporate Confidential Information**

**Corporate Confidential Information**

**Corporate Confidential Information**

**Corporate Confidential Information**

**Corporate Confidential Information**

**Corporate Confidential Information**

**Corporate Confidential Information**



**Corporate Confidential Information**

## Protocol synopsis

<b>Protocol number</b>	CLR325X2202
<b>Title</b>	A randomized, subject and investigator-blinded, placebo-controlled study of CLR325 in chronic stable heart failure patients
<b>Brief title</b>	Safety, pharmacokinetics and efficacy study of CLR325 in stable heart failure patients.
<b>Sponsor and Clinical Phase</b>	Novartis Phase IIa
<b>Intervention type</b>	Drug
<b>Study type</b>	Interventional
<b>Purpose and rationale</b>	The purpose of this study is to determine the safety and tolerability of CLR325 in heart failure patients to determine if further clinical development of the drug in this indication is warranted.
<b>Primary Objective(s)</b>	To determine the safety and tolerability of an 18-hour i.v. infusion of CLR325 in stable heart failure patients
<b>Secondary Objectives</b>	To determine the pharmacokinetics and immunogenicity of CLR325, and the active metabolite, CQJ295, during an 18-hour infusion of CLR325 in heart failure patients.
<b>Study design</b>	<p>This is a non-confirmatory, randomized, subject and investigator-blind, placebo-controlled study of an 18-hour infusion of CLR325 in stable heart failure patients (who have a clinically indicated pulmonary artery catheter in place) to assess safety, tolerability, and pharmacokinetics in this patient population. This study has an adaptive dose design. A maximum of 40 patients will be randomized. Initially, one cohort will be dosed: 6 patients with CLR325 and 2 with placebo.</p> <p style="text-align: center;"><b>Corporate Confidential Information</b></p>
<b>Population</b>	Patients with stable heart failure >18 years of age
<b>Inclusion criteria</b>	<p>Written informed consent must be obtained before any assessment is performed.</p> <p>Able to communicate well with the investigator, to understand and comply with the requirements of the study.</p> <p>Male and female patients &gt;18 years of age.</p> <p>Patients must weigh between 50 kg and 140 kg to participate in the study.</p> <p>Patients with a cardiac ejection fraction of <math>\leq 45\%</math> as assessed within the last 6 months.</p> <p>In the opinion of the investigator, heart failure patients who will not require a change in their dose of ACE, ARB, <math>\beta</math>-blocker, mineralocorticoid receptor antagonist, or diuretic for 24 hours after randomization.</p> <p>For PA catheter cohorts, patients who are planned to have a clinically indicated pulmonary artery catheter in place prior to randomization.</p>

	At baseline, vital signs (systolic and diastolic blood pressure and pulse rate) will be assessed in the supine position after the subject has rested for at least five minutes.
<b>Exclusion criteria</b>	<p>Presence of impaired renal function as indicated by clinically significant abnormal creatinine values (eGFR &lt; 30 ml/min/1.73m<sup>2</sup> calculated using the MDRD equation).</p> <p>Patients with a history of chronic hepatitis of any non-cardiac etiology.</p> <p>History of any active, clinically significant cardiac tachyarrhythmia, such as recurrent atrial fibrillation with rapid ventricular response within the last year. Note that patients with chronic atrial fibrillation with a pulse rate ≤ 100 bpm should not be excluded.</p> <p>Patients who have received an intravenous infusion of a cardiac inotrope (e.g., dobutamine or milrinone) in the last 24 hours prior to randomization.</p> <p>Patients with any significant change in their dose of their ACE, ARB, mineralocorticoid receptor antagonist, diuretic or β-blocker within the last 12 hours.</p> <p>Patients with known significant valvular heart disease as indicated by the following:</p> <ul style="list-style-type: none"> <li>• Severe aortic stenosis (Aortic Valve Area &lt; 1.0 cm<sup>2</sup> or peak gradient &gt; 50 mmHg as determined by echocardiography)</li> <li>• Severe mitral stenosis</li> </ul> <p>Patients with history of acute coronary syndrome within the last 60 days as determined by both clinical and enzymatic criteria.</p> <p>For PA catheter cohorts, patients with a pulmonary capillary wedge pressure of &lt;10 mm Hg at baseline. For echocardiographic cohorts, patients with a lateral E/E' ratio of &lt;7 on their baseline echocardiogram. For patients in whom a lateral E/E' ratio cannot be determined (e.g., patients in atrial fibrillation), a central venous pressure of &lt;5mm Hg on baseline echocardiogram as determined by inferior vena cava criteria.</p>
<b>Investigational and reference therapy</b>	CLR325
	<b>Corporate Confidential Information</b>
<b>Safety assessments</b>	Physical exam, vital sign measurements, hematology and blood chemistry, cardiac panel, ECG, cardiac and telemetry monitoring will be obtained.
	<b>Corporate Confidential Information</b>
<b>Data analysis</b>	The occurrence of adverse events will be examined by treatment. Both the total number of adverse events, and the preferred term for the adverse event will be compared by treatment group. Secondary analyses will be performed on pharmacokinetic parameters and presence of an immune response to CLR325 and anti-apelin antibodies.
<b>Key words</b>	Apelin, CLR325, chronic heart failure

## 1 Introduction

Acute decompensated heart failure is commonly caused by deteriorating cardiac function that leads to unplanned, often urgent, medical intervention. There are ~500,000 hospitalizations annually for acute decompensated heart failure in the USA and the 30-day mortality following hospitalization is up to 11% (Jong et al 2002). Patients typically experience fatigue, breathlessness, fluid retention, and chest pain. The common treatment approach of vasodilators and diuretics is ineffective in a substantial proportion of heart failure patients with a reduced ejection fraction due to a predominant deficit in cardiac contractility. Intravenous inotropes, including dobutamine ( $\beta$ -agonist), and milrinone/amrinone (PDEIII inhibitors) are used to augment cardiac output in these patients and are associated with increased risk of arrhythmia and mortality (Felker and O'Connor 2001). In contrast, digoxin does not increase all-cause mortality, and reduces heart failure hospitalizations in patients with heart failure (Digitalis Investigation 1997), but it is a weak inotrope with a narrow therapeutic index. Therefore, new inotropic therapies that can effectively enhance cardiac function without increasing the risk of arrhythmias and/or mortality are urgently needed for patients with heart failure.

### 1.1 Background

CLR325 is a synthetic cyclic peptide and an apelin-13 analog being developed for the treatment of patients with acute decompensated heart failure. Apelin is the natural ligand for APJ, a G-protein-coupled receptor that shares 31% homology with the human angiotensin II receptor (for review, see O'Carroll et al 2013). Apelin is produced as an inactive 77-amino-acid pre-pro-peptide that is cleaved into smaller, active fragments, such as apelin -36, -19, -17, -16, -13, and pyroglutamate-apelin-13 ((pyr<sup>1</sup>) apelin-13). Zhen et al 2013 recently identified (pyr<sup>1</sup>) apelin-13 as the predominant apelin isoform in human plasma. Apelin peptides increase contractility in isolated cardiomyocytes, *ex vivo* hearts, and *in vivo* (Ashley et al 2005; Farkasfalvi et al 2007). Plasma apelin levels are significantly decreased in heart failure patients (Chong et al 2006), and 6-hour infusion of (pyr<sup>1</sup>) apelin-13 into healthy volunteers or heart failure patients induced a sustained increase in cardiac index (Japp et al 2010; Barnes et al 2013). Also, low plasma apelin concentrations predicted arrhythmia recurrence in patients with persistent atrial fibrillation (Falcone et al 2010), suggesting that apelin peptides may be anti-arrhythmic. Taken together, these findings suggest that apelin peptides may increase cardiac contractility in heart failure patients without causing arrhythmias or increasing mortality.

### **1.1.1 Relevant data summary**

The most relevant data for the present study are summarized in the sections below. For detailed information, please refer to the CLR325 Investigators Brochure.

**Corporate Confidential Information**

**Corporate Confidential Information**

## **Corporate Confidential Information**

### **1.2 Study purpose**

The purpose of this study is to determine the safety and tolerability of CLR325 in stable heart failure patients to determine if further clinical development of the drug in a heart failure indication is warranted.

## 2 Study objectives

### 2.1 Primary objective(s)

Primary objective(s)	Endpoints related to primary objective(s)
<ul style="list-style-type: none"><li>To determine the safety and tolerability of an 18-hour i.v. infusion of CLR325 in stable heart failure patients.</li></ul>	<ul style="list-style-type: none"><li>Frequency of adverse events in CLR325 treated patients as compared to patients treated with placebo.</li><li>Frequency of clinically significant changes in hematology or clinical chemistry laboratories or changes in blood pressure or heart rate.</li></ul>

### 2.2 Secondary objective(s)

Secondary objective(s)	Endpoints related to secondary objective(s)
<ul style="list-style-type: none"><li>To determine the pharmacokinetics of CLR325, and the active metabolite, CQJ295, during an 18-hour i.v. infusion of CLR325 in heart failure patients.</li></ul>	<ul style="list-style-type: none"><li>Plasma and urine pharmacokinetic parameters of CLR325 and CQJ295 (C<sub>max</sub>, T<sub>max</sub>, AUC<sub>0-18h</sub>, AUC<sub>last</sub>, AUC<sub>0-28h</sub>, AUC<sub>inf</sub>, T<sub>1/2</sub>, CL (CLR325 only), V<sub>ss</sub> (CLR325 only), CL<sub>r</sub>, and Ae<sub>0-28</sub> during the course of an 18-hour infusion and post-infusion in heart failure patients.</li></ul>
<ul style="list-style-type: none"><li>To determine the immunogenicity of an 18-hour i.v. infusion of CLR325 in heart failure patients.</li></ul>	<ul style="list-style-type: none"><li>Treatment related titer of anti-CLR325 and anti-apelin antibodies after infusion of CLR325 in heart failure patients.</li></ul>



## **Corporate Confidential Information**

### **3 Investigational plan**

#### **3.1 Study design**

This is a non-confirmatory, randomized, subject and investigator-blind, placebo-controlled study of an 18-hour infusion of CLR325 in stable heart failure patients (who may have a clinically indicated pulmonary artery catheter in place) to assess safety, tolerability, and pharmacokinetics in this patient population. Initially, one PA catheter cohort will be dosed: 6 patients with CLR325 and 2 with placebo randomized using a block randomization scheme. The first cohort of patients will be those with chronic stable heart failure. For subsequent cohorts, patients with acute decompensation who have been stabilized following hospital admission may also be enrolled. Following completion of the first cohort, subsequent cohorts may enroll concurrently as determined by the sponsor.

## **Corporate Confidential Information**

The study will be comprised of a screening period of approximately 7 weeks (Day -45 to Day 1), a baseline period (Day 1), a treatment period (Day 1-2) and a follow up period (Days 3-28). Each treatment period will be comprised of a baseline pre-dosing interval (hour -1 to hour 0), an inpatient dosing interval (hour 1 to hour 18), and an inpatient post-dosing interval (hour 18 to hour 28). In addition, patients will have a follow-up visit (Day 10) and an end-of-study visit on Day 28.

