

Treatment with  $\beta_3$  adrenergic agonists reverses impaired cardiac myocyte  $\text{Na}^+$  export and improves severely decompensated heart failure– a clinical application of an experimental finding

Short title:  $\beta_3$  adrenergic agonists in congestive heart failure

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## **Abstract**

### **Background**

Raised intracellular Na<sup>+</sup> impairs cardiac contractility in severe heart failure (HF).  $\beta_3$  adrenoceptor ( $\beta_3$  AR) agonists activate cardiomyocyte Na<sup>+</sup>-K<sup>+</sup> pump-mediated Na<sup>+</sup> export. Treatment with a selective  $\beta_3$  AR agonist in patients with severely reduced left ventricular ejection fraction (LVEF) and stable HF improves LVEF. We examined effects of  $\beta_3$  AR agonists on Na<sup>+</sup>-K<sup>+</sup> pump function in experimental severe HF characterized by organ congestion and, in parallel, the clinical response in human treatment-refractory worsening chronic HF (WCHF).

### **Methods**

The circumflex coronary artery was ligated in rabbits to induce HF. Treatment with  $\beta_3$  AR agonists was started 2 weeks later and effects on congestive signs were assessed, including ascites and the primary outcome, lung: body weight ratio. Na<sup>+</sup>-K<sup>+</sup> pump current was measured in voltage-clamped myocytes from non-infarcted myocardium. The  $\beta_3$  AR agonist mirabegron was prescribed off-label to patients hospitalized with advanced HF (stage D) that was refractory to maximally tolerated guideline-directed treatment and intravenous furosemide, with or without inotropes, as assessed by  $\geq 2$  cardiologists.

### **Results**

Rabbits developed severe HF with markedly increased lung:body weight ratio and ascites. Na<sup>+</sup>-K<sup>+</sup> pump current was decreased in myocytes from rabbits with HF.  $\beta_3$  AR agonists eliminated pump inhibition, significantly reduced lung:body weight ratio and prevalence of ascites. Mirabegron was given to 9 patients aged  $74 \pm 7$  years (mean  $\pm$  SD) with advanced HF. LVEF was  $26 \pm 5\%$ . Signs and symptoms improved within 1-2 days of treatment. Mirabegron was well-tolerated and improvement of  $\geq 1$  NYHA Class maintained by all early after discharge. One patient died from HF at 16 months, 4 died from other causes at 2 – 30 months and 4 remain alive at  $30 \pm 5$  months with NYHA Class II symptoms.

### **Conclusion**

Treatment with  $\beta_3$  AR agonist in a pre-clinical model of severe HF can reverse Na<sup>+</sup>-K<sup>+</sup> pump inhibition and improve organ congestion implicating pump inhibition as a reversible myocardial dysfunction that is a useful treatment target. The changed in-hospital clinical trajectory and a more favourable post-discharge course than expected in advanced HF, suggests efficacy of  $\beta_3$  AR agonist shown in the animal model might translate into efficacy in advanced human HF.

### **Key words**

Adrenergic receptors, stage D heart failure, sodium, sodium-potassium ATPase.

## Introduction

For the last 20 years, attempts at improving outcomes of decompensated heart failure have generally been directed at unloading the heart by vasodilatation and have been largely unsuccessful<sup>1,2</sup>. An alternative strategy hypothesizes that cellular abnormalities in viable but dysfunctional myocardium, found even in end-stage heart failure (HF)<sup>1</sup>, might be useful treatment targets<sup>1,3</sup>. Contractile dysfunction in myocardium isolated from patients with severe HF with reduced ejection fraction (HFrEF) is in part due to a pathologically raised cytosolic Na<sup>+</sup> concentration ([Na<sup>+</sup>]<sub>i</sub>)<sup>4</sup> and drugs beneficial in chronic HFrEF stimulate sarcolemmal Na<sup>+</sup>-K<sup>+</sup> pump-mediated Na<sup>+</sup><sub>i</sub> export, as reviewed<sup>5</sup>.

