



GP-531: The New Paradigm in Heart Failure Treatment

Research Plan

July 2023

Research Plan

Executive Summary

Heart failure (HF) is the cardiovascular epidemic of the 21st century. Worldwide the prevalence of HF is estimated to exceed 60 million and its prevalence is rising, and is associated with a high risk for mortality and morbidity as well as resource utilization. Over the past two decades, major efforts have been dedicated to the investigation of the HF epidemic with the ultimate goal of reducing the burden of disease through prediction, prevention, and effective management of HF. Once developed, HF results in significant morbidity and mortality, with a 1-year mortality rate of 7.2% and a 1-year hospitalization rate of 31.9% in patients with chronic heart failure (CHF), and in patients hospitalized for acute heart failure (AHF), these rates increase to 17.4% and 43.9%.

In terms of the epidemiology, despite all of the progress made over the past 20 years in developing new therapies and devices for the treatment of CHF, the incidence of AHF has continued to rise as has the economic burden. According to the Global Burden of Disease (GBD) Collaborators, the current worldwide prevalence of HF is estimated at 64 million. Based on an American Heart Association (AHA) estimation of \$5,380 per HF case, the current worldwide economic burden of HF can be estimated at \$346 billion and is expected to increase by 2030 to an astounding worldwide expenditure of ~\$398 billion.

The mechanisms underlying the development of HF are multiple, complex, and not well understood, although considerable progress has been made as the result of intense research efforts over the past two decades. Chronic HF patients stratified by categories of left ventricular ejection fraction (EF) represent different phenotypes in terms of demographics, clinical presentation, etiology, and pharmacotherapies. HF patients are classified as HF with reduced EF (HFrEF; EF <40%), HF with mid-range EF (HFmrEF; EF 40–49%) and HF with preserved EF (HFpEF) (EF ≥50%). Approximately 47% of HF patients have HFrEF and approximately 53% have HFpEF. Guideline-directed evaluation and management (GDEM) for these patients includes renin-angiotensin system modulation (angiotensin-converting enzyme inhibitors [ACEi], angiotensin receptor blockers [ARB], or angiotensin receptor neprilysin inhibitors), beta-blockers, and mineralocorticoid receptor antagonists (MRA), with additional options like hydralazine/nitrates and ivabradine for specific clinical situations.

Despite considerable progress over the past 20 years, it is clear that the treatments for CHF have resulted in the paradox of achieving clinical success in treating acute coronary syndromes but this success has resulted in a greater incidence of cardiac dysfunction among survivors leading to decreased success treating ADHF. ViCardia is developing GP531, an adenosine mono-phosphate kinase (AMPK) agonist that targets mitochondrial dysfunction as the underlying cause of HF. In the Phase 2 clinical trial, ViCardia intends to show that GP531 increases survivability in patients hospitalized for ADHF, reduces incidences of rehospitalization, increases long term health, and improves quality of life for the HF patient hospitalized with reduced ejection fraction (HFrEF).

Summary

HF is a progressive syndrome that begins with a chronic condition that ultimately leads to ADHF, an unstable physiological condition requiring immediate medical treatment and hospitalization, marking a critical stage in the progression of HF. Currently, there is no therapy that reduces mortality risk in patients hospitalized for ADHF; and for that reason, ViCardia is developing GP531, the first therapy that is specifically designed to reduce mortality in ADHF patients. At the conclusion of the Phase 2 clinical trial, ViCardia expects to show GP531 improves clinical outcomes in ADHF, demonstrates a positive impact on treating in-hospital ADHF, and reduces length of stay without increasing 30-day events post-discharge. These goals would prove GP531 is a success, and thus demonstrating a clear pathway to a life-saving and cost-effective therapy for millions of patients worldwide.

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Specific Aims

Acute heart failure syndrome (AHFS) poses unique diagnostic and management challenges. This syndrome has recently received attention from researchers, clinicians, regulatory agencies, and the pharmaceutical industry. However, there is no consensus as to its definition, epidemiology, pathophysiology, appropriate therapeutic options, and directions for future research (1–3). The past decade of research provided convincing evidence that mitochondrial dysfunction is an important, if not the most significant event in the development of hypertrophy and HF (4). The prevailing view, supported by considerable clinical evidence, is the failing heart is “energy-deprived” and mitochondrial dysfunction is a driving force associated with this energy supply-demand imbalance in the myocardium (5). The energy imbalance is triggered by persistent episodes of cellular stress, tissue hypoxia and net adenosine triphosphate (ATP) catabolism and depletion. Eventually, myocardial dysfunction leads to reduced left ventricular ejection fraction (LVEF) and hypertrophy, ultimately resulting in CHF that progresses to ADHF.

At the present time, no treatment has been shown to improve patient outcomes (post-discharge mortality and rehospitalization) (6,7) in patients with ADHF. Existing HF therapies provide symptomatic relief by reducing cardiac workload through heart rate reduction and reduction of preload and afterload, but do not address the underlying causes of abnormal myocardial energetics nor directly target mitochondrial dysfunction. New approaches that have been evaluated over the past decade have either targeted symptoms, hemodynamics, or specific biochemical pathways of the disease process. All of these have failed to show efficacy in reducing the morbidity and mortality associated with ADHF, and some have even shown deleterious outcomes (7).

ViCardia is focused on completing the clinical development program for GP531, its lead compound, for the treatment of patients hospitalized for ADHF because there is no acute therapy that reduces mortality risk in these specific patients. GP531 is a second-generation adenosine regulating agent (ARA) (8) that acts as an AMPK agonist, that enhances the production of ATP in cardiomyocytes (9). As an energy regulator, AMPK not only improves energy supply to increase heart function, but also improves heart function by mediating various intracellular physiological functions, delaying myocardial fibrosis, and reducing heart damage. By enhancing endogenous adenosine to cardioprotective levels, GP531 has the therapeutic effect of improved bioenergetics in the myocardium which increases the contractile function of the heart and increases the LVEF, leading to higher quality clinical outcomes. Thus far, in two proof of concept canine studies with advanced HF, *GP531 has demonstrated improved global cardiac function and a significant increase in LVEF* (10). In humans, three separate Phase 1 studies, in 84 healthy volunteers, demonstrated safety and tolerability, and in a pilot Phase 2 clinical trial, with 18 patients with ischemic HF, GP531 exhibited excellent efficacy, safety, tolerability and evidence of reduced biomarkers for cardiac injury. The pre-clinical and clinical evidence demonstrate that GP531 has great potential to improve clinical outcomes in ADHF patients hospitalized for an acute event.

Specific Aim: Generating clinical evidence for the efficacy, safety and tolerability of GP531. ViCardia proposes conducting a Phase 2, multicenter, randomized, double-blind, placebo-controlled, dose-escalation study to evaluate the safety, tolerability, hemodynamic and symptomatic effects of GP531 in subjects hospitalized with ADHF and reduced LVEF. Subjects will be stratified according to ischemic vs. non-ischemic HF etiology. Approximately 150 subjects will be enrolled from approximately 15 sites in the United States. GP531 will be administered at 5 escalating doses vs. placebo. All subjects enrolled in the clinical trial will receive standard treatment in accordance with guideline-directed evaluation and management (GDEM).

**Efficacy endpoints of the trial will include:**

- Hemodynamic assessments of left ventricular function by echocardiographic imaging before the start of the infusion, and just prior to the end of infusion (approximately 20-24 hours post infusion start) or immediately following completion of infusion.
- Comparison of changes between treatment and control groups in cardiac injury biomarkers: BNP and Troponin I from baseline to 24h, 48h, 72h, 96h (or on discharge if before 96h) and 8 days post randomization.
- Follow-up telephone interviews will be conducted at day 30 and at day 60 post randomization with the subject, or a member of the subject's family, to confirm survival or clinical status (re-hospitalizations).

Success criteria:

- 1) Achieving reduced overall mortality in ADHF patients treated with GP531 – a significant reduction in the present mortality rate of approximately 20% within 60 days post discharge and a significant reduction in the rate of rehospitalization of 20% (11) within 30 days, and without mechanical assist device;
- 2) Improvement in LVEF from <35% after hospital admission to >41% after GP531 treatment;
- 3) Identification of the optimal GP531 dose(s) for the subsequent Phase 3 clinical trial.

Next Steps: Meeting the success criteria of the Phase 2 clinical trial will advance our discussions with the FDA and with financial and strategic partners for the preparation of the regulatory approval for a Phase 3 clinical trial.