On Day -45 to 1, screening evaluations will be performed. Study inclusion/exclusion criteria as well as medication use will be assessed during the screening and baseline periods. Patients who meet all of the inclusion and none of the exclusion criteria at screening will report to the study site on Day 1 of the baseline period, at a time specified by the investigator, and if they continue to meet all inclusion and none of the exclusion criteria, will remain in-house until the final 28 hour inpatient assessment in the treatment period.

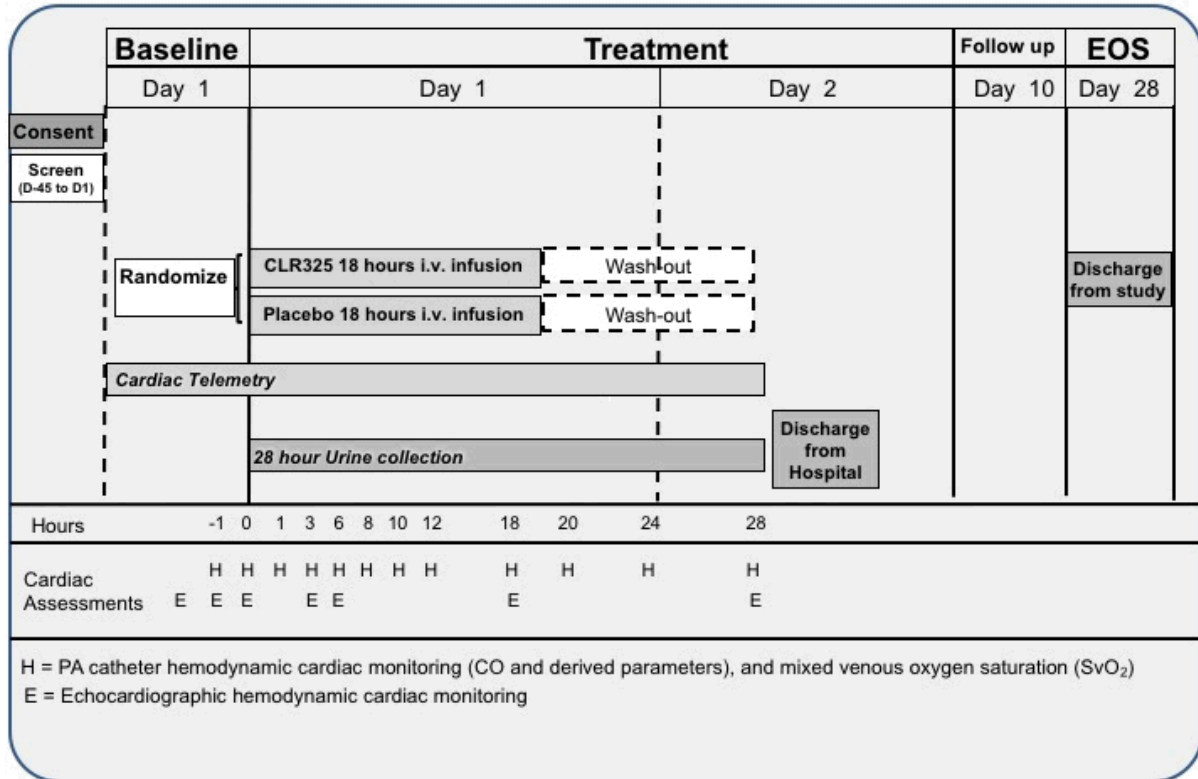
On Day 1, pre-dose laboratory evaluations, cardiac hemodynamic monitoring, and 3-lead continuous telemetry monitoring will be performed. The sponsor will pre-specify if hemodynamics are to be monitored by a PA catheter, echocardiography, or both for each cohort. For PA catheter cohorts, cardiac hemodynamics will be monitored continuously using a pulmonary artery catheter (that will have been placed for standard of care clinical indications by the subject's caregiver prior to randomization in the study)

**Corporate Confidential Information** Intracardiac pressures (right atrial, pulmonary artery) will be recorded every hour during the treatment period. Cardiac outputs will be obtained at hours -1, 0, 1, 3, 6, 8, 10, 12, 18, 20, 24, and 28 of the treatment period. If not needed for standard of care clinical indications, the pulmonary artery catheter may be removed after the final cardiac output measurement at hour 28. For echocardiographic cohorts, echocardiograms will be obtained during the treatment period as specified in the SOM. Note that the sponsor's decision regarding obtaining the echocardiogram will be applied uniformly across all patients in the cohort.

#### **Corporate Confidential Information**

Following the pre-dose procedures, eligible patients will be dosed intravenously. Post-dose evaluations will include routine laboratory evaluations, cardiac monitoring, continuous telemetry monitoring, **Corporate Confidential Information**. Patients may be released from the study site on Day 2, following completion of all 28-hour assessments, at the discretion of the investigator and if there are no safety concerns. The patients will be asked to return to the study site on Day 10 for a follow-up visit that will include a physical exam, routine laboratory evaluations, ECG evaluation **Corporate Confidential Information**. An end-of-study visit will be conducted on Day 28 and will include a physical exam, routine laboratory evaluations, ECG evaluation **Corporate Confidential Information**

**Figure 3-1 Study Design**



**Corporate Confidential Information**

### **3.4 Rationale for choice of comparator**

A normal saline intravenous infusion will be used as a comparator to CLR325 in this study. Normal saline was chosen as this is visually indistinguishable from the CLR325 infusion and is the excipient for the CLR325 infusion. Also, the total volume of normal saline or CLR325 infusion given in this study over 18 hours will have minimal effects on cardiac hemodynamics of study subjects.

**Corporate Confidential Information**

### **3.6 Risks and benefits**

This study will be conducted in patients with stable heart failure to assess the status of their disease.

**Corporate Confidential Information**

Based on a human trial of (pyr1) apelin-13 ([Barnes et al 2013](#)), it is also possible that CLR325 could lead to an increase in blood pressure in treated subjects which could lead to symptoms of chest pain, shortness of breath, or headache in affected subjects. In addition, it is possible that by increasing cardiac contractility, cardiac filling pressures may drop, leading to a paradoxical drop in blood pressure. This might result in subjects developing symptoms of dizziness, lightheadedness, chest pain, or shortness of breath. Finally, it is possible that CLR325 could induce sustained cardiac arrhythmias such as ventricular tachycardia or supraventricular tachycardia leading to symptoms of palpitations, dizziness, lightheadedness, shortness of breath, chest pain, or trigger a subject's AICD to discharge.

### **Corporate Confidential Information**

The risk to subjects in this trial will be minimized by adherence to the inclusion/exclusion criteria, close clinical monitoring, in-patient status, and study "stopping rules". In addition to the potential risks outlined below, there are unknown risks to CLR325, which may be serious and unforeseen.

The 18 hours i.v. infusion requiring an indwelling catheter could potentially lead to discomfort, development of hematoma or in rare cases of phlebitis.

#### **3.6.1 Potential risks associated with blood collection**

Blood samples will be collected frequently during the study either via venipuncture or i.v. cannula. The total volume to be collected over the course of the study is approximately 251 mL. Risks associated with blood collection include pain, swelling and/or bruising at the insertion site of the needle. Although rare, localized clot formation, infections and nerve damage may occur. Lightheadedness and/or fainting may also occur during or shortly after the blood draw.

### **Corporate Confidential Information**

### 3.6.2 Potential risks associated with immunogenicity

The presence of anti-CLR325 antibodies and anti-apelin antibodies will be determined in all study subjects. The risk of developing anti-CLR325 and anti-apelin antibodies in humans is unknown;

#### Corporate Confidential Information

Since CLR325 is an apelin analog, it is possible that antibodies directed against CLR325 could cross-react with endogenous apelin. The development of neutralizing antibodies towards endogenous apelin might be associated with adverse events. Mice deficient in apelin have an increased propensity to develop heart failure following aortic banding or aging (Kuba et al 2007); however, it is unknown what the consequences of neutralizing endogenous apelin are in normal mice or humans. Subjects that develop anti-apelin antibodies will be followed with repeat immunogenicity assessments until their levels of anti-apelin antibodies are undetectable. The need for medical intervention will be dependent on the study subject's condition and the standard of medical care. The timing of follow up laboratory assessments, their interpretation and need for medical intervention will be made jointly by the Investigator and Sponsor.

### 3.6.3 Potential risks associated with infusion-related reactions

#### Corporate Confidential Information

Hypersensitivity reactions can manifest as fever, chills, urticaria, dyspnea, headaches, myalgia, and/or hypotension. If a severe hypersensitivity reaction occurs, CLR325 should be discontinued and appropriate therapy should be initiated (e.g., antihistamines, corticosteroids, airway support, etc.).

#### Corporate Confidential Information

infusion sites should be closely monitored during the study for signs of an allergic response, inflammation or thrombosis. Infusions should be discontinued in any subject with such signs and appropriate therapy should be initiated as needed (e.g., antihistamines, corticosteroids, etc.).

### 3.6.4 Potential risks associated with systemic inflammation

#### Corporate Confidential Information

Subjects showing signs or symptoms of systemic inflammation (elevated white blood cell count above 2.5x the ULN, fever) should be discontinued from CLR325 infusion and supportive care initiated as clinically indicated.

### **3.6.5 Potential risks associated with a PA catheter**

Risks associated with continued invasive hemodynamic monitoring by a PA catheter may include complications such as bleeding and infection. The risk of infections will be limited by sterile procedures. The rate of bleeding complications from the PA catheter has been reported at 1.9% to 4%, and PA catheter infections with an incidence of 1.9%. Through stimulation of the myocardium, atrial and ventricular arrhythmias can occur which are generally without hemodynamic relevance and self-limiting, occurring with an incidence of 0.5-3%. Pulmonary infarction has also been reported at an incidence of approximately 1% ([Binanay et al 2005](#), [Harvey et al 2005](#)).

## **4 Population**

The study population will be composed of adult male and female patients with stable heart failure with reduced ejection fraction. These patients will have been diagnosed with heart failure with reduced ejection fraction at least 6 months prior to study enrollment.

Studies to assess reproductive toxicology have not yet been performed. Women who are of child bearing potential, currently pregnant, or have been pregnant in the last 6 months, are excluded from this study. This exclusion is in place to prevent women with a newly diagnosed peri-partum cardiomyopathy from being included in this study.

### **4.1 Inclusion criteria**

1. Written informed consent must be obtained before any assessment is performed.
2. Able to communicate well with the investigator, to understand and comply with the requirements of the study.
3. Male and female patients >18 years of age.
4. Patients must weigh between 50 kg and 140 kg to participate in the study.
5. Patients with a cardiac ejection fraction of  $\leq 45\%$  assessed within the last 6 months.
6. For PA catheter cohorts, patients who are planned to have a clinically indicated pulmonary artery catheter in place prior to randomization.
7. In the opinion of the investigator, heart failure patients who will not require a change in their dose of ACE, ARB,  $\beta$ -blocker, mineralocorticoid receptor antagonist, or diuretic for 24 hours after randomization.
8. At baseline, vital signs (systolic and diastolic blood pressure and pulse rate) will be assessed in the supine position with the head of the bed at 30° after the subject has rested for at least five minutes. Supine vital signs should be within the following ranges:
  - oral body temperature between 35.0-37.5 °C
  - systolic blood pressure, 90-140 mm Hg
  - diastolic blood pressure, 50-90 mm Hg
  - pulse rate, 50 - 100 bpm

If blood pressure or heart-rate is out-of-range at screening or baseline, the Investigator may obtain two additional readings, so that a total of up to three consecutive assessments are made, with the subject resting quietly in supine position for approximately 5 minutes preceding each



repeat assessment. At least the last reading must meet the inclusion criteria in order for the subject to qualify.