The Na<sup>+</sup>-K<sup>+</sup> pump can be activated by agonists for the β<sub>3</sub> adrenoceptor (β<sub>3</sub> AR)<sup>6</sup>. We have previously reported a decrease in cardiac contractility indicated by hemodynamic indices when a β<sub>3</sub> AR agonist was given acutely by intravenous infusion to normal sheep<sup>6</sup>. However, after severe HFrEF was experimentally induced, the agonist caused a shift towards an increase in contractility<sup>6</sup>. While this shift suggests treatment with β<sub>3</sub> AR agonists is beneficial in HFrEF, hemodynamic indices are poor predictors of treatment outcomes in decompensated heart failure<sup>2</sup>. To address more relevant clinical objectives we have studied effects of longer durations of treatment with two β<sub>3</sub> AR agonists on congestive indices in a rabbit model of HFrEF.

Mono-therapy with β<sub>3</sub> AR agonists in the rabbit model was efficacious. However, this does not indicate the agonists also add efficacy to guideline-directed drugs up-titrated to maximal tolerated doses. No animal model can reproduce this standard clinical trial practice or the setting of co-morbidities that often limits treatment options in decompensated human HF. Here we complement our studies on the rabbits by reporting on outcomes of adding the β<sub>3</sub> AR agonist mirabegron to treatment of patients with advanced, stage D HF.

The rationale for prescribing mirabegron was in part based on our previous study on patients with stable, mostly Class II NYHA HFrEF <sup>7</sup>. Mirabegron seemed safe during treatment for 6 months, and an exploratory secondary analysis suggested the treatment increased left ventricular ejection fraction (LVEF) in patients with a particularly low baseline EF, but not in similar placebo treated patients <sup>7</sup>. We felt this, combined with a mechanistically plausible rationale and beneficial effects of  $\beta_3$  AR agonists on organ congestion we identified in the rabbits, justified prescribing mirabegron to our patients. We also took into account that the prognosis for advanced HF is very poor and that no effective medical treatment is available <sup>8, 9</sup>.

Mirabegron is approved for treatment of overactive bladder and as such was prescribed off-label to 9 patients who were hospitalized with advanced HF. At least two cardiologists had to agree the HF was refractory to maximal tolerated conventional guideline-directed treatment and that it was unlikely that the patients could be discharged from hospital. We report in-hospital clinical trajectories before and after we started treatment with mirabegron and post-discharge outcomes during maintained treatment.

## **Methods**

Rabbits were used because  $\beta_3$  AR agonist affinities in rabbit cardiac myocytes and functional effects of activation of it are similar to those in the human myocardium <sup>10</sup>. HFrEF was induced by ligating the circumflex coronary artery. Details of anaesthesia and postoperative analgesia for rabbits and experimental protocols are available in Expanded Methods in Online Data Supplements. Protocols were designed to minimise the number of rabbits and were approved by the Royal North Shore Hospital Animal Ethics Committee.

Echocardiography was performed in anesthetised rabbits before coronary artery ligation or sham operations and 7 days after surgery to measure LVEF. LVEF <25% was used to determine if rabbits should enter the treatment protocols because we had found a value <25% to be a reliable marker of heart failure at subsequent autopsy as indicated by organ congestion and an increase in RNA levels for brain natriuretic peptide (BNP) in the LV.

We used pulmonary congestion as reflected by lung: body weight ratio as the primary endpoint. This is supported by a strong correlation between lung weight at autopsy and extravascular lung water which reflects pulmonary congestion in vivo and is robust across diverse co-morbidities in humans <sup>11</sup>. We also recorded liver and heart weights and presence or absence of ascites. We expected rates of change in the BNP mRNA levels to be too slow for detection within treatment periods of 3 or 14 days that were used. Furthermore, serum BNP levels in clinical studies are poor surrogates for treatment efficacy in decompensated HF <sup>2</sup>. Therefore, BNP mRNA level in the LV was not used as an outcome variable.