Research Strategy

1. Significance

The burden of acute decompensated heart failure: high mortality rates following hospitalization.

As described in the Executive Summary, Heart Failure is the cardiovascular epidemic of the 21st century (12–14). HF also constitutes a major clinical problem because it is not a disease but a syndrome with an elusive pathophysiology (11,15) associated with a high risk for mortality and morbidity as well as high resource utilization (14). Acute decompensated heart failure (ADHF) is the new onset of severe HF or the sudden intensification of chronic HF, and is a life-threatening condition that usually requires hospitalization (3). ADHF is considered an unstable condition where standard treatment is, by definition, insufficient, and additional interventions must be considered (16,17). Current treatment of ADHF is mostly symptomatic, centred on decongestive drugs, at best tailored according to the initial haemodynamic status with little regard to the underlying pathophysiological particularities (15).

In the United States, HF affects approximately 6.2 million people, of which 1.1 million are admitted annually to the hospital with ADHF, accounting for 6.5 million hospital days and a substantial portion of the estimated \$45.2 billion that is spent each year on HF in the United States (18,19).

Patients with a primary diagnosis of HF now make >3 million physician visits per year. The direct and indirect costs of HF in the United States are staggering: in 2017 they were estimated to be US \$31 billion and expected to increase to \$70 billion by 2030 (Fig. 1.1) (3,20).

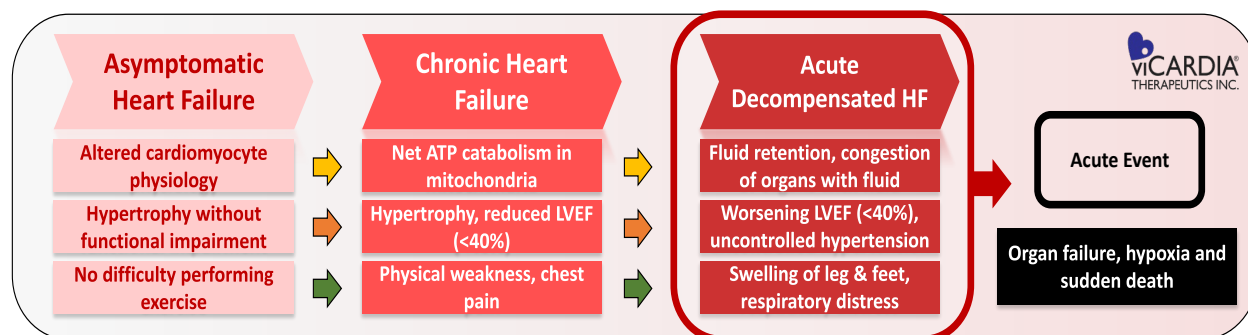


Figure 1.1: The progression of heart failure into acute decompensated heart failure and acute events.

Approximately 47% of hospitalized patients have HF with reduced ejection fraction (HFrEF) and 53% have preserved or relatively preserved ejection fraction (HFpEF). However, both classifications have similar post-discharge event rates (21). Once developed, HF results in significant morbidity and mortality, with a 1-year mortality rate of 7.2% and a 1-year hospitalization rate of 31.9% in patients with chronic heart failure, and in patients hospitalized for acute heart failure, these rates increase to 17.4% and 43.9% (6). The estimated lifetime cost of HF per individual patient is \$110,000/year, with more than three-fourths of this cost consumed by in-hospital care (22).

ADHF presents a major challenge to patients, physicians, and healthcare systems. Patients with ADHF experience a progressive deterioration in their quality of life (QoL), with worsening clinical status that requires frequent and sometimes prolonged hospitalizations. Progression of HF leads to prolongation of hospital stays, with consequences for the cost of care, while hospitalizations for ADHF are significant predictors of increased mortality risk per se. Each time a patient is hospitalized for ADHF there is a risk of further worsening of myocardial function, leading to further episodes of hospitalization. The growing complexities of case management as this cycle repeats itself add further to the cost of care. Cardiovascular and non-cardiovascular conditions combine to increase rates of ADHF hospital readmission at 30 days (23).



Rehospitalization after the initial hospitalization for an acute event is one of the main issues affecting patients' short- and long-term prognosis.

Although some progress has been made in reducing mortality in patients hospitalized with ADHF, rates of rehospitalization for ADHF continue to rise in the US, and approach 30% within 60 to 90 days of discharge (24). This is despite the advances of treating in-hospital patients with therapies such as diuretics, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), β blockers, and mineralocorticoid receptor antagonists (MRAs). Attempts at developing novel therapeutic agents for ADHF over the past two decades have been marked by disappointment (25).

Despite the efficacy of many therapies for patients with HF, to date, hospital admissions for ADHF continue to increase and no new therapies have improved clinical outcomes. There is an unmet need for increased individualized in-hospital management, including treatments targeting the causative factors, and continuation of treatment after hospital discharge to improve long-term outcomes.

An engine out of fuel: insufficient ATP as an important driver of HF.

The primary goal of treating HF patients is restoration of cardiac function. Recent studies show that heart function can be successfully recovered in patients with HF, even after structural alterations have occurred. The heart cycles about 13.4 lbs between Adenosine Tri-Phosphate (ATP) and Adenosine Di-Phosphate (ADP) every day (26). Adequate amounts of ATP must be generated to support the heart's contractile demands and maintain viability for the other cells and organs in the body. **Abnormalities in cardiac metabolism, such as a lack of ATP-ADP metabolism, causes mechanical failure of the heart** (27). Although virtually all aspects of myocyte physiology are altered in HF, the past decade of research provided convincing evidence that mitochondrial dysfunction may be one of the most important events in the development of hypertrophy and HF (4). HF is the result of a variety of pathophysiological processes originating from dysfunctional metabolism, mitochondrial dysfunction, inflammation, and apoptosis. As an energy regulator, AMPK not only improves energy supply to increase heart function, but also improves HF and heart function by mediating various intracellular physiological functions, delaying myocardial fibrosis, and reducing heart damage (28).

Current therapies for HF produce benefit by reducing cardiac workload, by lowering heart rate and loading conditions, which reduces myocardial energy demands. The recent recognition that the failing heart is "energy deprived" and that mitochondrial dysfunction is a driving force associated with this energy imbalance in the heart has led to the evaluation of mitochondria as a therapeutic target in HF. Mitochondrial dysfunction in the heart leads to reduced ATP synthesis and excessive formation of damaging ROS (5).

Current therapeutics for HF, including various ACE inhibitors, aldosterone antagonists, beta-receptor blockers, diuretics, and combinations thereof, all target HF symptoms such as hypertension, congestion and arrhythmias. However, none of these approved therapies improve functional capacity of the heart by addressing the critical underlying mitochondrial pathophysiology responsible for mechanical failure.

Therefore, therapies that modulate cardiac metabolism, and increase ATP output, are pivotal in effectively addressing HF and ADHF in particular. The principal cause of limited ATP supply to the heart is found in malfunctioning mitochondria of cardiomyocytes. Dysfunctional mitochondria produce less cellular energy (ATP) and produce increased levels of reactive oxygen species (ROS), both of which lead to oxidative stress and ultimately to HF (9,29). Moreover, beyond their role as a failed 'power plant', other pathophysiological mechanisms of the mitochondria that contribute towards HF include bottlenecks of metabolic flux, redox imbalance, protein modification, ROS-induced ROS generation, impaired mitochondrial Ca^{2+} homeostasis and inflammation (30).

GP531, like Acadesine, its parent drug, activates AMPK by functioning as an AMP mimetic. Acadesine is taken up by adenosine transporters in cells and then converted to ZMP, which mimics all of the effects of AMP on AMPK. However, unlike Acadesine, GP531 is taken up without being converted to ZMP. This is reflected in the fact that GP531 is largely expelled intact in the urine without being metabolized to uric acid, as is the case with Acadesine. As shown in Figure 1.3 on the next page, GP531 acts directly on AMPK and on AMP.

Mitochondrial biogenesis: a potential HF remedy with cardioprotective effects.