## 4.2 Exclusion criteria

1. Use of other investigational drugs at the time of enrollment, or within 5 half-lives of enrollment, or until the expected PD effect has returned to baseline, whichever is longer; or longer if required by local regulations.
2. History of hypersensitivity to the study drug or to drugs of similar chemical classes.
3. As per the investigator's judgement, a history of tobacco, drug or alcohol abuse that will compromise the patient's ability to participate in this study.
4. Currently pregnant, pregnant within the last 6 months, or nursing (lactating) women.
5. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant. Women are considered post-menopausal and not of child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child bearing potential.
6. Sexually active males must use a condom during intercourse while taking drug and for 10 hours (*= 5 times the terminal half-life of investigational medication*) after stopping CLR325 and should not father a child in this period. A condom is required to be used also by vasectomized men in order to prevent delivery of the drug via seminal fluid.
7. Chronic infection with Hepatitis B (HBV) or Hepatitis C (HCV). A positive Hepatitis B surface antigen (HBsAg) test excludes a patient. Patients with a positive Hepatitis C antibody test should have HCV RNA levels measured. Patients with positive (detectable) HCV RNA should be excluded.
8. Patients with a history of chronic hepatitis of any non-cardiac etiology.
9. Patients with values of AST or ALT >100 U/L measured within the last 3 months before randomization.
10. Presence of impaired renal function as indicated by clinically significant abnormal creatinine values (eGFR < 30 ml/min/1.73m<sup>2</sup> calculated using the MDRD equation).
11. Known family history or known presence of long QT syndrome.
12. History of any active, clinically significant cardiac tachyarrhythmia (such as recurrent atrial fibrillation with rapid ventricular response) within the last year. Note that patients with chronic atrial fibrillation with a pulse rate ≤ 100 bpm should not be excluded. Anticoagulation for patients with atrial fibrillation should be managed per usual clinical practice for patients undergoing right heart catheterization.
13. Patients with known significant valvular heart diseases indicated by the following:
  - severe aortic stenosis (Aortic Valve Area < 1.0 cm<sup>2</sup> or peak gradient > 50 mm Hg as determined by echocardiography)
  - severe mitral stenosis

14. Patients with history of acute coronary syndrome within the last 60 days as determined by both clinical and enzymatic criteria.
15. For echocardiography-based cohorts only, patients admitted to an inpatient setting for acute decompensated heart failure within the last 30 days.
16. Patients who have received an intravenous infusion of a cardiac inotrope (e.g., dobutamine or milrinone) in the last 24 hours prior to randomization.
17. For PA catheter cohorts, patients with a pulmonary capillary wedge pressure of <10 mm Hg at baseline. For echocardiographic cohorts, patients with a lateral E/E' ratio of <7 on their baseline echocardiogram. For patients in whom a lateral E/E' ratio cannot be determined (e.g., patients in atrial fibrillation), a central venous pressure of <5mm Hg on baseline echocardiogram as determined by inferior vena cava criteria.
18. Patients with any significant change in their dose of their ACE, ARB, mineralocorticoid receptor antagonist, diuretic, or  $\beta$ -blocker within the last 12 hours. Patients with minor changes in their heart failure regimen may be eligible if deemed clinically stable by both the investigator and sponsor.
19. Any surgical or medical condition which in the opinion of the investigator may place the subject at higher risk from his/her participation in the study.

No additional exclusions may be applied by the investigator, in order to ensure that the study population will be representative of all eligible patients.

## **5 Restrictions for Study Subjects**

During recruitment, screening/informed consent review, and baseline visit, the subjects must be informed and reminded of the following restrictions:

### **5.1 Contraception requirements**

Please refer to exclusion criteria ([Section 4](#)) for details of contraception requirements for the study.

### **5.2 Prohibited treatment**

Any cardiac intravenous inotrope such as dobutamine or milrinone is prohibited. Digitalis is allowed if the patient has been on a stable dose for at least 2 weeks prior to entry into the study.

### **5.3 Dietary restrictions and smoking**

Patients will be domiciled in a hospital setting during the treatment period, and therefore will not be allowed to drink alcohol or smoke during this time.

### **5.4 General restrictions**

Patients will have limited mobility due to the PA catheter during the treatment period.

## **6 Treatment**

### **6.1 Study treatment**

Details on the storage and management of study medication, randomization and instructions for prescribing and taking study treatment are outlined in Section 3 of the Site Operations Manual.

#### **6.1.1 Investigational treatment**

The investigational treatment, CLR325 **Corporate Confidential Information** will be prepared by Novartis and supplied to an unblinded pharmacist at the site as open label bulk medication. The unblinded pharmacist will prepare the appropriate dose depending on the cohort (detailed instructions will be provided in the pharmacy manual). Normal saline will be used as a placebo (will be arranged by sites locally).

Study drugs must be received at the study site by a designated person, handled and stored safely and properly, and kept in a secure location to which only the Investigator and designated staff have access.

Upon receipt, the study drugs should be stored according to the instructions specified on the drug labels. Storage conditions must be adequately monitored and appropriate temperature logs maintained as source data. Appropriate documentation of the subject specific dispensing process must be maintained.

Medication labels will be in the local language, will comply with the legal requirements of each country, and will include storage conditions for the drug but no information about the patient

### **6.2 Treatment arms**

Patients will be assigned to one of the following 2 treatment arms in fixed randomization ratio (CLR325:Placebo):

- Single dose of CLR 325 (i.v.)
- Single dose of Placebo (i.v.)

### **6.3 Permitted dose adjustments and interruptions of study treatment**

Should there be any technical problems with the infusion line (i.e. infiltration, infusion pump malfunction) the infusion must be restarted within 30 minutes or the patient will be considered discontinued from treatment and the infusion shall not be restarted.

Dosage may be discontinued if required to address any safety concerns and should not be restarted. Dose adjustments of study treatment will not be permitted.

### **6.4 Treatment assignment**

Randomization numbers will be assigned in ascending, sequential order to eligible subjects (see Site Operation Manual for details). The randomization number becomes the definitive subject number as soon as a subject receives the first dose of the respective study treatment.

The investigator will enter the randomization number in the eCRF system. The randomization numbers will be generated using the following procedure to ensure that treatment assignment is unbiased and concealed from subjects and investigator staff. A randomization list will be produced by or under the responsibility of Novartis Drug Supply Management using a validated system that automates the random assignment of treatment arms to randomization numbers in the specified ratio. The randomization scheme for subjects will be reviewed and approved by a member of the Novartis IIS Randomization Group.

## **6.5 Treatment blinding**

The sponsor will be unblinded to treatment during this study. Patients, investigator staff, and persons performing the assessments will remain blind to the identity of the treatment from the time of randomization until database lock, using the following methods:

1. Randomization data are kept strictly confidential until the time of unblinding, and will not be accessible by patients, site staff, and study investigators with the exception of the unblinded site pharmacy staff.
2. The identity of the treatments will be concealed by the use of study drugs that are all identical in packaging, labeling, and schedule of administration.

See the Site Operation Manual for further details on blinding.

Unblinding will only occur in the case of subject emergencies ([Section 6.6](#)) and at the time of the interim analysis (see [Section 3.5](#)) and at the conclusion of the study. An assessment will be done by the appropriate site personnel and the sponsor for any subject whose treatment code has been broken inadvertently or for any non-emergency reason to assess whether or not study drug should be discontinued.

## **6.6 Emergency breaking of assigned treatment code**

Emergency unblinding should only be undertaken when it is essential to treat the subject safely and efficaciously. Most often, study treatment discontinuation and knowledge of the possible treatment assignments are sufficient to treat a study subject who presents with an emergency condition. A complete set of emergency code break cards will be provided to the investigator site(s) and a complete set will be available at Novartis. All code break cards must be retained until the end of the study and returned to Novartis. They must be stored in a secure place but be accessible in case of emergency. The investigator will receive a blinded code break card for each subject, with the details of drug treatment covered by a removable, scratch-off cover. In an emergency, the scratch-off cover can be removed to determine the treatment. The scratch-off covers are not to be removed for any reason other than an emergency. When the investigator removes the scratch-off cover he/she must note the date, time, and reason for removing it and retain this information with the case report form documentation. The unblinded treatment code should not be recorded on the CRF. The investigator must also immediately inform the Novartis local monitor that the code has been broken.

It is the investigator's responsibility to ensure that there is a procedure in place to allow access to the code break cards in case of emergency. If appropriate, the investigator will inform the subject how to contact his/her backup in cases of emergency when he/she is unavailable.

An assessment will be done by the appropriate site personnel and the sponsor after an emergency unblinding to assess whether or not study drug should be discontinued for a given patient and, if applicable, whether the patient can continue. Patients who undergo emergency unblinding and are discontinued cannot be re-enrolled in the study.

## **6.7 Treatment exposure and compliance**

Patients will receive all study medication at the Investigator site. Study medication will be administered by site personnel, compliance will be ensured by appropriate training of site personnel. The date and time of administration of study drug will be recorded in the dosage administration record section of the eCRF.

Pharmacokinetic parameters (i.e., measures of treatment exposure) will be determined in all subjects treated with CLR325.

## **6.8 Recommended treatment of adverse events**

At present there is insufficient information to provide specific recommendations regarding treatment of adverse events. Medication used to treat AEs must be recorded on the Concomitant medications/Significant non-drug therapies section of the source documentation.

Medication used to treat AEs must be recorded on the Concomitant medications/Significant non-drug therapies CRF.

## **6.9 Rescue medication**

Rescue medications are not applicable given that all patients in this study will have stable disease.

## **6.10 Concomitant treatment**

All prescription medications, over-the-counter drugs and significant non-drug therapies (including physical therapy and blood transfusions) administered or taken within the timeframe defined in the entry criteria prior to the start of the study and during the study, must be recorded on the Concomitant medications/ Significant non-drug therapies section of the (e)CRF. Medication entries should be specific to trade name, the single dose and unit, the frequency and route of administration, the start and discontinuation date and the reason for therapy.

While it is preferred that changes in prescription medications should not be made during the treatment period, changes in patient's baseline medications are allowed if deemed necessary by the investigator.

# **7 Discontinuation and study completion**

## **7.1 Discontinuation of study treatment**

Patients may voluntarily discontinue study treatment for any reason at any time.

The investigator must discontinue study treatment for a given patient if, on balance, he/she believes that continuation would be detrimental to the patient's well-being.