We started treatment with the  $\beta_3$  AR agonists CL316,243 infused via subcutaneous osmotic minipumps at 2 mg/kg/24 hours  $\geq 2$  weeks after coronary ligation. CL316,243 is a selective agonist for the rabbit  $\beta_3$  AR <sup>10</sup> and stimulates the Na<sup>+</sup>-K<sup>+</sup> pump in isolated rabbit cardiac myocytes <sup>6</sup>. Duration of treatment was limited to 3 days due to prohibitive cost of the drug in a medium sized animal like rabbit. We treated another group of rabbits for 2 weeks with the  $\beta_3$  AR agonist ASP9531 (a gift from Astellas Pharma) via osmotic minipumps at a rate of 250  $\mu$ g/kg/day.

Electrogenic Na<sup>+</sup>-K<sup>+</sup> pump current ( $I_p$ , arising from the 3: 2 Na<sup>+</sup>: K<sup>+</sup> exchange ratio) was measured in myocytes from non-infarcted myocardium using the whole-cell patch clamp technique. Patch pipette solutions perfusing the intracellular compartment included 10 mmol/L Na<sup>+</sup>. Myocytes were voltage clamped at -40 mV and  $I_p$  was identified as the shift in

holding current induced by 100  $\mu\text{mol/L}$  ouabain according to pre-determined quality criteria

<sup>12</sup>.  $I_p$  (in pA) is reported normalized to membrane capacitance (in pF) and hence myocyte size.

### *Patients*

Patients had been admitted to hospital with worsening chronic HF (WCHF). They had no valvular stenosis or regurgitation as the primary cause of HF and, as independently assessed by  $\geq 2$  cardiologists, decompensated heart failure was refractory to treatment short of i.v. inotropes or had relapsed after such treatment. At the treating physician's discretion and taking into account adverse effects the inotropes have on long-term outcomes <sup>13, 14</sup>, most patients were not treated with inotropes before mirabegron was prescribed.

Mirabegron, which is approved by the Food and Drug Administration in the United States and the Therapeutic Goods Administration in Australia for treatment of urinary incontinence and overactive bladder, was prescribed off-label. A drug may be prescribed off-label in Australia if, in the opinion of the prescriber, it is in the best interest of the patient and is supported by reasonable evidence <sup>15</sup>. Approval by the institutional review board was not required.

Reasoning for proposing treatment was explained to the patients and, when possible, to their close relatives, and patients consented to treatment. Consent for publication of de-identified case histories was obtained in accordance with institutional guidelines.

Mirabegron was given orally in a dose of 25 mg b.d. if the glomerular filtration rate (GFR) was at least 30 ml/min/1.73 m<sup>2</sup>. With a GFR <30- but  $\geq 15$  ml/min/1.73 m<sup>2</sup> we reduced the dose to 25 mg daily and did not use the drug at GFRs <15 ml/min/1.73 m<sup>2</sup>. Clinical data presented is observational, reflects the practice of the patients' usual cardiologist and was not acquired according to pre-determined protocols.

### *Statistics*

Results are expressed as mean  $\pm$  standard error (SE) unless indicated otherwise. Comparisons are made with analysis of variance (ANOVA). A post-hoc Bonferroni test is used for multiple comparisons as appropriate. Comparisons for categorical variables are made with a Fisher's exact test.

Expanded Methods are available in Online Data Supplements.

## Results

### *Hemodynamic Variables and Clinical Indices of Heart Failure in Rabbits*

Treatment with CL316,243 did not alter heart rate or blood pressure significantly in sham-operated rabbits or in rabbits with HF and there was no significant effect of HF or treatment with CL316,243 on serum creatinine (Table 1).