Mitochondria are dynamic organelles, continuously undergoing biogenesis through fission, fusion, and autophagy (31). In addition to effects on metabolism, AMPK also regulates mitochondrial biogenesis, autophagy, cell polarity, cell growth and proliferation (32,33). Severe energetic stress is harmful to mitochondria and, over time, all mitochondria become damaged and need to be replaced. The metabolic changes observed in the mitochondria are considered a critical landmark in the development of HF. Therefore, mitochondrial pathophysiology provides an important therapeutic target for reviving the contractile function of the myocardium, reversing events leading up to HF (4). Currently no drugs specifically target mitochondrial biogenesis in HF, and GP531 as an AMPK activator represents a promising therapeutic approach (4).

GP531: a small-molecule drug that enhances the energy output of the mitochondria in HF patients.

GP531 is a second-generation member of a novel class of compounds known as “Adenosine Regulating Agents” (ARAs). The principal effects of ARAs are mediated by the localized augmentation of endogenous adenosine levels (Figure 1.2). Importantly, their effects are not mediated by conversion of the drug to adenosine or by any direct activity at the adenosine receptors. Endogenous adenosine is a natural defense against myocardial injury via a number of mechanisms. It is released from the breakdown of ATP during episodes of cellular stress, as occurs in ischemia and/or hypoxia (34). At the molecular level, endogenous adenosine acts as a retaliatory metabolite that counters ATP catabolism and depletion, and as such, acts as a key regulator of cellular energetics (35). At the cellular level, endogenous adenosine protects the cell from multiple pathways of injury, including inflammation, apoptosis and necrosis, all of which are major contributors to myocardial injury and global myocardial dysfunction (34,36). Myocardial injury is evidenced by elevations in troponin in a majority of patients hospitalized with HF (37), and troponin release is associated with a worsening prognosis (38).

Elevated endogenous adenosine levels have been identified in HF patients, but levels are not sufficient to be cardioprotective (39). **The clinical utility of exogenous adenosine administered systemically is limited by undesirable peripheral hemodynamic effects and adverse effects on cardiac conduction (40).**

Improved transplant-free survival has been observed in HF patients with a mutant adenosine monophosphate deaminase1 (AMPD1) gene that is associated with enhanced endogenous adenosine production (41). ARAs are compounds that selectively increase endogenous adenosine levels during episodes of cellular stress in which the breakdown of ATP exceeds ATP synthesis. As a consequence of adenosine’s extremely short half-life (<1 second), increases in endogenous adenosine levels elicited by ARAs are restricted to their site of formation, where they are most needed (42), resulting in fewer side effects.

In addition, ARAs are only activated in the event of ATP catabolism, when cardiomyocytes do not produce sufficient energy to power myofibrillar contractions, such as in an episode of ischemia and/or tissue hypoxia (43). In the event of homeostasis and a healthy ADP-ATP, ARAs are pharmacologically silent.

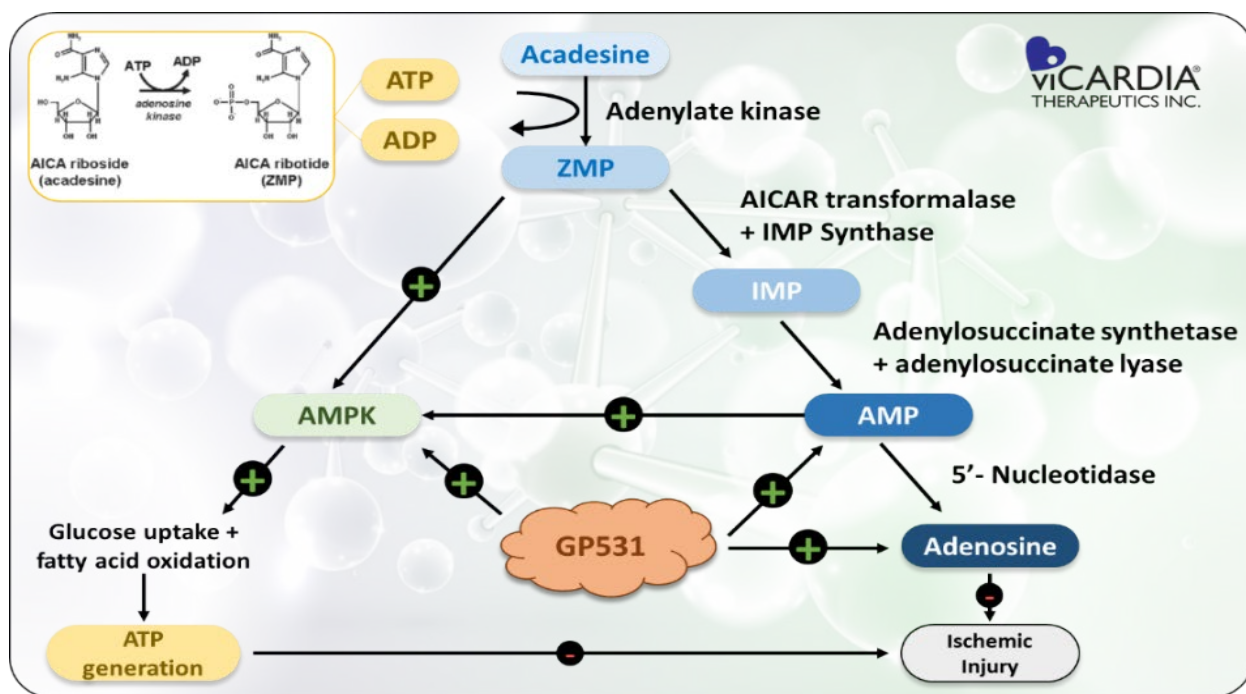


Figure 1.2: Mechanism of action for GP531, an acadesine analogue, including direct activation of AMPK and indirect augmentation of endogenous adenosine, reducing cardiac injury. GP531 is not metabolized to a great extent and does not bind to plasma protein. Unlike acadesine, it is largely expelled intact in urine without being metabolized to uric acid.

Target Product Profile (TPP) of GP531.

ViCardia developed a preliminary TPP for GP531, which specifies the labeling concepts that are the goals of its drug development program (Table 1.1).

Table 1.1: Target product profile of GP531.

Product Name	GP531 is the laboratory code designation for 5-amino-1-β-D-(5-benzylamino- 5-deoxyribofuranosyl) imidazole-4-carboxamide L-tartrate.
Molecular Formula	$C_{16}H_{21}N_5O_4 \cdot C_4H_6O_6$
Molecular Weight	497.97 (anhydrous)
Mechanism of Action (MoA)	<p>GP531 is a potent adenosine mono-phosphate kinase (AMPK) agonist that is administered intravenously, stimulates the production of ATP and promotes mitochondrial biogenesis in the myocardium.</p> <p>Unlike acadesine, the parent drug which is first converted to AICA ribotide (ZMP) by adenylate kinase (44,45), which then through dual pathways activates AMPK, <u>GP531 directly activates AMPK and as a result leads to increased ATP output and improved mechanical function of the heart.</u></p>



Indication	<i>Acute Decompensated Heart Failure (ADHF)</i>		
Primary Efficacy Endpoints: <i>baseline by echo-cardiography</i>	Minimal: Documented ejection fraction within the last 12 months or during current hospitalization of $\leq 35\%$ as measured by any method.	Target: Documented ejection fraction during current hospitalization or immediately prior to discharge (usually at 24 hours post-dosing) of $>41\%$ as measured by any method.	Optimal: Documented EF during current hospitalization or immediately prior to discharge (usually at 24 hours post-dosing) of $>49\%$ as measured by any method.
Primary Efficacy Endpoints <i>Survivability</i>	<i>Achieving a significant reduction of post discharge mortality in patients suffering from ADHF treated with GP531 versus the standard-of-care (i.e. loop diuretics and vasodilators) baseline mortality, at least 60 days post discharge without rehospitalization and without mechanical assist device is a primary efficacy endpoint. At day 30 and again at day 60, post randomization, study personnel at each clinical site will conduct a telephone interview with the Subject, a relative of the Subject, or a guardian/caregiver to determine the survival status (and the timing and cause of the subject's death if applicable) or re-admission to hospital and the reason for re-hospitalization if applicable. SAEs will be collected out to the 30 day telephone contact. The day 60 information collected will be utilized for endpoint reasons.</i>		
Secondary Efficacy Endpoints <i>Biomarkers</i>	Minimal: <ul style="list-style-type: none"> BNP ≥ 400 pg/mL NT-proBNP ≥ 1600 pg/mL Troponin I – baseline measurement 	Target: Comparison of changes between GP531 and placebo for each cohort in cardiac injury biomarkers assessed by BNP and Troponin I from baseline to 24h, 48h, 72h, 96h (or on discharge if before 96h) and 8 days post randomization will be assessed using t-tests.	Optimal: Comparison of changes between GP531 and placebo for each cohort in cardiac injury biomarkers assessed by BNP and Troponin I from baseline to 24h, 48h, 72h, 96h (or on discharge if before 96h) and 8 days post randomization will be assessed using t-tests.
Secondary Efficacy Endpoints <i>Methodology</i>	<ul style="list-style-type: none"> Cardiac biomarkers of myocardial injury (Troponin I and BNP) will be measured from blood samples taken at baseline, 8 hours after the start of infusion, at the end of infusion (~24 hours), and again at approximately 48, 72 and 96 hours after the start of the infusion or at discharge from the hospital and again at 8 days post-randomization. Rescue medications (IV inotropes and/or IV vasodilators) initiated after initiation of the infusion will be recorded. Comparison of changes between treatment and control groups in cardiac injury biomarkers assessed by BNP and Troponin I from baseline to 24h, 48h, 72h, 96h (or on discharge if before 96h) and 8 days post randomization. 		