Study treatment must be discontinued under any of the following circumstances:

- Patient withdraws consent
- Any protocol deviation that results in a significant risk to the subject's safety
- Emergence of the following adverse events:
  - Acute myocardial infarction
  - Worsening myocardial ischemia
  - Sustained atrial or ventricular tachycardia
  - AICD discharge or anti-tachycardia pacing by the AICD in a patient with an AICD in place
  - An increase in non-sustained ventricular tachycardia or ventricular ectopy deemed clinically concerning by the investigator
  - Severe allergic reaction (e.g., angioedema)
- Occurrence of persistent elevation of systolic blood pressure to >180 mmHg or diastolic blood pressure >100 mm Hg
- Occurrence of symptomatic hypotension requiring persistent medical intervention (i.e. fluid replacement or inotropic therapy). Note that change of patient position does not qualify as an intervention if the need for this change in position is not persistent
- A drop in cardiac index of > 30% as compared to the average of all cardiac index measurements obtained prior to the start of infusion. All cardiac index values showing a 30% drop from baseline should be repeated and study drug terminated only if values are confirmed.
- Any clinically significant cardiac arrhythmia resulting in hemodynamic instability or requiring medical intervention to terminate
- Any of the following laboratory abnormalities:
  - Elevation in white blood cell count to greater than 2.5x above the ULN
  - Elevated AST or ALT > 8x above the ULN
  - Elevated AST or ALT > 3x above the ULN and TBL > 2x the ULN but without an increase in ALP to >2x the ULN
  - Elevated AST or ALT > 3x above the ULN and symptoms of malaise, fatigue, abdominal pain, nausea, vomiting, or rash with eosinophilia.

The appropriate personnel from the site and Novartis will assess whether study treatment should be discontinued for any patient whose treatment code has been broken inadvertently for any reason.

Patients who discontinue study treatment or who decide they do not wish to participate in the study further should NOT be considered withdrawn from the study UNLESS they withdraw their consent (see protocol [Section 7.3](#)). They should return to the clinic as soon as possible for the end of study visit assessments indicated in the [Assessment Schedule](#). If they fail to return for these assessments for unknown reasons, every effort (e.g. telephone, e-mail, letter) should be made to contact them as specified in protocol [Section 7.2.1](#).

Subjects who are withdrawn from the study for reasons other than safety, the decision to replace them by an equal number of newly enrolled subjects will be taken by the Sponsor.

## **7.2 Study completion and post-study treatment**

Each patient will be required to complete the study in its entirety and thereafter no further study treatment will be made available to them. Study completion is defined as when the last patient completes their End of Study visit, and any repeat assessments associated with this visit have been documented and followed-up appropriately by the Investigator, or in the event of an early study termination decision, the date of that decision.

### **7.2.1 Lost to follow-up**

For patients whose status is unclear because they fail to appear for study visits without stating an intention to discontinue or withdraw, the investigator should show "due diligence" by documenting in the source documents steps taken to contact the subject, e.g. dates of telephone calls, registered letters, etc. A subject should not be formally considered lost to follow-up until his/her scheduled end of study visit would have occurred.

## **7.3 Withdrawal of consent**

Subjects may voluntarily withdraw consent to participate in the study for any reason at any time. Withdrawal of consent occurs only when a subject:

- Does not want to participate in the study anymore, and
- Does not allow further collection of personal data

In this situation, the investigator should make a reasonable effort (e.g. telephone, e-mail, letter) to understand the primary reason for the subject's decision to withdraw his/her consent and record this information.

Study treatment must be discontinued and no further assessments conducted, and the data that would have been collected at subsequent visits will be considered missing.

Further attempts to contact the subject are not allowed unless safety findings require communicating or follow-up.

All efforts should be made to complete the assessments prior to study withdrawal. A final evaluation at the time of the subject's study withdrawal should be made as detailed in the assessment table.

Novartis will continue to keep and use collected study information (including any data resulting from the analysis of a subject's samples until their time of withdrawal) according to applicable law.

For US sites: All biological samples not yet analyzed at the time of withdrawal may still be used for further testing/analysis in accordance with the terms of this protocol and of the informed consent form.

For EU and RoW sites: All biological samples not yet analyzed at the time of withdrawal will no longer be used, unless permitted by applicable law. They will be stored according to applicable legal requirements.

#### **7.4 Study Stopping rules**

The study will be paused and no further dosing will be conducted pending a full, unblinded safety review if any of the following criteria are met:

- One or more SAE's are reported
- If 2 or more patients experience an AE that is similar and of severe grade
- If 2 or more patients have their infusion discontinued for safety or tolerability reasons
- A CLR325-treated patient develops anti-apelin antibodies
- The principal investigator and the sponsor consider that the number and/or severity of adverse events justify discontinuation of the study
- The sponsor unilaterally requests it

#### **7.5 Early study termination**

The study can be terminated at any time for any reason by Novartis. Should this be necessary, patients should be seen as soon as possible and treated as a prematurely withdrawn patient. The investigator may be informed of additional procedures to be followed in order to ensure that adequate consideration is given to the protection of the patient's interests.

The investigator will be responsible for informing IRBs/IECs of the early termination of the trial.



## 8 Procedures and assessments

**Table 8-1 Assessment Schedule**

Study Phase	Screening	Baseline	Treatment												Follow-up	EOS
Visit Numbers <sup>1</sup>	V1	V2	V3												V4	V777
Study Day(s) (windows)	-45 to 1	1	1 to 2												10 (-3 +3)	28 (-5 +5)
Time (post-dose)	-	-	-1h <sup>2</sup>	0h	1h	3h	6h	8h	10h	12h	18h	20h	24h	28h	-	-
Informed consent	X															
<b>CCI</b>		X														
Inclusion / Exclusion criteria	X	X														
Medical history/current medical conditions	X	X														
Concomitant therapies	X	X														
Demography	X															
Alcohol Test and Drug Screen	X															
Hepatitis <sup>3</sup>	X															
Pregnancy test	X	X <sup>4</sup>														
Body weight	X	X											X		X	X
Fluid Input and Output			X													
Vital Signs	X	X	X <sup>5</sup>												X	X
Physical examination	X	X											X <sup>6</sup>		X	X
Hematology	X	X					X			X	X		X		X	X
Blood chemistry	X	X					X			X	X		X		X	X
Coagulation	X	X								X			X		X	X
Urinalysis	X	X											X		X	X
28-hr urine collection			X <sup>7</sup>													

Study Phase	Screening	Baseline	Treatment												Follow-up	EOS	
Visit Numbers <sup>1</sup>	V1	V2	V3												V4	V777	
Study Day(s) (windows)	-45 to 1	1	1 to 2												10 (-3 +3)	28 (-5 +5)	
Time (post-dose)	-	-	-1h <sup>2</sup>	0h	1h	3h	6h	8h	10h	12h	18h	20h	24h	28h	-	-	
ECG evaluation	X	X		X <sup>8</sup>			X <sup>9</sup>			X <sup>9</sup>	X <sup>9</sup>		X <sup>9</sup>	X <sup>9</sup>	X	X	
<b>CCI</b>		X															
PK blood collection <sup>10</sup>	See table below																
<b>CCI</b>	See table below																
	See table below																
	See table below																
	See table below																
Immunogenicity		X													X	X	
Cardiac Hemodynamic monitoring <sup>11</sup>		X	X														
PA catheter derived Cardiac Output <sup>12</sup>			X														
Mixed venous blood gas <sup>12</sup>			X														
Telemetry cardiac monitoring <sup>13</sup>			X														
Echocardiogram <sup>14</sup>		X	X														
AICD Interrogation <sup>15</sup>		X														X	
Study drug administration				X													
Subjects domiciled			X														
Study completion information																X	
AEs and SAEs	X <sup>16</sup>																

<sup>1</sup> Visit structure given for internal programming purpose only

<sup>2</sup> (-) 1 hour assessments need to be at LEAST 1 hour prior to dose administration.

Study Phase	Screening	Baseline	Treatment												Follow-up	EOS
Visit Numbers <sup>1</sup>	V1	V2	V3												V4	V777
Study Day(s) (windows)	-45 to 1	1	1 to 2												10 (-3 +3)	28 (-5 +5)
Time (post-dose)	-	-	-1h <sup>2</sup>	0h	1h	3h	6h	8h	10h	12h	18h	20h	24h	28h	-	-

<sup>3</sup> If obtained within the past 2 months, sites may confirm with proper documentation that Hepatitis serologie's have been evaluated.

<sup>4</sup> Urine dipstick

<sup>5</sup> Vital signs will be measured manually by staff. At hour 0 a complete set of vital signs will be measured. Blood pressure and pulse rate will be measured during treatment period timepoint: 15 minutes, 30 minutes and 45 minutes post dose. A complete set of vital signs will be measured hourly thereafter, from hour 1 to hour 28. Complete vital signs consist of Blood Pressure, Pulse Rate, Body Temperature, Respiratory Rate and Oxygen Saturation. For the hourly vital sign assessments there is a window of +/- 10 minutes.

<sup>6</sup>For the physical exam performed during treatment period (at 24h), a +/- 30 min window is allowed.

<sup>7</sup> Patient should void prior to the start of the infusion. Urine collection for 28 hours will begin at the start of infusion.

<sup>8</sup> ECG may be evaluated up to 45 minutes prior to t=0.

<sup>9</sup> Assessment windows for ECGs during Treatment may be +/- 30 minutes.

<sup>10</sup> Assessment window for PK blood collection during Treatment period at 0 hour may be within 60 min prior to start of infusion. A +/- 5 min window is allowed post start of infusion up to 28 hours post infusion. See Table 6-1 of SOM.

<sup>11</sup> Continuous Cardiac Hemodynamics will be monitored using a pulmonary artery catheter, every 15 minutes from hour -1 to hour 1 in the treatment period, and hourly for the remainder of the treatment period. See SOM for more details.

### Corporate Confidential Information

<sup>13</sup> 3 lead continuous telemetry to begin pre-dose and will be discontinued up to 28 hours post infusion.

<sup>14</sup> For echocardiography cohorts only. Sites will be notified if echo will be performed (for the full cohort).

<sup>15</sup> If patient has AICD in place.

<sup>16</sup> As required

**Details for highly repetitive assessments**

Study Phase	Visit	Day	Time (post-dose)	PK blood collection		Corporate Confidential Information		
Screening	V1	-45 to 1	-				X	
Baseline	V2	1	-					
Treatment	V3	1 to 2	-1h <sup>1</sup>					
			0h	X	X	X	X	X
			0.5h	X				
			1h					
			3h	X				
			5h	X				
			6h					
			8h	X				
			10h	X				
			12h	X				
			18h	X	X	X	X	X
			20h	X				
			24h	X				
			28h	X	X	X	X	X
Follow-up	V4	10 -3 +3	-	X	X	X	X	X
EOS	V777	28 -5 +5	-	X	X	X	X	X

<sup>1</sup> (-) 1 hour assessments need to be at LEAST 1 hour prior to dose administration.

## **8.1 Informed consent procedures**

Eligible patients may only be included in the study after providing written (witnessed, where required by law or regulation), IRB/IEC-approved informed consent.

If incapable of doing so, in cases where 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 or a separate assent form.