Coronary ligation led to a severe HF phenotype with significantly increased lung: body weight- (Figure 1A), heart: body weight- (Figure 1B) and liver: body weight ratios (Figure 1C). Most rabbits that had coronary ligation and were not treated with CL316,243 had ascites (Figure 1D). None of the sham-operated rabbits or sham-operated rabbits treated with CL316,243 had ascites (Figure 1D). Treatment with CL316,243 of rabbits that had coronary ligation reduced lung- (Figure 1A), heart- (Figure 1B) and liver weight and reduced the prevalence of ascites (Figure 1D).

### *Na<sup>+</sup>-K<sup>+</sup> Pump Function in Heart Failure and Response to CL316,243 Treatment*

Measurements of membrane currents of a myocyte from a rabbit with HF and from a rabbit with HF treated with CL316,243 are illustrated in Figure 2A.  $I_p$  of myocytes from rabbits with HF was significantly lower than  $I_p$  of myocytes from sham-operated rabbits.  $I_p$  of myocytes from rabbits with HF treated with CL316,243 was significantly higher than that of myocytes from untreated rabbits (Figure 2B). In vivo treatment with CL316,243 also increased  $I_p$  of

myocytes from sham-operated rabbits free of HF (Figure 2B) consistent with an increase in  $I_p$  after in vitro exposure to CL316,243 of myocytes isolated from normal rabbits that had not undergone surgery <sup>6</sup>.

The main  $\text{Na}^+\text{-K}^+$  pump constituents are a large catalytic  $\alpha$  subunit and a smaller  $\beta$  subunit with the  $\alpha 1$  and  $\beta 1$  isoforms the most abundant in rabbit heart. There was no significant difference between expression of  $\alpha 1$ - or  $\beta 1$  subunits (Figure 2D) in myocardial homogenate from sham-operated rabbits or from rabbits with HF, with or without CL316,243 treatment, indicating that CL316,243 increased  $\text{Na}^+\text{-K}^+$  pump turnover rather than abundance.

#### *Treatment with ASP9531*

ASP9531 is highly potent and specific for the human  $\beta 3$  AR. We ascertained it stimulates the  $\text{Na}^+\text{-K}^+$  pump in rabbit cardiac myocytes in vitro, similar to the stimulation caused by other  $\beta 3$  AR agonists we previously examined <sup>6</sup>. Myocytes were isolated from normal rabbits as they were in the previous study <sup>6</sup>. After establishing the whole-cell voltage clamp configuration, myocytes were exposed to 200 nmol/L ASP9531 for ~5 min before  $I_p$  was measured. Mean  $I_p$  was  $0.52 \pm 0.07$  pA/pF for 6 myocytes exposed to ASP9531 and  $0.30 \pm 0.02$  pA/pF for 8 control myocytes ( $P < 0.01$ ).

We also measured  $I_p$  in myocytes from normal rabbits that had been given ASP9531 (30 mg/kg/24 hours) orally for 7 days. Mean  $I_p$  of myocytes isolated from 5 rabbits given ASP9531 was  $0.47 \pm 0.06$  pA/pF and  $0.30 \pm 0.02$  pA/pF in myocytes from 5 control rabbits ( $p < 0.05$ ). These results indicate in vitro or in vivo exposure to ASP9531 stimulates the  $\text{Na}^+\text{-K}^+$  pump. Oral treatment proved impractical (Online Data Supplements) and for rabbits with HF we gave ASP9531 via osmotic minipumps.

Duration of treatment with ASP9531 was longer than that of treatment with CL316,243 (14 vs. 3 days) and accordingly there was a longer time between coronary ligation of the rabbits



and the time of their sacrifice (28 days). To limit the number of rabbits undergoing thoracotomy, a sham-operated control group was not used again.

ASP9531 did not significantly alter heart rate, blood pressure or serum creatinine (Table 1). The lung: body weight ratio in rabbits with HF for 28 days (Figure 3A) was similar to that after 14 days (Figure 2A) suggesting that severity of HF was stable after day 14. Treatment with ASP9531 for 14 days reduced the lung: body- (Figure 3A) and heart: body weight ratios (Figure 3B) but there was no statistically significant effect on the liver: body weight ratio (Figure 3C -see comment to this in legend). Treatment eliminated ascites (Figure 3D).