Preclinical supportive evidence for the effectiveness of GP531.

GP531 has shown impressive activity in a canine model of advanced HF. Six dogs with intracoronary microembolization-induced HF received GP531 (constant intravenous infusion, 10 µg/kg/min or normal saline vehicle) for 6 hours in random order and demonstrated significantly decreased LV end-diastolic pressure, end-diastolic volume, end-systolic volume and end-diastolic wall stress. In addition, GP531 significantly increased LVEF (27 ± 1 at baseline to 34 ± 1 after 6 hours of drug infusion, $p < 0.05$) deceleration time of early mitral inflow velocity and the slope of end-systolic PVR without increasing MVO₂ (Fig. 1.3) (10). In addition, elevated HF serum-biomarkers such as NT-pro and BNP and Troponin I were decreased. These results implicate that the local release of adenosine (by ARAs) in the LV myocardium enhance the energy output and thus the LV performance (10). **Importantly, GP531 did not cause arrhythmias.**

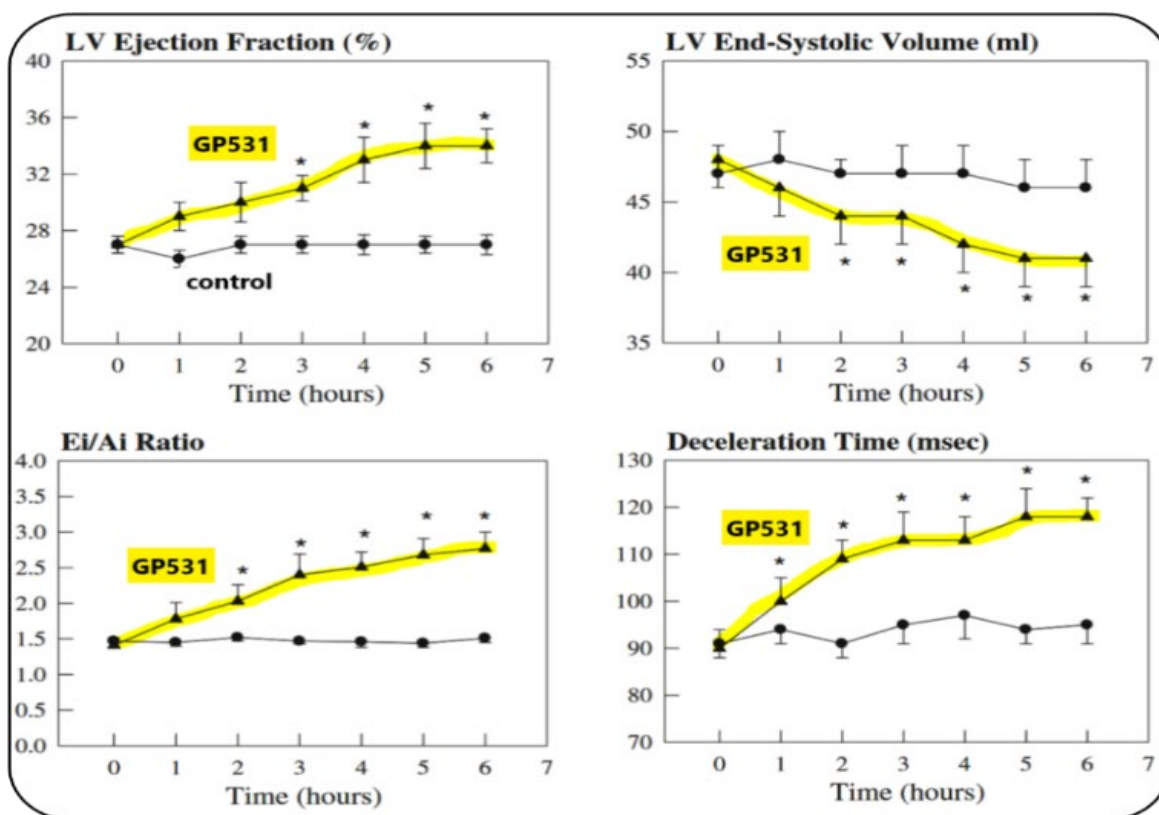


Figure 1.3: Acute intravenous infusion of GP531 improves myocardial function in dogs with advanced heart failure (adapted from Wang et al., 2013).

Clinical supportive evidence for the safety and tolerability of GP531.

Four clinical studies were conducted to evaluate the safety and tolerability of GP531 in humans (Table 1.2).

Table 1.2: Summary of Phase 1-2 clinical trials that evaluated GP531.

Study #, & Location	Study Description	Objectives	Design	# of Subjects
CS1301 Phase 1 (UK)	<i>Dose-ranging study to assess safety and tolerability of GP531 administered IV for 6 hours</i>	<ul style="list-style-type: none"> Assess the safety and tolerability of IV GP-531 in normal subjects Assess the pharmacokinetics of IV GP-531 in normal subjects. 	<i>Single-center, single-blind, randomized, placebo-controlled, ascending dose study in normal subjects</i>	40 (male) 6 (female)
CS1302 Phase 1 (UK)	<i>Safety and tolerability of GP531 following oral administration and at steady state plasma levels using a loading dose and extended duration of IV administration</i>	<ul style="list-style-type: none"> Assess the safety and tolerability of Oral and IV GP531 Assess the pharmacokinetics of oral and IV GP531 Assess the absolute oral bioavailability of GP531 Assess the effect of a high fat meal on the pharmacokinetics of oral GP531 	<i>Single-center, single-blind, randomized, placebo-controlled, crossover, ascending dose study in normal subjects</i>	30 (male)
CS1310 Phase 1 (Baltimore, MD)	<i>A study the absolute oral bioavailability of GP531 in normal, fed, male subjects</i>	<ul style="list-style-type: none"> Assess the absolute oral bioavailability of GP531 in normal, fed, male subjects, determined following a 2-hour IV infusion of 150mg of GP531 and oral administration of 1500mg of the drug solution To assess the effect of GP531 on bleeding time 	<i>Single-center, open-label, randomized, crossover study</i>	9 (male)
CS1304 Phase 2a (Gainesville, FL)	<i>Dose finding study in CAD subjects with chronic stable angina undergoing exercise stress testing</i>	<ul style="list-style-type: none"> Assess the effects of IV GP531 on exercise induced ischemia in subjects with stable exertional angina Assess safety and tolerability of IV GP531 Assess the pharmacokinetics of IV GP531 in subjects with CAD 	<i>Single-center, pilot study</i>	18 (male)

Three separate clinical phase 1 studies were completed in 84 healthy volunteers to evaluate GP531, showing an excellent safety and tolerability profile:

- In **study 1301**, a total of 36 normal subjects received an IV infusion of GP531 at doses of 50, 150, 300, 450 and 600 µg/kg/min for six hours. All subjects received the entire infusion of the study drug and there were no deaths, serious adverse events (SAEs) or premature withdrawals due to AEs.
- In **study 1302**, eighteen normal subjects received a 72-hour IV infusion of GP531 at doses of 50, 100, and 200 µg/kg/min one week following a single oral dose of 50, 1500, and 3000mg, respectively. All subjects received the entire infusion of the study drug, and all three doses were well tolerated.
- In **study 1310**, eight male subjects were randomized to receive 150 mg of GP531 or placebo administered IV over two hours and, in a separate dosing, 1500mg of GP531 or placebo administered orally in solution. IV and oral dosing was separated by seven days and their order was randomly assigned. Both the oral and the IV doses were each preceded by the ingestion of a fatty meal. There were no deaths, SAEs or discontinuations due to an AE in this study.