Informed consent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the patient source documents.

The date of signing of informed consent (and withdrawal, if later withdrawn) should be documented in the CRF.

Novartis will provide to investigators a proposed informed consent form that complies with the ICH GCP guideline and regulatory requirements and is considered appropriate for this study. Any changes to the proposed consent form suggested by the investigator must be agreed to by Novartis before submission to the IRB/IEC.

Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

## **Corporate Confidential Information**

## **8.2 Subject demographics/other baseline characteristics**

Demographic and baseline characteristic data will be collected on all patients.

Relevant medical history/current medical conditions data includes data until signature of informed consent. Where possible, diagnoses and not symptoms will be recorded.

Investigators have the discretion to record abnormal test findings on the medical history CRF whenever in their judgment, the test abnormality occurred prior to the informed consent signature.

## Corporate Confidential Information

### 8.3.2 Invasive hemodynamic monitoring

For PA catheter cohorts, the clinically indicated pulmonary artery catheter placed prior to entry into the study will remain in place until the end of the treatment period. The following parameters are measured:

- Right atrial pressure (RAP) in mmHg
- Systolic and diastolic right ventricular pressure (RVP) in mmHg – only required in patients with a PA catheter with RV ports capable of measuring RV pressures.
- Systolic and diastolic pulmonary arterial pressure (PAP) in mmHg
- Pulmonary capillary wedge pressure (PCWP) in mmHg
- Cardiac output (CO) in L/min
- Brachial systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate - measured with each set of hemodynamic measurements

In addition pulse oximetry will be used to measure

- Peripheral arterial oxygen saturation SaO<sub>2</sub> in %

Derived hemodynamic parameters are:

- Systemic vascular resistance (SVR) in dynes•sec/cm<sup>5</sup>
- Pulmonary vascular resistance (PVR) in dynes•sec/cm<sup>5</sup>
- Mean PAP in mmHg
- Stroke volume (SV) in mL
- Cardiac index (CI) in L/min/m<sup>2</sup>

The hemodynamic measurements are repeated as indicated in the Site Operations Manual. In the event of any clinically significant change in hemodynamic measurements, additional hemodynamic measurements will be obtained at greater frequency as outlined in the Site Operations Manual. The hemodynamic measurements are done immediately before blood

collection at each time point. All hemodynamic parameters will be entered in the eCRF. Details of the hemodynamic measurements will be described in the Site Operations Manual.

### **8.3.3 Echocardiogram**

A limited echocardiogram will be performed for an entire dosing cohort for those cohorts designated by the sponsor as echocardiography cohorts. If the site enrolls a subject in the echocardiographic cohort, the baseline scan will be evaluated locally to confirm patients with a lateral E/E' ratio of  $<7$  will be excluded.

The complete technical details of the equipment and machine settings will be captured in a separate echocardiographic manual prepared by an imaging CRO who will perform all analysis for determining cardiac output, ventricular volumes, and left ventricular ejection fraction. In brief, a Doppler flow evaluation to determine the blood flow velocity across the aortic valve will be acquired over multiple cardiac cycles which, along with heart rate, will be used to determine cardiac output. Additional measurements of ventricular volumes (systole and diastole) and left ventricular ejection fraction will also be captured. The image data will be collected from sites by an imaging CRO for central reading and reporting of the quantitative image analysis. A detailed Echocardiogram Manual will be provided to all sites participating in this imaging assessment which includes the acquisition and image transfer details.

### **8.3.4 Mixed venous blood gas (SvO<sub>2</sub>)**

For PA catheter cohorts, a blood sample will be withdrawn from the PA catheter for a measurement of oxygen saturation (SvO<sub>2</sub>) at the times indicated in the [Assessment Schedule](#).

## **8.4 Safety**

Safety assessments are specified below; methods for assessment and recording are specified in the Site Operations Manual, with the [Assessment Schedule](#) detailing when each assessment is to be performed.

### **8.4.1 Physical examination**

A complete physical examination will include the examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, vascular and neurological. If indicated based on medical history and/or symptoms, rectal, external genitalia, breast, and/or pelvic exams may be performed.

Information for all physical examinations should be included in the source documents and will not be transferred to the sponsor as part of the study analysis. Significant findings that are present prior to informed consent are included in the Relevant Medical History section of the source documents. Significant findings observed after informed consent signature which meet the definition of an Adverse Event must be appropriately recorded on the Adverse Event eCRF section.

### 8.4.2 Vital signs

Vital signs include blood pressure (BP), pulse rate, respiratory rate, body temperature and peripheral oxygen saturation (SaO<sub>2</sub>). After the subject has been supine for approximately 5 minutes systolic and diastolic BP will be measured once using an automated validated device, with an appropriately sized cuff. In case the cuff sizes available are not large enough for the subject's arm circumference, a sphygmomanometer with an appropriately sized cuff may be used.

### 8.4.3 Height and weight

Height in centimeters (cm) (without shoes) will be measured.

Body weight (to the nearest 0.1 kilogram [kg] in indoor clothing, but without shoes) will be measured.

Body mass index (BMI) will be calculated using the following formula:

- $BMI = \text{Body weight (kg)} / [\text{Height (m)}]^2$

### 8.4.4 Laboratory evaluations

Clinically relevant deviations of laboratory test results occurring during or at completion of the study must be reported and discussed with Novartis personnel. The results should be evaluated for criteria defining an adverse event and reported as such if the criteria are met. Repeated evaluations are mandatory until normalization of the result(s) or until the change is no longer clinically relevant. In case of doubt, Novartis personnel should again be contacted.

#### Hematology

Hemoglobin, hematocrit, erythrocyte sedimentation rate, red blood cell count, white blood cell count with differential and platelet count will be measured.

#### Clinical chemistry

#### Corporate Confidential Information

Sodium, potassium, uric acid, chloride, albumin, calcium, magnesium, phosphate, alkaline phosphatase, amylase, bicarbonate, indirect bilirubin, direct bilirubin, total bilirubin, blood urea nitrogen, LDH, GGT, AST, ALT, , glucose, total cholesterol, triglycerides, C-reactive protein, t

If the total bilirubin concentration is increased above 1.5 times the upper limit of normal, direct and indirect reacting bilirubin should be differentiated.

#### Coagulation

PT (reported as INR), aPTT.



## Urinalysis

Urine test by dipstick e.g. Combur9: leucocytes, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood/ hemoglobin, specific gravity.

If the dipstick result is positive for protein, nitrite, leucocytes and/or blood, the sample will be sent for microscopic analysis of WBC, RBC and casts.

## Hepatitis screen

All subjects will be tested for Hepatitis B and Hepatitis C seropositivity at the screening visit. Hepatitis B will be evaluated using the Hepatitis B surface antigen (HBsAg). Screening for Hepatitis C will be based on HCV antibodies. Patients with a positive Hepatitis C antibody test should have HCV RNA levels measured.

Results will be available as source data and will not be recorded within the clinical database.

### 8.4.5 Electrocardiogram (ECG)

ECG evaluations will be obtained at specific time points specified in the [Assessment Schedules](#).

A standard 12-lead ECG will be performed with the subject in a supine position. Interpretation of the tracing must be made by a qualified physician and documented on the ECG and in the ECG section of the source document. Each ECG tracing should be labeled with the

- study number
- patient initials
- patient number
- date

and kept in the source documents at the study site. Clinically significant abnormalities should be recorded on the relevant medical history eCRF page prior to informed consent signature and on the Adverse Events page thereafter. Clinically significant findings must be discussed with the sponsor.

### 8.4.6 Immunogenicity

All patients dosed with CLR325 will have blood samples analyzed for antibodies targeting CLR325. In addition, cross-reactivity of anti-drug antibodies towards endogenous apelin will be assessed. Anti-CLR325 and anti-apelin antibodies will be assessed in serum by an immunoassay. Due to the size of CLR325 and apelin, all anti-drug antibodies are expected to possess neutralizing capacity.

#### Corporate Confidential Information

The timing of immunogenicity sampling is provided in the [Assessment Schedule](#), and details of sample collection are provided in the Laboratory Manual.

All blood samples will be taken by either direct venipuncture (other than the vein used for dose administration) or an indwelling cannula inserted in a forearm vein. All samples will be given a unique sample number and a collection number (as listed in Blood log within the

SOM). The actual sample collection date and time will be entered on the PK blood collection page of the source documentation. Sampling problems will be commented in the eCRF system.

#### **8.4.7 Alcohol test and drug screen**

Patients will be tested for substances of abuse (e.g., alcohol, amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, and opiates).

Results will be available as Source data and will not be recorded within the clinical database.

#### **8.4.8 AICD interrogation**

If patients have an automatic implantable cardioverter defibrillator (AICD) in place, the device will be interrogated at the baseline visit and at the end of study visit to assess any arrhythmias that occurred during the entire study period. The information gathered from the interrogation (e.g., number of runs of ventricular tachycardia) will be recorded in the eCRF.

#### **8.4.9 Cardiac Holter and telemetry monitoring**

Patients will be connected to a Holter monitor to gather data on heart rate and cardiac arrhythmias that occur during the treatment period of the study. The monitoring will begin at approximately 1 hour prior to the start of study drug infusion and disconnected prior to inpatient discharge. Given the time required to analyze Holter recordings, Holter results will not be available to site investigators prior to patient discharge. However, in addition to Holter monitoring, patients will also be monitored via real time cardiac telemetry to allow site staff to immediately detect the occurrence of any cardiac arrhythmias.

#### **8.4.10 Fluid input and output**

Fluid intake and output during the treatment period will be recorded in the appropriate section of the eCRF.

### **8.5 Pharmacokinetics**

Pharmacokinetic (PK) samples will be obtained and evaluated in all subjects. Untreated samples (placebo) will not be analyzed. The plasma samples will be analyzed to quantify CLR325 using a validated LC-MS/MS method.

For standard pharmacokinetic abbreviations and definitions see the list provided at the beginning of this protocol. The following pharmacokinetic parameters will be determined using the actual recorded sampling times and non-compartmental method(s) with Phoenix WinNonlin (Version 6.4 or higher): C<sub>max</sub>, T<sub>max</sub>, AUC<sub>0-18h</sub>, AUC<sub>last</sub>, AUC<sub>0-28h</sub>, AUC<sub>inf</sub>, T<sub>1/2</sub>, CL (CLR325 only), V<sub>ss</sub> (CLR325 only), CL<sub>r</sub>, and Ae<sub>0-28</sub> from the plasma concentration-time or urine concentration and collection volume data. The renal clearance (CL<sub>r</sub>) will be determined based on AUC and Ae available for the same time period.

The linear trapezoidal rule will be used for AUC calculation. Regression analysis of the terminal plasma elimination phase for the determination of T<sub>1/2</sub> will include at least 3 data points after C<sub>max</sub>. If the adjusted R<sup>2</sup> value of the regression analysis of the terminal phase will be less than 0.75, no values will be reported for T<sub>1/2</sub>, AUC<sub>inf</sub>, V<sub>ss</sub> (CLR325 only), and CL (CLR325 only).