#### *Mirabegron in severe treatment-resistant human HFrEF*

Nine patients aged  $74 \pm 7$  years (all clinical data here presented as mean  $\pm$  standard deviation) had been admitted to hospital with WCHF. A cause precipitating cardiac decompensation was not identified in any of them. They had been treated for HFrEF for  $\geq 6$  months. All had symptoms and clinical signs of pulmonary congestion or edema. Eight had leg and/or sacral edema and 5 had abdominal distension attributed to ascites. Three were in New York Heart Association (NYHA) Class III and 6 in NYHA Class IV and all had advanced, stage D HF<sup>8</sup>.<sup>9</sup> BNP, available for 7 patients, was  $2385 \pm 2167$  ng/L.

Four patients had bi-ventricular pacing and an implantable cardiac defibrillator. Two had a defibrillator alone but not bi-ventricular pacing. The most recent LVEF measured by echocardiography was  $26 \pm 5\%$ . The systolic blood pressure before starting treatment with mirabegron was  $110 \pm 13$  mm Hg. This value includes the blood pressures of 2 patients while they had a second course of i.v. inotropic support when early relapse of severe decompensation had occurred after a first course. Mirabegron was introduced during a bridging period before inotropes were weaned off in these patients. Their serum creatinine was raised to 221 and 310  $\mu\text{mol/L}$  before they were given the second course of inotropes. The inotropes reduced these to levels that allowed use of mirabegron according to guidelines for the

approved indication. The averaged serum creatinine for all 9 patients, including the 221 and 310  $\mu\text{mol/L}$  levels, was  $153 \pm 84 \mu\text{mol/L}$ . One additional patient had inotropic support during an admission 4 weeks previously but was not given inotropes before mirabegron was started during the index admission.

HF had been refractory to escalating treatment in hospital that included i.v. furosemide. Eight patients were also treated with spironolactone and 2 additionally with a thiazide diuretic. ACE inhibitors and  $\beta_1$  AR antagonists had been prescribed in maximal tolerated doses before admission to hospital but ACE inhibitors were discontinued in 7 patients before mirabegron was used due to low blood pressure and/or renal impairment. One patient had an ACE inhibitor replaced with sacubitril/losartan in hospital. This caused marked symptomatic hypotension needing immediate treatment with i.v. saline. Sacubitril/losartan was discontinued before mirabegron was introduced.

With the addition of mirabegron to treatment there was no decrease in blood pressure in any of the patients, physical signs and symptoms improved and all were stabilized and discharged from hospital. This was achieved with a lower daily dose of furosemide than at the start of treatment with mirabegron and with no increase in serum creatinine levels. Three patients aged 68, 73 and 76 years resumed full- or part time work.

Five patients died  $17 \pm 13$  months after starting treatment with mirabegron. One was readmitted with WCHF 10 months after discharge and subsequently died from heart failure 16 months after treatment with mirabegron had been started. One died at 30 months from pneumonia that he declined treatment for because serious co-morbidities markedly limited his quality of life. Two have died from cancer and one had kidney disease that progressed to end-stage. In contrast to what had been the case during his index admission, renal function had not responded to i.v. inotrope treatment. He had required hemodialysis and died from a cardiac

arrest 10 months after treatment with mirabegron was initiated. Mirabegron had been discontinued before he died.

Four patients remain alive  $32 \pm 5$  months after treatment with mirabegron was initiated. While co-morbidities for some patients preclude accurate assessment of cardiac limitation, improvement  $\geq 1$  NYHA Class was maintained post-discharge for all except for the patient who died from heart failure 16 months after starting treatment with mirabegron and the patient who had progressive kidney disease.