The pharmacokinetic data obtained from these initial studies showed that GP531 exhibits good dose proportionality. The half-life ($T_{1/2}$) of the IV drug is between 6 and 8 hours. Approximately 84% of theoretical steady state of GP531 in plasma was achieved at the end of a 6-hour infusion. Renal clearance (CL_R) of GP531 accounted for approximately 83% of the total CL of the drug in the Phase 1 study 1301 and ranged from 59%-97% in study 1302. *Oral bioavailability of GP531 (~9%)* is poor due to poor absorption. In addition, a pilot double-blind, placebo-controlled **phase 2 pilot clinical trial** was completed to evaluate the effects of GP531 on exercise-induced ischemia in 18 patients with Coronary Artery Disease (CAD) and stable exertional angina. Fifteen subjects received doses of GP531 of 5, 50 or 150 µg/kg/min given IV for 18 hours after a 10-minute loading dose that was given at a rate five times the maintenance rate. Three subjects received placebo. All eighteen subjects completed the study and all three doses of GP531 were well tolerated. There were no deaths, SAEs, or discontinuations due to an AE in this study. Plasma concentrations of GP531 in the Phase 2a study were dose proportional in subjects and were slightly higher than plasma concentrations seen in normal subjects enrolled in Phase 1 studies at similar doses. Plasma clearance of GP531 was reduced by about 25% in subjects with CAD in comparison to clearance in younger normal subjects. CL_R accounted for 56-60% of total CL. These subjects with CAD were substantially older (mean 59.3 ± 8.0 years, range 48-70 years) than the normal subjects studied in the earlier Phase 1 studies, mean age was 33 ± 10 (range 20-58).

Impact on clinical practice: reducing hospital re-admissions and improving quality-of-life for HF patients.

In the Phase 2 clinical trial, ViCardia intends to show that GP531 increases survivability in patients hospitalized for ADHF, reduces incidences of rehospitalization, increases long term health, and improves quality of life for the ADHF patient hospitalized for an acute event (46–49). Due to the therapeutic effect of GP531, ViCardia expects to demonstrate a significant increase in the LVEF from <35% at enrolment in the study to >41% at discharge following treatment with GP531. This effect is expected to be durable due to GP531's underlying mechanism-of-action, reviving the myocardium and increasing the mechanical function of the myocardium. The immediate benefit of the treatment with GP531, is expected to result in fewer patients being rehospitalized at 30 days and at 60 days. At the conclusion of the Phase 2 clinical trial, ViCardia expects to show GP531 improves clinical outcomes in ADHF, demonstrates a positive impact on treating in-hospital ADHF, and reduces length of stay without increasing 30-day events post-discharge. Meeting these important clinical outcomes will support the commercial success of GP531, and thus demonstrating a clear pathway to a life-saving and cost-effective therapy for millions of patients worldwide (24).

Scientific impact: targeting of mitochondrial dysfunction in a broader context.

Successful completion of the proposed Phase 2 study will enable ViCardia to further improve the understanding of the pathophysiology of HF and better explain the mechanism of ADHF (28). The successful completion will also lay the groundwork for the Phase 3 clinical trial. Because under pathological conditions, AMPK in HF patients is activated as a response to stress and helps restore energy supply as part of the mitochondrial function, clinical knowledge gained regarding the efficacy of GP531 may be applied in a broader context to increase our understanding of mitochondrial dysfunction in other disease areas (50,51). Since the discovery of AMP-activated protein kinase (AMPK) as a central regulator of energy homeostasis, many exciting insights into its structure, regulation and physiological roles have been revealed (33). Over the last 30 years since the formal naming of AMPK by Dr. Hardie and his colleagues (52), AMPK has been shown to be the primary energy sensor and regulator of energy homeostasis in eukaryotes. It is activated by energy stress in response to increased ATP consumption (e.g., exercise, cell proliferation, anabolism) or decreased ATP production (e.g., low glucose levels, oxidative stress, hypoxia), which are sensed as low ratios of ATP to AMP and ADP (44,53). Due to its central roles in cellular metabolism, AMPK activation and mitochondrial dysfunction are associated with various disease conditions beyond HF, including neurological disorders, metabolic disorders and the general aging process of individual cells with further consequences to overall health and longevity. For example, AMPK is dysregulated in diabetes, obesity, cardiometabolic disease, and cancer. Because mitochondrial dysfunction plays a central role in abnormal glucose metabolism and insulin resistance in **type 2 diabetes** (54), AMPK is a promising pharmacological target for the treatment of type 2 diabetes (55,56). While the relationship between the mitochondria and diabetes is complex, therapeutic interventions that stimulate mitochondrial biogenesis may be important for effective management of diabetes (57). In addition, the relationship between mitochondrial dysfunction and **Parkinson's Disease (PD)** has been studied for decades, in particular the central role of oxidative stress in the pathogenesis of PD (58). Although therapeutics that target mitochondrial defects in PD have been clinically evaluated these have failed to show benefit (59). However, this lack of success was attributed to a limited number of appropriate biomarkers to measure drug efficacy and the incomplete understanding of molecular pathways in emerging PD pathogenesis.

2. Innovation

Limitations of contemporary ADHF treatment.

Treatment of ADHF has progressed little over the past 30 years (15), and neither have the chances of survival following hospital admission, both directly and one year after follow-up (60). To date, no drug has been shown to improve the survival rate in patients with ADHF (15). An overview of contemporary treatment options is provided below and in Figure 2.1.

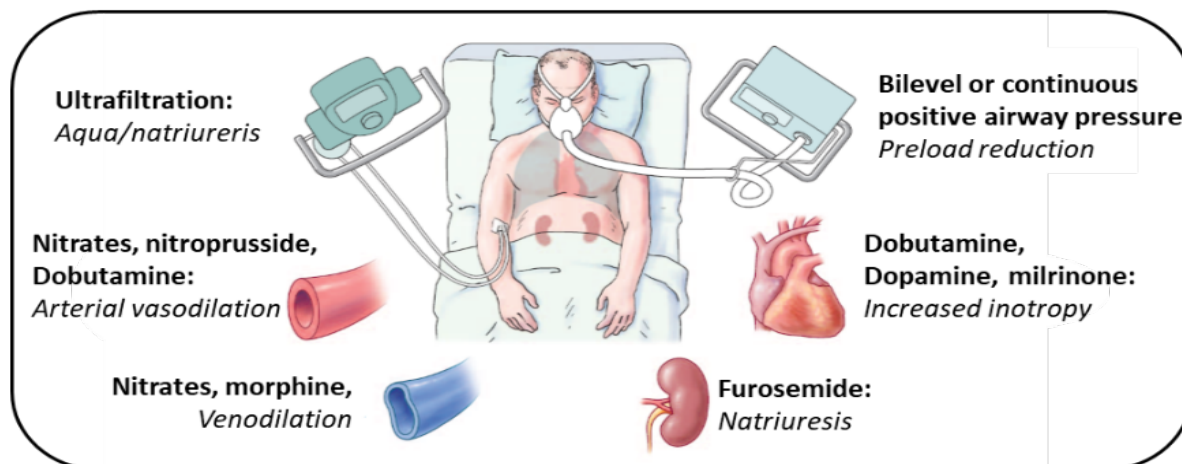


Figure 2.1: Therapies and targets used for management of ADHF (adapted from Allen & O'Connor, 2007).

The clinical presentation of ADHF is characterized mostly by symptoms and signs related to systemic congestion (that is, extracellular fluid accumulation, initiated by increased biventricular cardiac filling pressures). Accordingly, the initial treatment in most patients with AHF consists of non-invasive ventilation and intravenous diuretics, often in combination with short-acting vasodilators. Despite our expanding knowledge on the pathophysiology of HF as it progresses to ADHF, current therapeutic approaches are largely limited to inhibition of neuroendocrine activation, reduction of myocardial oxygen consumption through ventricular unloading, and reduction of heart rate (31). The most commonly prescribed therapies for patients with HFrEF are angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), β blockers, and mineralocorticoid receptor antagonists (MRAs) (61).