### 8.5.1 PK blood collection

For details on PK blood collection and processing, labeling, and shipment instructions, see laboratory manual. The laboratory manual must be strictly adhered to. PK sample collection times are described in the [Assessment Schedule](#). Samples will be collected via syringe that will be connected to an i.v. catheter or butterfly needle and transferred into a customized tube that will be supplied by the central laboratory. These customized tubes are not sterile and must not be filled by direct phlebotomy.

The exact clock time of dosing, as well as actual sample collection date and time will be entered on the PK blood collection summary page of the source documentation. Sampling problems will be noted in the relevant field of the source documentation.

### 8.5.2 PK assessments

See Sample Blood log tables in the Site Operations Manual.

Deviations for the following assessment times are acceptable based on logistical and operational considerations:

**Table 8-2 Permitted assessment windows**

Activity	0hr	Post start of infusion
PK blood collection time-points	Within 60 min prior to start of infusion	± 5 min up to 28 hours

Every effort will be made to take the pharmacokinetic sample at the protocol specified time. Other assessments, e.g., ECG, vital signs etc. should be taken after the PK sample.

### 8.5.3 PK urine collection (28-hour urine collection)

PK urine samples will be collected to assess renal clearance of CLR325 and its active metabolite, CQJ295. Twenty-eight (28) hour cumulative urine will be collected post the start of the infusion. The urine collected from each voiding will be refrigerated (4°C). The entire collection over 28 hours will be made homogenous and the final volume and pH value will be recorded before a sample for PK analysis is taken.

For details on the sample numbering, collection, processing, labeling, and shipment instructions, see laboratory manual. The collection period is also described in the [Assessment Schedule](#). Relevant sample information will be entered on the PK urine collection summary page of the source documentation. Sampling problems will be noted in the relevant field of the source documentation.

## **8.6 Other assessments**

No additional tests will be performed on subjects entered into this study.

**Corporate Confidential Information**

## Corporate Confidential Information

### 9 Safety monitoring

#### 9.1 Adverse events

An adverse event (AE) is any untoward medical occurrence (i.e., any unfavorable and unintended sign [including abnormal laboratory findings], symptom or disease) in a subject or clinical investigation subject after providing written informed consent for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal (investigational) product.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments.

Abnormal laboratory values or test results constitute adverse events only if they fulfill at least one of the following criteria:

- they induce clinical signs or symptoms,
- they are considered clinically significant,
- they require therapy.

Clinically significant abnormal laboratory values or test results should be identified through a review of values outside of normal ranges/clinically notable ranges, significant changes from baseline or the previous visit, or values which are considered to be non-typical in patients with underlying disease. Investigators have the responsibility for managing the safety of individual subject and identifying adverse events. Alert ranges for liver related events are included in [Section 9.3](#).

Adverse events must be recorded on the Adverse Events eCRF for patients that pass screening and enter into the study. The adverse events should be reported according to the signs, symptoms or diagnosis associated with them, and accompanied by the following information:

1. The severity grade AE grade
  - mild: usually transient in nature and generally not interfering with normal activities
  - moderate: sufficiently discomforting to interfere with normal activities
  - severe: prevents normal activities
2. Its relationship to the study treatment

3. Its duration (start and end dates) or if the event is ongoing an outcome of not recovered/not resolved should be reported.
4. Whether it constitutes a serious adverse event (SAE). See [Section 9.2](#) for definition of SAE
5. Action taken regarding study treatment. All adverse events should be treated appropriately. Treatment may include one or more of the following:
  - no action taken (i.e. further observation only)
  - study treatment dosage adjusted/temporarily interrupted
  - study treatment permanently discontinued due to this adverse event
  - concomitant medication given
  - non-drug therapy given
  - subject hospitalized/subject's hospitalization prolonged
6. Its outcome (not recovered/not resolved; recovered/resolved; recovering/resolving, recovered/resolved with sequelae; fatal; or unknown)

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 drug, the interventions required to treat it, and the outcome.

Information about common side effects already known about the investigational drug can be found in the Investigator Brochure (IB) or will be communicated between IB updates in the form of Investigator Notifications. This information will be included in the subject informed consent and should be discussed with the subject during the study as needed.

The investigator should also instruct each subject to report any new adverse event (beyond the protocol observation period) that the subject, or the subject's personal physician, believes might reasonably be related to study treatment. This information should be recorded in the investigator's source documents, however, if the AE meets the criteria of an SAE, it must be reported to Novartis.

## **9.2 Serious adverse event reporting**

### **9.2.1 Definition of SAE**

An SAE is defined as any adverse event (appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s) or medical conditions(s) which meets any one of the following criteria:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly/birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
  - routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (e.g., worsening of heart failure symptoms)
  - elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug

- treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
- social reasons and respite care in the absence of any deterioration in the patient's general condition
- is medically significant, i.e. defined as an event that jeopardizes the subject or may require medical or surgical intervention.

Life-threatening in the context of a SAE refers to a reaction in which the subject was at risk of death at the time of the reaction; it does not refer to a reaction that hypothetically might have caused death if more severe.

All malignant neoplasms will be assessed as serious under “medically significant” if other seriousness criteria are not met.

Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse reaction.

Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious reactions, such as important medical events that might not be immediately life threatening or result in death or hospitalization but might jeopardize the patient or might require intervention to prevent one of the other outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization or development of dependency or abuse.

All AEs (serious and non-serious) are captured on the CRF, SAEs also require individual reporting to DS&E as per [Section 9.2.2](#).

### **9.2.2 SAE reporting**

To ensure patient safety, every SAE, regardless of causality, occurring after the subject has provided informed consent and until 30 days after the patient has stopped study participation (defined as time of last dose of study drug taken or last visit whichever is the later) must be reported to Novartis within 24 hours of learning of its occurrence as described below. Any SAEs experienced after this should only be reported to Novartis if the investigator suspects a causal relationship to study treatment.

Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode, regardless of when the event occurs. This report must be submitted within 24 hours of the investigator receiving the follow-up information. An SAE that is considered completely unrelated to a previously reported one should be reported separately as a new event.

Information about all SAEs (either initial or follow up information) is collected and recorded on the paper Serious Adverse Event Report Form. The investigator must assess the relationship to each specific component of study treatment (if study treatment consists of several drugs) complete the SAE Report Form in English, and send the completed, signed form by fax within 24 hours after awareness of the SAE to the local Novartis Drug Safety and Epidemiology Department, notifying the Study Lead. Contact information is listed in the Site Operations Manual.

The original copy of the SAE Report Form and the fax confirmation sheet must be kept with the source documentation at the study site. Follow-up information should be provided using a new paper SAE Report Form stating that this is a follow-up to a previously reported SAE.

SAEs (initial and follow-up) that are recorded electronically in the Electronic Data Capture system should be entered, saved and e-signed within 24 hours of awareness of the SAE or changes to an existing SAE. These data will automatically be submitted to Novartis Drug Safety & Epidemiology immediately after investigator signature or 24 hours after entry, whichever occurs first. Study site personnel must also inform the Study Lead.

Follow-up information provided should describe whether the event has resolved or continues, if and how it was treated, whether the treatment code was broken or not and whether the subject continued or withdrew from study participation. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs.

If the SAE is not previously documented in the Investigator's Brochure (new occurrence) and is thought to be related to the investigational treatment a Drug Safety and Epidemiology Department associate may urgently require further information from the investigator for Health Authority reporting. Novartis may need to issue an Investigator Notification (IN) to inform all investigators involved in any study with the same investigational treatment that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with EU Guidance 2011/C 172/01 or as per national regulatory requirements in participating countries.

### **9.3 Liver safety monitoring**

To ensure subject safety and enhance reliability in determining the hepatotoxic potential of an investigational drug, a standardized process for identification, monitoring and evaluation of liver events has to be followed.

The following two categories of abnormalities / adverse events have to be considered during the course of the study:

- Liver laboratory triggers, which will require repeated assessments of the abnormal laboratory parameter
- Liver events, which will require close observation, follow-up monitoring and completion of the standard base liver CRF pages

Please refer to [Table 9-1](#) for complete definitions of liver laboratory triggers and liver events.

Every liver laboratory trigger or liver event should be followed up by the investigator or designated personal at the trial site, as summarized below and detailed in in [Table 9-1](#).

For the liver laboratory trigger:

- Repeating the LFT within the next week to confirm elevation.



Repeat laboratory tests should be entered on the appropriate unscheduled local laboratory CRF page.

- If the elevation is confirmed, close observation of the patient will be initiated, including consideration of treatment interruption if deemed appropriate.

For the liver events:

- Repeating the LFT to confirm elevation as appropriate
- Discontinuation of the investigational drug (refer to [Section 7.1](#), if appropriate)
- Hospitalization of the subject if appropriate
- A causality assessment of the liver event via exclusion of alternative causes (e.g. disease, co-medications)
- An investigation of the liver event which needs to be followed until resolution.

These investigations can include serology tests, imaging and pathology assessments, hepatologist’s consultancy, based on investigator’s discretion. All follow-up information, and the procedures performed should be recorded as appropriate in the CRF, including the liver event overview CRF pages.

**Table 9-1 Liver Event and Laboratory Trigger Definitions**

	<b>Definition/ threshold</b>
Liver laboratory triggers	3 x ULN < ALT / AST < 5 x ULN 1.5 x ULN < TBL < 2 x ULN
Liver events	ALT or AST > 5 x ULN ALP > 2 x ULN (in the absence of known bone pathology) TBL > 2 x ULN (in the absence of known Gilbert syndrome) ALT or AST > 3 x ULN and INR > 1.5 Potential Hy’s Law cases (defined as ALT or AST > 3 x ULN and TBL > 2 x ULN [mainly conjugated fraction] without notable increase in ALP to > 2 x ULN) Any clinical event of jaundice (or equivalent term) ALT or AST > 3 x ULN accompanied by (general) malaise, fatigue, abdominal pain, nausea, or vomiting, or rash with eosinophilia Any adverse event potentially indicative of a liver toxicity *