Loop diuretics are prescribed in reducing elevated pressures that are central to ADHF pathophysiology. However, while effective in treatment of congestive HF, loop diuretics do not show significantly improved health outcomes in ADHF treatment to date (62). **Vasodilators** reduce cardiac oxygen demand and congestion, thereby achieving acute improvements in an ADHF setting. Other than its acute benefits, vasodilators have not been able to lower mortality rates nor improve post-discharge outcomes in the ADHF setting (63). **Natriuretic peptides** are an additive therapy for ADHF patients receiving loop diuretics. While natriuretic peptides significantly reduce elevated blood pressures, their use is controversial due to questionable efficacy and safety (7). **Inotropes** are effective in improving short-term cardiac output and stabilizing hemodynamics, but their use is controversial due to increased long-term mortality and a high potential for adverse events (64).

Even with the best of modern therapy, hospitalization for ADHF is still associated with high 30-day, 60-day and 1-year mortality rates (61). The search for better treatments is one of the major challenges in cardiology (26).

AMPK: a key-enabler of healthy bioenergetics in the mitochondria of cardiomyocytes.

Importantly, current treatments provide short-term stabilization and symptomatic relieve of ADHF, reducing the need for mechanical ventilatory support, minimizing risks of cardiac ischemia and re-hospitalization. Nonetheless, underlying pathology remains unaddressed so that rehospitalization and mortality over the next 3-12 months remain unacceptably and unnecessarily high.

GP531 acts as an AMP-activated protein kinase (AMPK) agonist that stimulates metabolic substrate uptake (44,45). AMPK phosphorylation is directly promoted by AMP and ADP binding, and therefore linked to the cellular energy status that is reflected by the AMP/ATP and the ADP/ATP ratios. AMPK is the molecular sensor that monitors the energy status of cells by sensing the relative levels of AMP, ADP and ATP (9), is activated as cellular energy falls, and is also a key node governing the heart's response to exercise, regulating energy utilization and energy-requiring cellular functions such as cell division, and autophagy. Additionally, AMPK acts as a glucose sensor, becoming activated as cellular glucose is depleted independent of adenine nucleotide concentrations. Its overall effect is the discrete regulation of cellular metabolism to the benefit of the cell (65). As such, AMPK provides an excellent drug target and various preclinical models have been established (33).

Importantly, the mechanism of GP531's action is site-specific, whereby endogenous adenosine levels are increased only in areas of ischemia/hypoxia where ATP undergoes net catabolism (43). GP531 is also event-specific, augmenting adenosine only during episodes of cellular stress leading to net ATP catabolism as occurs under conditions of ischemia and/or hypoxia. The significance of these observations is that therapeutic levels of GP531 are pharmacologically silent in normally metabolizing tissues, resulting in no

direct cardiac or systemic hemodynamic effects. Therefore, AMPK agonists, and in particular GP531, may hold the premise for a new generation of effective, targeted, disease-reversing HF therapeutics, significantly shifting the current clinical practice paradigm that currently focusses on short-term stabilization and symptomatic relieve, resulting in a high risk of relapse and hospital re-admissions.

Historical efforts and barriers of addressing AMPK as a therapeutic target for HF.

Acadesine is the prototype in this class of therapeutic agents, i.e., ARAs functioning as AMPK agonists. First-generation acadesine compounds have been evaluated in five randomized, placebo-controlled trials in an attempt to address mitochondrial dysfunction in HF. These trials were conducted in the setting of coronary artery bypass graft (CABG) surgery and enrolled a total of 4,043 patients. In a meta-analysis of these trials, there was statistically significant 26% reduction in composite events (cardiac death, myocardial infarction, and stroke) in patients undergoing CABG ($p=0.01$) (66). These beneficial effects were observed in the absence of direct effects on pre-ischemic cardiac function or peripheral hemodynamics. The clinical experience with acadesine documents a remarkable safety profile of the mechanistic approach (66,67). Acadesine therapy has also shown to reduce the severity of acute post-reperfusion myocardial infarction (MI), substantially reducing the risk of death over the two years after the infarction by 4.3-fold, from 27.8% in the control group to 6.5% in the acadesine group ($p=0.006$) (40). This is the first study of this size to demonstrate an important reduction in mortality associated with reperfusion-induced MI in any setting of clinical revascularization and the first to show a sustained benefit over the long-term. Nonetheless, commercialization of acadesine as a therapeutic for CAD was discontinued in 2010 due to an interim futility analysis by study sponsor Merck (68). Following further analysis of these results, GP531 and other second-generation ARAs were synthesized to provide more site-specific activity and reduce the risk of systemic hemodynamic side effects, such as a transient increase in serum uric acid, providing a significant limitation in dose duration. Relative to acadesine, GP531 has been shown to have a longer half-life ($T_{1/2}$), better oral bioavailability, and somewhat greater potency (69,70). Leveraging more profound insights on the central role of AMPK in mitochondrial homeostasis, promotion of mitochondrial biogenesis and impairment of its activation in HF (29), ViCardia set out to demonstrate the clinical benefits of its AMPK agonist, GP531, as a treatment for ADHF.

3. Approach

Clinical Investigation: GP531 as a treatment for acute decompensated heart failure.

GP531 is being investigated for the treatment of ADHF. Our plan is to conduct a Phase 2, multicenter, randomized, double-blind, placebo-controlled, dose-escalation study to assess GP531's potential to reduce post-discharge mortality and rehospitalization. We will evaluate the efficacy, safety, tolerability, hemodynamic and symptomatic effects of GP531 in subjects hospitalized for an acute event as a result of ADHF and reduced ejection fraction. Subjects will be stratified according to ischemic vs. non-ischemic HF etiology.

Clinical Phase 2 Study Design.

Approximately 150 subjects will be enrolled from approximately 15 sites in the United States. It is expected that each site will enroll approximately 8-12 subjects. Study subjects will be subjects with worsening heart failure, also known as ADHF, admitted to hospital with worsening symptoms and who meet ALL of the inclusion criteria and NONE of the exclusion criteria (see 'Human Subjects' form attached to this application). GP531 as administered in all clinical trials is 5-amino-1- β -D-(5-benzylamino-5-deoxyribofuranosyl) imidazole-4-carboxamide L-tartrate, 100 mg/mL and 0.1 mg/mL sodium metabisulfite. The reference therapy for the control group, i.e., placebo, will be normal saline and 0.1

mg/mL sodium metabisulfite. All subjects enrolled in the clinical trial will receive standard treatment in accordance with guideline-directed evaluation and management (GDEM).

GP531 is administered post-randomization as an IV infusion over approximately 24 hours, at 5 escalating doses of 2, 6, 18, 54 and 100 µg/kg/min. After a screening phase upon admission to hospital, subjects will be randomized and infusion begun within 48 hours of admission using a central randomization system in a 4:1 ratio to either GP531 or placebo. Subjects will be stratified based on the etiology of their cardiomyopathy, being either ischemic or non-ischemic.

- Stratified, randomized dosing will begin with the first cohort receiving a 24-hour infusion at the lowest dose of 2 µg/kg/min or placebo. Subsequent cohorts will receive an infusion over 24 hours at escalating doses. Subjects entering the second, third, fourth and fifth cohorts will receive doses of 6 µg/kg/min or placebo, 18 µg/kg/min or placebo, 54 µg/kg/min, and 100 µg/kg/min or placebo, respectively.
- Each cohort will consist of 30 subjects.
- An interim safety analysis will be performed by the Data Safety Monitoring Board (DSMB) upon completion of the follow-up clinic visit (Day 8) for each cohort. Dose escalation to the next cohort will occur only after the completion of the interim safety analysis for the previous cohort. In addition, at the end of cohort 4 an interim dose-response analysis of hemodynamic parameters and cardiac biomarkers will be performed prior to proceeding to cohort 5. If a descriptive plateau is seen in the dose-response effect, then the DSMB may recommend not to progress to cohort 5.

Subjects will be treated according to the standard medical practice of the institution to which they have been admitted and in compliance with the guidelines of the American College of Cardiology/American Heart Association/Heart Failure Society of America (ACC/AHA/HFSA) most recently updated in May 2017.

- All subjects will continue to receive conventional therapy, which may include diuretics, digoxin, angiotensin converting enzyme (ACE) inhibitors, angiotensin-II receptor blockers (ARBs), beta blockers, and aldosterone inhibitors.
- Due to the potential for pharmacological interactions, there are certain exceptions to concomitant medications that are included in the exclusion criteria.