**Table 9-2 Follow Up Requirements for Liver Events and Laboratory Triggers**

<b>Criteria</b>	<b>Actions required</b>	<b>Follow-up monitoring</b>
Potential Hy's Law case <sup>a</sup>	<ul style="list-style-type: none"> <li>Discontinue the study drug immediately</li> <li>Hospitalize, if clinically appropriate</li> <li>Establish causality</li> <li>Complete liver CRF</li> </ul>	ALT, AST, TBL, Alb, PT/INR, ALP and $\gamma$ GT until resolution <sup>c</sup> (frequency at investigator discretion)
<b>ALT or AST</b>		
> 8 × ULN	<ul style="list-style-type: none"> <li>Discontinue the study drug immediately</li> <li>Hospitalize if clinically appropriate</li> <li>Establish causality</li> <li>Complete liver CRF</li> </ul>	ALT, AST, TBL, Alb, PT/INR, ALP and $\gamma$ GT until resolution <sup>c</sup> (frequency at investigator discretion)
> 3 × ULN and INR > 1.5	<ul style="list-style-type: none"> <li>Discontinue the study drug immediately</li> <li>Hospitalize, if clinically appropriate</li> <li>Establish causality</li> <li>Complete liver CRF</li> </ul>	ALT, AST, TBL, Alb, PT/INR, ALP and $\gamma$ GT until resolution <sup>c</sup> (frequency at investigator discretion)
> 5 to ≤ 8 × ULN	<ul style="list-style-type: none"> <li>Repeat LFT within 48 hours</li> <li>If elevation persists, continue follow-up monitoring</li> <li>If elevation persists for <i>more than 2 weeks</i>, discontinue the study drug</li> <li>Establish causality</li> <li>Complete liver CRF</li> </ul>	ALT, AST, TBL, Alb, PT/INR, ALP and $\gamma$ GT until resolution <sup>c</sup> (frequency at investigator discretion)
> 3 × ULN accompanied by symptoms <sup>b</sup>	<ul style="list-style-type: none"> <li>Discontinue the study drug immediately</li> <li>Hospitalize if clinically appropriate</li> <li>Establish causality</li> <li>Complete liver CRF</li> </ul>	ALT, AST, TBL, Alb, PT/INR, ALP and $\gamma$ GT until resolution <sup>c</sup> (frequency at investigator discretion)
> 3 to ≤ 5 × ULN (patient is asymptomatic)	<ul style="list-style-type: none"> <li>Repeat LFT within the next week</li> <li>If elevation is confirmed, initiate close observation of the patient</li> </ul>	Investigator discretion Monitor LFT within 1 to 4 weeks

<b>Criteria</b>	<b>Actions required</b>	<b>Follow-up monitoring</b>
<b>ALP (isolated)</b>		
> 2 × ULN (in the absence of known bone pathology)	<ul style="list-style-type: none"> <li>Repeat LFT within 48 hours</li> <li>If elevation persists, establish causality</li> <li>Complete liver CRF</li> </ul>	<p>Investigator discretion</p> <p>Monitor LFT within 1 to 4 weeks or at next visit</p>
<b>TBL (isolated)</b>		
> 2 × ULN (in the absence of known Gilbert syndrome)	<ul style="list-style-type: none"> <li>Repeat LFT within 48 hours</li> <li>If elevation persists, discontinue the study drug immediately</li> <li>Hospitalize if clinically appropriate</li> <li>Establish causality</li> <li>Complete liver CRF</li> </ul>	<p>ALT, AST, TBL, Alb, PT/INR, ALP and γGT until resolution<sup>c</sup> (frequency at investigator discretion)</p> <p>Test for hemolysis (e.g. reticulocytes, haptoglobin, unconjugated [indirect] bilirubin)</p>
> 1.5 to ≤ 2 × ULN (patient is asymptomatic)	<ul style="list-style-type: none"> <li>Repeat LFT within the next week</li> <li>If elevation is confirmed, initiate close observation of the patient</li> </ul>	<p>Investigator discretion</p> <p>Monitor LFT within 1 to 4 weeks or at next visit</p>
Jaundice	<ul style="list-style-type: none"> <li>Discontinue the study drug immediately</li> <li>Hospitalize the patient</li> <li>Establish causality</li> <li>Complete liver CRF</li> </ul>	<p>ALT, AST, TBL, Alb, PT/INR, ALP and γGT until resolution<sup>c</sup> (frequency at investigator discretion)</p>
Any AE potentially indicative of a liver toxicity*	<ul style="list-style-type: none"> <li>Consider study drug interruption or discontinuation</li> <li>Hospitalization if clinically appropriate</li> <li>Establish causality</li> <li>Complete liver CRF</li> </ul>	<p>Investigator discretion</p>

\*These events cover the following: hepatic failure, fibrosis and cirrhosis, and other liver damage-related conditions; the non-infectious hepatitis; the benign, malignant and unspecified liver neoplasms

a Elevated ALT/AST > 3 × ULN and TBL > 2 × ULN but without notable increase in ALP to > 2 × ULN

b (General) malaise, fatigue, abdominal pain, nausea, or vomiting, or rash with eosinophilia

c Resolution is defined as an outcome of one of the following: (1) return to baseline values, (2) stable values at three subsequent monitoring visits at least 2 weeks apart, (3) remain at elevated level after a maximum of 6 months, (4) liver transplantation, and (5) death.

## 9.4 Renal safety monitoring

Renal events are defined as one of the following:

- confirmed (after  $\geq 24$ h) increase in serum creatinine of  $\geq 25\%$  compared to baseline during normal hydration status
- new onset ( $\geq 1+$ ) proteinuria, hematuria or glucosuria; or as a
- doubling in the urinary albumin-creatinine ratio (ACR) or urinary protein-creatinine ratio (PCR) (if applicable).

The following two categories of abnormalities/adverse events have to be considered during the course of the study:

- Serum creatinine triggers that will require follow up and repeat assessments of the abnormal laboratory parameter
- Urine dipstick triggers that will require follow up and repeat assessments of the abnormal laboratory parameter

**Table 9-3 Specific Renal Alert Criteria and Actions**

Renal Event	Actions
Serum creatinine increase 25 – 49% compared to baseline	Confirm 25% increase after 24-48h Follow up within 2-5 days
Serum creatinine increase $\geq 50\%$ compared to baseline	Follow up within 24-48h if possible Consider drug interruption Consider patient hospitalization /specialized treatment
Albumin- or Protein-creatinine ratio increase $\geq 2$ -fold Albumin-creatinine ratio (ACR) $\geq 30$ mg/g or $\geq 3$ mg/mmol; New dipstick proteinuria $\geq 1+$ Protein-creatinine ratio (PCR) $\geq 150$ mg/g or $>15$ mg/mmol	Confirm value after 24-48h Perform urine microscopy Consider drug interruption / discontinuation
New dipstick glucosuria $\geq 1+$ not due to diabetes	Blood glucose (fasting) Perform serum creatinine, ACR
New dipstick hematuria not due to trauma	Urine sediment microscopy Perform serum creatinine, ACR
Document contributing factors: co-medication, other co-morbid conditions, and additional diagnostic procedures performed in the CRF	

Monitor patient regularly (frequency at investigator's discretion) until one of the following:

Event resolution: (sCr within 10% of baseline or protein-creatinine ratio within 50% of baseline)

Event stabilization: sCr level with  $\pm 10\%$  variability over last 6 months or protein-creatinine ratio stabilization at a new level with  $\pm 50\%$  variability over last 6 months.

## **9.5 Pregnancy reporting**

To ensure patient safety, each pregnancy occurring after signing the informed consent must be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. The study drug must be discontinued, though the patient may stay in the study, if she wishes to do so. All assessments that are considered as a risk during pregnancy must not be performed. The patients may continue all other protocol assessments.

Pregnancy must be recorded on a Pharmacovigilance Pregnancy Form and reported by the investigator to the local Novartis Drug Safety and Epidemiology Department. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the Novartis study drug of any pregnancy outcome. Any SAE experienced during pregnancy must be reported on an SAE Report Form.

Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother. (Prepare and submit for EC/IRB approval the ICF to cover this with the main study ICFs).

Procedures to be followed in this case are described in the attached link:

[Link to Guideline on Prevention of Pregnancies in Clinical Trials \(go/CSSG\)](#)

## **9.6 Prospective suicidality assessment**

Not applicable.

## **9.7 Early phase safety monitoring**

The Investigator will monitor adverse events in an ongoing manner and inform the Sponsor of any clinically relevant observations. Any required safety reviews will be made jointly between medically qualified personnel representing the Sponsor and Investigator. Such evaluations may occur verbally, but the outcome and key discussion points will be summarized in writing (e-mail) and made available to both Sponsor and all Investigator(s). Criteria pertaining to stopping the study/treatment or adapting the study design are presented above.

When two or more clinical site(s) are participating in the clinical study, the Sponsor will advise the Investigator(s) at all sites in writing (e-mail) (and by telephone if possible) of any new, clinically relevant safety information reported from another site during the conduct of the study in a timely manner.

## **10 Data review and database management**

### **10.1 Site monitoring**

Before study initiation, at a site initiation visit or at an investigator's meeting, a Novartis representative will review the protocol and CRFs with the investigators and their staff. During the study Novartis employs several methods of ensuring protocol and GCP compliance and the quality/integrity of the sites' data. The monitor will visit the site to check the completeness of patients records, the accuracy of entries on the CRFs, the adherence to the protocol and to Good Clinical Practice, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications. Key study personnel must be available to assist the monitor during these visits.

### **10.2 Data collection**

Designated investigator staff will enter the data required by the protocol into the Electronic Case Report Forms using fully validated software that conforms to 21 CFR Part 11 requirements. Designated investigator site staff will not be given access to the EDC system until they have been trained. Automatic validation programs check for data discrepancies and, by generating appropriate error messages, allow the data to be confirmed or corrected before transfer of the data to CMED clinical database, Timaeus. The Investigator must certify that the data entered into the Electronic Case Report Forms are complete and accurate. After database lock, the investigator will receive a CD-ROM or paper copies of the subject data for archiving at the investigational site.

Data not requiring a separate written record will be defined in the Site Operations Manual and assessment schedule and can be recorded directly on the CRFs. All other data captured for this study will have an external originating source (either written or electronic) with the CRF not being considered as source.

### **10.3 Database management and quality control**

All data should be recorded, handled and stored in a way that allows its accurate reporting, interpretation and verification.

CMED Data Management review the data entered into the eCRFs by investigational staff for completeness and accuracy and instruct the site personnel to make any required corrections or additions. Queries are sent to the investigational site using an electronic data query. Designated investigator site staff is required to respond to the query and confirm or correct the data.

Concomitant medications entered into the database will be coded using the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system. Medical history/current medical conditions and adverse events will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

Select laboratory samples will be processed centrally and the results will be sent electronically to Novartis (or a designated CRO)

### **Corporate Confidential Information**

Holter data will be processed centrally and results will be sent electronically to Novartis (or designated CRO).

For echocardiograms, a dedicated imaging CRO will collect all echo data from the sites and send for quantitative analysis to a blinded expert reader. The analysis results will then be collected by the imaging CRO and transformed into a Novartis format defined by a data transfer specification. The transformed output will then be sent to Novartis data management for incorporation into the Clinical Study Report (CSR). The imaging CRO will be responsible for all image data clarification forms and missing data at all sites.

At the conclusion of the study, the occurrence of any emergency code breaks will be determined after return of all code break reports and unused drug supplies to Novartis.

### **Corporate Confidential Information**

#### **10.4 Data Monitoring Committee**

Not required.

#### **10.5 Adjudication Committee**

Not required

### **11 Data analysis**

Data analyses will be conducted under the supervision of Novartis personnel.

#### **11.1 Analysis sets**

For all analysis sets, subjects will be analyzed according to the study treatment(s) received.

The full analysis set will include all subjects that received any study drug or placebo.

The safety analysis set will be the same as the full analysis set.

The primary PK analysis set will include all subjects with available PK data. The secondary PK analysis set will include all subjects with available PK data and no protocol deviations with relevant impact on PK data.

## **Corporate Confidential Information**

### **11.2 Subject demographics and other baseline characteristics**

All data for background and demographic variables will be listed by treatment group and subject. Summary statistics will be provided by treatment group.