The Data Safety Monitoring Board (DSMB) will monitor PK and safety data to ensure the safety of the subjects enrolled in the trial, and to conduct the Interim Safety Evaluation upon the completion of the 8-day follow-up visit of the last subject in each of the first four dosing cohorts.

The subject is considered to have completed the study upon the completion of the last protocol-specified contact, e.g., telephone contact by physician or qualified designee at 60 days post randomization.

Specific Aims: Generating clinical evidence for the efficacy of GP531.

To assess the efficacy, safety and tolerability of GP531 at 5 escalating doses when added to standard therapy vs. placebo plus standard therapy in subjects with worsening chronic HF and reduced EF who require hospitalization. Parameters to evaluate efficacy, safety and tolerability include:

- Overall mortality at 7 days, 30 days and 60 days after GP531 treatment.
- Safety, e.g., AEs, SAEs, renal function, clinically significant arrhythmias, (AF, VT, etc.).
- Renal safety: serum creatinine, serum BUN, eGFR, serum cystatin C, urinary
- NGAL, urinary β2-microglobulin, urinary KIM-1, and urinalysis to include microscopy
- Cardiac biomarkers: BNP, Troponin I
- Left ventricular performance and hemodynamic parameters assessed by echocardiography with tissue Doppler imaging
- Signs and symptoms, (e.g. dyspnea, clinical status, and body weight)
- Pharmacokinetics

Objectives, milestones and success criteria.

The study will begin with a low dose in 30 patients (24 will be given drug and 6 will be given placebo). If this is safe, a higher dose (equal to three times the low dose) will be given to the second group of 24 patients with another 6 patients receiving placebo, and if this is safe another dose (equal to three times the previous dose) will be given to the third group of another 24 patients with another 6 patients receiving placebo. If this dose is safe, a fourth group of patients will receive another dose (equal to three times the previous dose) in 24 patients and another 6 patients will receive placebo. And if this dose is safe, a fifth group of patients will receive another dose (equal to approximately two times the previous dose) in 24 patients and another 6 patients will receive placebo. The infusion will last up to 24 hours. Blood and urine samples will be taken to measure chemicals that will detect unwanted (or adverse) effects as well as other chemicals in your blood that can show changes in the stress on your heart that can cause or alleviate injury to it. Please see below for specific details of what procedures and assessments will occur during the entire study.

Clinical Phase 2 study – objectives, milestones and outcome measures	
Objective 1: Assessment of GP531's safety and tolerability.	Start: month 1 End: month 18
<p>Success criterium 1.1: All safety parameters are met with minimal adverse event (AE) or serious adverse events SAE) during the administration of GP531 or the placebo, while the subject is in the hospital for treatment. The safety parameters are: AEs, SAEs, renal function, clinically significant arrhythmias, (AF, VT, etc.). Renal safety includes monitoring of serum creatinine, serum BUN, eGFR, serum cystatin C, urinary NGAL, urinary β2-microglobulin, urinary KIM-1, and urinalysis to include microscopy.</p> <p>Success criterium 1.2: Collection of cardiac biomarkers, including BNP, Troponin I, collection of left ventricular performance and hemodynamic parameters assessed by echocardiography with tissue Doppler imaging.</p> <p>Success criterium 1.3: Achieving tolerability by observing minimal signs and symptoms of dyspnea, deterioration of clinical status and changes in body weight.</p>	
<p>Methods:</p> <p>A. Documentation of adverse events and serious adverse events.</p> <p>B. Chemistry, hematology. and cardiac biomarkers (Troponin I and BNP) measurements will be performed at baseline, 24, 48, 72, and 96 hours or discharge if before 96 hours post randomization and at the follow-up visit 8 days post randomization. Renal function will be assessed by eGFR, BUN, Creatinine, and urinalysis with microscopy locally and Cystatin-C, NGAL, β2-microglobulin and KIM-1, at a central laboratory will be done at 8 and 32 hours post start of infusion.</p> <p>In addition to the time points above, bedside telemetry monitoring will occur during the entire infusion and a minimum 24 hours post infusion (48-hour visit).</p> <p>Safety will be monitored from the start of the infusion through discharge, in a follow-up visit 8 days following randomization, and in telephone interviews at 30 and 60 days from the day of randomization.</p> <ul style="list-style-type: none"> Particular attention will be given to renal function. The following will be measured: eGFR, BUN, creatinine, serum cystatin C, urinary NGAL, urinary β2-microglobulin and urinary KIM-1. In addition, urinalysis and urine microscopy will be performed. Markers of renal dysfunction and/or injury will be monitored/assessed at baseline, 8 hours after the start of infusion, at the end of infusion (~24 hours), and again at approximately 32, 48, 72 and 96 hours after the start of the infusion or at discharge from the hospital and again at 8 days post-randomization. In addition, a urinalysis, urinary NGAL, urinary β2-microglobulin and urinary KIM-1 will be assessed at 32 hours. 	

- Cardiac biomarkers of myocardial injury (Troponin I and BNP) will be measured from blood samples taken at baseline, 8 hours after the start of infusion, at the end of infusion (~24 hours), and again at approximately 48, 72 and 96 hours after the start of the infusion or at discharge from the hospital and again at 8 days post-randomization. Rescue medications (IV inotropes and/or IV vasodilators) initiated after initiation of the infusion will be recorded.

All AEs will be recorded from the time of informed consent signing through the day 8 Clinic visit. SAEs will be collected through the day 30 telephone contact.

Risks & mitigation:

As is true of all medicines and especially true of those in the early stages of drug development, not all risks of the medicine are known. To date, GP531 has been given to 54 normal volunteers and 15 patients with chest pain during exercise (angina pectoris). There were no serious adverse events reported. The most common non-serious side effect reported was headache, which was reported at about the same rate as by those taking a placebo. This symptom was not considered severe, resolved within 24 hours and did not result in stopping the treatment.

The investigator or qualified designee will explain the study to the subject, answer all of his/her questions, and obtain written informed consent before performing any study-related procedure. A copy of the signed informed consent will be given to the subject.

Biomarkers of kidney injury will be measured during this study and the following additional precautions have been taken to protect you in the present study:

- 1) The study will begin at a very low dose, and this will be determined to be safe before proceeding to the next higher dose and so on.
- 2) The starting dose will be about 50 times lower than the one which showed an increase in the early marker of kidney dysfunction.

If you become one of the second group of patients, then you will know that the drug was safe in the patients who previously received the lower dose. If you are part of the third group of patients, then you will know that the drug was safe in the patients who previously received the lower doses. The person obtaining this informed consent will tell you which group you will be in.

All new drugs are tested for safety in animals before being given to people. In the case of GP531, the animal studies showed no adverse findings except at very large doses damage to the kidney was seen.

Both the nonclinical and human data identify the renal tubule as the major safety concern and the most important consideration in setting the initial dose in the Phase 2 dose-escalation study as discussed in (Section 8.4.2 of the Investigator's Brochure).

Objective 2: Assessment of GP531's efficacy.

Start: month 6
End: month 18

Success criterium 2.1: Reduction in mortality at 30 days post discharge from ~20% to <20%

Success criterium 2.2: Improvement in LVEF from <35% after hospital admission to >41%

Methods:

Follow up will be a telephone interview with subject, member of subject's family, or other designated person to assess the clinical status of the subject, (the cause and date of death or re-hospitalization of the subject and the reason for hospital admission).

The left ventricular ejection fraction can be measured by any method (echocardiography, radionuclide imaging, MRI, angiography, etc.), but must have been performed within the previous 12 months. LVEF of <35% within the previous 12 months is required to qualify for the study. If EF ≤35% occurred in the setting of an acute event (e.g., acute MI), a repeat measurement at least 30 days later with documented EF ≤35% is required.

Hemodynamic assessments will be made by echocardiography with images acquired at baseline and at the end of infusion utilizing an imaging protocol provided by the core laboratory. It is preferred that the second echocardiogram be obtained just prior to end of infusion (approximately 20-24 h post start of infusion - preferred) or immediately following. Echocardiographic images will be sent to the echo core laboratory for evaluation.

- A. Comparison of changes in hemodynamic measurements between treatment and control groups by echocardiography from baseline to approximately 24 hours post infusion start to include:
 1. Left ventricular function assessed by left ventricular ejection fraction, cardiac output, fractional shortening, left ventricular end diastolic and end systolic dimensions;
 2. Left ventricular diastolic function and hemodynamics assessed by left atrial pressure, mitral E/E' ratio, mitral E wave/A wave velocity ratio, mitral inflow E wave deceleration time;
 3. Pulmonary artery systolic pressure;
 4. Valvular function assessed by mitral and tricuspid regurgitation grade.
- B. Comparison of changes between treatment and control groups in cardiac injury biomarkers assessed by BNP and Troponin I from baseline to 24h, 48h, 72h, 96h (or on discharge if before 96h) and 8 days post randomization.
- C. Comparison between treatment and control groups in signs and symptoms to include:
 1. Dyspnea assessed by a self-administered, 7-point Likert dyspnea scale at approximately 24h post infusion.
 2. Changes in clinical status assessed by self-administered visual analog scale (VAS) from baseline to approximately 24h
 3. Changes in body weight from baseline to approximately 24h, 48h, 72h, 96h (or on discharge if before 96h)
- D. Comparison of differences between treatment and control groups in incidence rates at 30 days and 60 days post randomization of:
 1. Heart failure re-hospitalization
 2. Other cardiovascular re-hospitalization
 3. Cardiovascular mortality
 4. All-cause mortality

Efficacy endpoints will include:

- Hemodynamic assessments of left ventricular function by echocardiographic imaging before the start of the infusion, and just prior to the end of infusion (approximately 20- 24 hours post infusion start) or immediately following completion of infusion.
- Comparison of changes between treatment and control groups in cardiac injury biomarkers assessed by BNP and Troponin I from baseline to 24h, 48h, 72h, 96h (or on discharge if before 96h) and 8 days post randomization.

Key safety data will be collected through day 30. A post-discharge office/clinic visit will be conducted by a physician at 8 days post randomization to include safety measurements and the assessment of renal and cardiac biomarkers. Follow-up telephone interviews will be conducted at day 30 and at day 60 post randomization with the subject, a member of the subject's family, or other person designated by the subject to confirm survival or clinical status (re-hospitalizations).

Risks & mitigation:

The DSMB will monitor PK and safety data to ensure the safety of the subjects enrolled in the trial, and to conduct the Interim Safety Evaluation upon the completion of the 8-day follow- up visit of the last subject in each of the first four dosing cohorts.

The subject is considered to have completed the study upon the completion of the last protocol-specified contact, e.g., telephone contact by physician or qualified designee at 60 days post randomization



<p>Objective 3: To identify GP531's pharmacokinetic parameters.</p>	<p>Start: month 6 End: month 18</p>
<p>Success criterium 3.1: Concentration at Steady State (Css): end of infusion measured plasma concentration</p>	
<p>Success criterium 3.2: Total drug clearance: infusion rate divided by Css end of infusion</p>	
<p>Methods:</p> <p>Blood (approximately 45 mL or 3 tablespoons) will be drawn to measure the number and quality of the subject's blood cells (blood count), blood chemicals, blood hormones, kidney function, inflammation, and heart muscle injury.</p> <p>Blood will also be drawn to measure study drug levels in the subject's blood and an additional tube of blood will be drawn at the end of the infusion. This blood will be frozen and stored for future measurement of appropriate and novel biomarkers of renal function as they are developed. Future studies involving genetic measurements will not be performed on your stored blood.</p> <ul style="list-style-type: none"> • A urine sample will be collected. • The subject will be asked to answer (2) questionnaires; regarding their symptoms and how they feel. • The subject will have an echocardiogram (ultrasound of the heart) to assess various measures • of your heart's ability to pump blood • Any adverse events that have occurred will be recorded <p>While in the hospital, at approximately 32, 48, 72 and 96hours after the start of study infusion, the following will be performed:</p> <ul style="list-style-type: none"> • Weight, blood pressure, heart rate, and breathing rate will be measured • All new or any changes in medications will be recorded • Blood (approximately 30 mL or 2 tablespoons) will be drawn to measure the number and quality of the subject's blood cells (blood count), blood chemicals, blood hormones, kidney function, inflammation, and heart muscle injury. • A urine sample will be collected. <p>Any adverse events that have occurred will be recorded.</p>	
<p>Risks & mitigation:</p> <p>Patient recruitment is challenging as it involves multiple stakeholders, including patients, healthcare providers, office staff, sponsors, and clinical trial research teams. Subjects for the Phase 2 clinical trial will be recruited through the emergency department of the participating study sites. ViCardia plans to include recommended best practices for successful patient recruitment and retention for its Phase 2 clinical trial.</p> <p>Once patients are enrolled in ViCardia's Phase 2 study, our emphasis will shift to retaining patients in the trial and ensuring they are compliant with all aspects and timelines of the protocol. Specifically, in ViCardia's Phase 2 study, the patients are followed for 60 days following hospital discharge. This requirement is part of the protocol and is one of the most significant endpoints for the study. Thus, patient retention is crucial to ViCardia's success.</p> <p>Follow up will be a telephone interview with subject, member of subject's family, or other designated person to assess the clinical status of the subject, (the cause and date of death or re-hospitalization of the subject and the reason for hospital admission). Centralized communications that streamline the information flow and provide after-hours support for questions, follow up with patients, appointment rescheduling, and sharing information directly with site coordinators. Providing patients with immediate access to operators for questions and easy-to-use tools to complete study-related tasks increase patient retention in clinical trials.</p>	

Objective 4: To identify of the optimal GP531 dose(s) for subsequent Phase 3 clinical trial.	Start: month 1 End: month 18
Success criterium 4.1: Identification of the optimal GP531 dose of the five doses administered to patients during the Phase 2 clinical trial.	
<p>Methods:</p> <p>An Interim PK and Safety Evaluation will be performed by the DSMB soon after the 8- day follow-up clinic visit following Cohorts 1, 2, 3 and 4. This analysis is required for dose escalation to Cohorts 2, 3, 4, and 5 respectively. In addition, at the end of cohort 4 an interim hemodynamic and cardiac biomarker dose-response analysis will be performed prior to proceeding to cohort 5.</p> <p>For subjects who are randomized to GP531, GP531 will be administered as follows:</p> <ul style="list-style-type: none"> • <u>In Cohort 1:</u> 2.88 mg/kg (2 µg/kg/min x 60 minutes x 24 hours = 2,880 µg/kg or 2.88 mg/kg body weight) diluted in normal saline to a total of 250 mL, delivered as an IV infusion at a rate of 2 µg/kg/min (0.17 mL/min), which will last approximately 24 hours. • <u>In Cohort 2:</u> 8.64 mg/kg (6 µg/kg/min x 60 minutes x 24 hours = 8,640 µg/kg or 8.64 mg/kg body weight) diluted in normal saline to a total of 250 mL, delivered as an IV infusion at a rate of 6 µg/kg/min (0.17 mL/min), which will last approximately 24 hours • <u>In Cohort 3:</u> 25.92 mg/kg (18 µg/kg/min x 60 minutes x 24 hours = 25,920 µg/kg or 25.92 mg/kg body weight) diluted in normal saline to a total of 250 mL, delivered as an IV infusion at a rate of 18 µg/kg/min (0.17 mL/min), which will last approximately 24 hours. • <u>In Cohort 4:</u> 77.76 mg/kg (54 µg/kg/min x 60 minutes x 24 hours = 77,760 µg/kg or 77.76 mg/kg body weight) diluted in normal saline to a total of 250 mL, delivered as an IV infusion at a rate of 54 µg/kg/min (0.17 mL/min), which will last approximately 24 hours. • <u>In Cohort 5:</u> 144 mg/kg (100 µg/kg/min x 60 minutes x 24 hours = 144,000 µg/kg or 144 mg/kg body weight) <p>For subjects who are randomized to placebo, placebo will be administered as 250 mL of normal saline delivered as an IV infusion over approximately 24 hours at a rate of 0.17 mL/min.</p>	
<p>Risks & mitigation:</p> <p>Both the GP531 and placebo formulations contain sodium metabisulfite, a sulfite that may cause allergic type reactions including life-threatening anaphylactic and asthmatic symptoms in certain susceptible people. The overall prevalence of sulfite sensitivity in the general population is unknown and probably low. Sulfite sensitivity is seen more frequently in people with asthma. As a mitigation strategy, subjects with a history of allergic bronchospasm or asthma are, at present, excluded from study participation. (see Human Subjects form included with this application for a full list of exclusion criteria).</p>	

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