Relevant medical history, current medical conditions, results of laboratory screens, drug tests and any other relevant information will be listed by treatment group and subject.

### **11.3 Treatments (study drug, rescue medication, other concomitant therapies, compliance)**

Data for total dose of infusion, infusion rate and total time of infusion will be listed by treatment group and subject.

Data for concomitant therapies will be listed by treatment group and subject.

### **11.4 Analysis of the primary variable(s)**

The primary analyses for this study will characterize the safety of 18 hour infusion of CLR325 in patients with stableheart failure.

#### **11.4.1 Variable(s)**

The primary variable will be the occurrence of adverse events in patients in the study.

#### **11.4.2 Statistical model, hypothesis, and method of analysis**

All information obtained on adverse events will be displayed by treatment and subject.

The number and percentage of subjects with adverse events will be tabulated by body system and preferred term with a breakdown by treatment. A subject with multiple adverse events within a body system is only counted once towards the total of this body system.

The occurrence of adverse events will be examined by treatment. Both the total number of adverse events, and the preferred term for the adverse event will be compared by treatment group.

It is hypothesized that the estimated rate of serious adverse events in the treated group equal to that in the placebo group. The estimated probability of serious adverse events will be examined with 90% confidence limits around the estimated probability. Summary tables with the number, percentage, and severity of adverse events will be provided to assess safety and tolerability per treatment groups. The number and percentage of patients with adverse events will be tabulated by body system and preferred term with a breakdown by treatment group. The difference in the occurrence of adverse effects will be quantified by using the Fisher's Exact Test. Separate tables and listings will be presented indicating event severity and study drug relationship. All safety laboratory and other continuous data will be listed, and descriptive statistics will be generated.



All data for vital signs, cardiac monitoring, ECG evaluations, hematology, blood chemistry and urinalysis will be listed for each subject and summarized by descriptive statistics per treatment and for time interval where appropriate. For all these safety variables, if ranges are available, abnormalities will be flagged. The individual data and descriptive statistics will be also shown as graphical outputs. Cardiac monitoring data will be listed and descriptive statistics generated by treatment and time. Graphical presentations will be generated to show the individual time course of the data, including concentration-response plots.

Pharmacokinetic results will be summarized by descriptive statistics per treatment.

#### **11.4.3 Handling of missing values/censoring/discontinuations**

All drug concentrations below the lower limit of quantification (LLOQ) will be reported as “zero” and will be treated as zero for calculation of PK parameters.

#### **11.4.4 Supportive analyses**

Not applicable.

### **11.5 Analysis of secondary and exploratory variables**

## **Corporate Confidential Information**

#### **11.5.2 Safety**

##### **Vital signs**

All vital signs data will be listed by treatment, subject, and visit/time and if ranges are available abnormalities (and relevant orthostatic changes) will be flagged. Summary statistics will be provided by treatment and visit/time.

##### **ECG evaluations**

All ECG data will be listed by treatment, subject and visit/time, abnormalities will be flagged. Summary statistics will be provided by treatment and visit/time.

## **Clinical laboratory evaluations**

All laboratory data will be listed by treatment, subject, and visit/time and if normal ranges are available abnormalities will be flagged. Summary statistics will be provided by treatment and visit/time. The values of the laboratory evaluations will be compared by treatment.

## **Adverse events**

All information obtained on adverse events will be displayed by treatment and subject.

The number and percentage of subjects with adverse events will be tabulated by body system and preferred term with a breakdown by treatment. A subject with multiple adverse events within a body system is only counted once towards the total of this body system.

The occurrence of adverse events will be examined by treatment. Both the total number of adverse events, and the preferred term for the adverse event will be compared by treatment group.

## **Other safety evaluations**

Graphs of cardiac and safety variables will be produced by time from baseline to the end of treatment (hour 28).

## **Immunogenicity**

All immunogenicity results will be listed by subject and visit/time.

### **11.5.3 Pharmacokinetics**

CLR325 plasma concentration data will be listed by treatment, subject, and visit/sampling time point. Descriptive summary statistics will be provided by treatment and visit/sampling time point, including the frequency (n, %) of concentrations below the LLOQ and reported as zero.

Summary statistics will include mean (arithmetic and geometric), SD, CV (arithmetic and geometric), median, minimum and maximum. An exception to this is Tmax where median, minimum and maximum will be presented. Concentrations below LLOQ will be treated as zero in summary statistics and for PK parameter calculations. A geometric mean will not be reported if the dataset includes zero values. Pharmacokinetic parameters will be calculated as described in [Section 8.5](#) and will be listed by treatment and subject.

### **11.5.4 Pharmacokinetic / pharmacodynamic interactions**

Not applicable.

### **11.5.5 Other assessments**

**Corporate Confidential Information**

### **11.5.5.2 Immunogenicity**

Anti-CLR325 and anti-apelin antibodies in serum will be analyzed pre-dose, day 10 and day 28. Summary statistics will be provided for anti-CLR325 and anti-apelin antibodies. Immunogenicity will be reported using the binary assessment “Yes” or “No”. For immunogenicity positive samples (“Yes”), the corresponding titer will be reported.

### **11.6 Sample size calculation**

The sample size is based on clinical considerations for safety review of this study. With 6 active subjects in a dose cohort, there is a 74% chance of at least one subject having a particular adverse event when the event rate is 20%. With 4 active subjects in a dose cohort, this chance is 60%.

### **11.7 Power for analysis of key secondary variables**

Not applicable.

## **Corporate Confidential Information**

## **12 Ethical considerations**

### **12.1 Regulatory and ethical compliance**

This clinical study was designed and shall be implemented and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local

regulations (including European Directive 2001/20/EC, US Code of Federal Regulations Title 21, and Japanese Ministry of Health, Labor, and Welfare), and with the ethical principles laid down in the Declaration of Helsinki.

## **12.2 Responsibilities of the investigator and IRB/IEC**

Before initiating a trial, the investigator/institution should obtain approval/favorable opinion from the Institutional Review Board/Independent Ethics Committee (IRB/IEC) for the trial protocol, written informed consent form, consent form updates, subject recruitment procedures (e.g. advertisements) and any other written information to be provided to subjects. Prior to study start, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to Novartis monitors, auditors, Novartis Quality Assurance representatives, designated agents of Novartis, IRBs/IECs, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulatory authority, the investigator must inform Novartis immediately that this request has been made.

For multi-center trials, a Coordinating Investigator will be selected by Novartis around the time of Last Patient Last Visit to be a reviewer and signatory for the clinical study report.

## **12.3 Publication of study protocol and results**

Novartis assures that the key design elements of this protocol will be posted in a publicly accessible database such as [clinicaltrials.gov](http://clinicaltrials.gov). In addition, upon study completion and finalization of the study report the results of this trial will be either submitted for publication and/or posted in a publicly accessible database of clinical trial results.

## **13 Protocol adherence**

This protocol defines the study objectives, the study procedures and the data to be collected on study participants. Additional assessments required to ensure safety of subjects should be administered as deemed necessary on a case by case basis. Under no circumstances should an investigator collect additional data or conduct any additional procedures for any research related purpose involving any investigational drugs.

Investigators must apply due diligence to avoid protocol deviations. If the investigator feels a protocol deviation would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by Novartis and approved by the IRB/IEC/REB it cannot be implemented. All significant protocol deviations will be recorded and reported in the CSR.

### **13.1 Protocol Amendments**

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by Novartis, Health Authorities where required, and the IRB/IEC prior to implementation.

Only amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented, provided the Health Authorities and the reviewing IRB/IEC are subsequently notified by protocol amendment.

Notwithstanding the need for approval of formal protocol amendments, the investigator is expected to take any immediate action required for the safety of any subject included in this study, even if this action represents a deviation from the protocol. In such cases, the Study Lead should be informed and (serious) adverse event reporting requirements ([Section 9](#)) followed as appropriate.

## 14 References

- Ashley EA, Powers J, Chen M, et al. (2005) The endogenous peptide apelin potently improves cardiac contractility and reduces cardiac loading in vivo. *Cardiovasc. Res.* p. 73-82.
- Barnes GD, Alam S, Carter G, et al. (2013) Sustained cardiovascular actions of APJ agonism during renin-angiotensin system activation and in patients with heart failure. *Circ Heart Fail* p. 482-91.
- Binanay C, Califf RM, Hasselblad V, et al. (2005) Evaluation study of congestive heart failure and pulmonary artery catheterization effectiveness: the ESCAPE trial. *JAMA* p. 1625-33.
- Chong KS, Gardner RS, Morton JJ, et al. (2006) Plasma concentrations of the novel peptide apelin are decreased in patients with chronic heart failure. *Eur. J. Heart Fail.* p. 355-60.
- Digitalis Investigation Group (1997) The effect of digoxin on mortality and morbidity in patients with heart failure. *N. Engl. J. Med.* p. 525-33
- Falcone C, Buzzi MP, D'Angelo A, et al. (2010) Apelin plasma levels predict arrhythmia recurrence in patients with persistent atrial fibrillation. *Int J Immunopathol Pharmacol* p. 917-25.
- Farkasfalvi K, Stagg MA, Coppen SR, et al. (2007) Direct effects of apelin on cardiomyocyte contractility and electrophysiology. *Biochem. Biophys. Res. Commun.* p. 889-95.
- Felker GM, O'Connor CM (2001) Between Scylla and Charybdis: The choice of inotropic agent for decompensated heart failure. *Am. Heart J.* p. 932-3
- Harvey S, Harrison DA, Singer M, et al. (2005) Assessment of the clinical effectiveness of pulmonary artery catheters in management of patients in intensive care (PAC-Man): a randomised controlled trial. *Lancet* p. 472-7.
- Japp AG, Cruden NL, Barnes G, et al. (2010) Acute cardiovascular effects of apelin in humans: potential role in patients with chronic heart failure. *Circulation* p. 1818-27.
- Jong P, Vowinckel E, Liu PP, et al. (2002) Prognosis and determinants of survival in patients newly hospitalized for heart failure: a population-based study. *Arch. Intern. Med.* p. 1689-94.
- Kuba K, Zhang L, Imai Y, et al. (2007) Impaired heart contractility in Apelin gene-deficient mice associated with aging and pressure overload. *Circ. Res.* p. e32-42.
- Nagueh, AF, Middleton, KJ, Kopelen, HA, et al. (1997) Doppler tissue imaging: a noninvasive technique for evaluation of left ventricular relaxation and estimation of filling pressures. *JACC* p. 1527-33.
- O'Carroll AM, Lolait SJ, Harris LE, et al. (2013) The apelin receptor APJ: journey from an orphan to a multifaceted regulator of homeostasis. *J. Endocrinol.* p. R13-35.
- Yang, P, Maguire, JT, and Davenport, AP (2015) Apelin, Elabela/Toddler, and biased agonists as novel therapeutic agents in the cardiovascular system. *Trends in Pharmacological Sciences* p. 560-567.

Zhen EY, Higgs RE, Gutierrez JA (2013) Pyroglutamyl apelin-13 identified as the major apelin isoform in human plasma. *Anal. Biochem.* p. 1-9.