Clinical characteristics of the patients, their treatments and outcomes are summarized in Table 2. Case histories and co-morbidities are presented in Expanded Methods and Results in the Online Data Supplements.

## **Discussion**

Benefit of treating HFrEF with  $\beta_3$  AR agonists was previously suggested experimentally by a dose-dependent improvement of hemodynamic indices when i.v. bolus injections of an agonist were given to sheep <sup>6</sup>. Here, clinically relevant lung congestion and ascites <sup>2</sup> were reversed in rabbits given  $\beta_3$  AR agonists continuously at a fixed rate over 3- or 14 days.

The mechanistic rationale for benefit of  $\beta_3$  AR agonists is based on the  $\text{Na}^+$ - $\text{K}^+$  pump stimulation they cause and the adverse effects of a raised  $[\text{Na}^+]_i$  on cardiac contractility. While a modest increase above baseline increases contractility of normal human myocardium in vitro, there is an inverse relationship between  $[\text{Na}^+]_i$  beyond already raised levels and contractility in myocardium from patients with severe HFrEF <sup>4</sup>. This increases the intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) due to reduced export via  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchange, and dysregulation of  $\text{Ca}^{2+}$ -dependent excitation-contraction coupling causes contractile dysfunction <sup>16</sup>. The high  $[\text{Na}^+]_i$  also causes mitochondrial dysfunction and energy deficiency

<sup>17</sup>.

Raised  $[Na^+]_i$  in the myocardium of patients with severe HFrEF is in part due to influx through the late  $Na^+$  current<sup>18</sup>. Reduced  $Na^+$ - $K^+$  pump-mediated  $Na^+_i$  efflux, here estimated by ~40% in rabbit myocytes with  $[Na^+]_i$  clamped at 10 mmol/L, also contributes. Direct measurement of  $[Na^+]_i$  and the relative contributions the late  $Na^+$  current and  $Na^+$ - $K^+$  pump inhibition make to an increase of  $[Na^+]_i$  in the beating heart is not experimentally possible. However, from in silico modelling<sup>19</sup>, we estimate a decrease in  $Na^+$ - $K^+$  pump-mediated  $Na^+_i$  efflux by 40% contributes much more than increased  $Na^+$  influx *via* the late  $Na^+$  current.

Raised  $[Na^+]_i$  could become self-sustaining because it stimulates mitochondrial synthesis of reactive oxygen species (ROS)<sup>20</sup> that in turn can activate the late  $Na^+$  current<sup>21</sup> and inhibit the  $Na^+$ - $K^+$  pump<sup>22</sup>.  $Na^+$ - $K^+$  pump inhibition is expected to be countered by activation of the  $\beta_3$  AR that mediates antioxidant effects via the classical NO-dependent signalling pathway<sup>23</sup>. This is experimentally supported by the reversal of pump inhibition caused by oxidative stress with exposure of cardiac myocytes to CL316,243<sup>6, 22</sup>. Reversal should mitigate  $Na^+_i$ -dependent  $Ca^{2+}_i$  dysregulation and energy deficiency and hence contractile dysfunction.

The parallel reversal of  $Na^+$ - $K^+$  pump inhibition and organ congestion in rabbits treated with CL316,243 is in keeping with the hypothesis that cellular and molecular abnormalities in severe HFrEF are potential treatment targets<sup>3</sup> and with an inhibited  $Na^+$ - $K^+$  pump as one such target. However,  $Na^+$ - $K^+$  pump inhibition might not have remained a useful target in this study if CL316,243 had been added to treatment with drugs used in guideline-directed clinical practice because the most commonly used of these agents prevent  $Na^+$ - $K^+$  pump inhibition caused by angiotensin II receptor-<sup>22</sup>  $\beta_1$  AR-<sup>22</sup> and aldosterone receptor activation<sup>24</sup>, effectively causing  $Na^+$ - $K^+$  pump stimulation, as reviewed<sup>5</sup>.

The patients we report were given guideline-directed device therapy and drugs up-titrated as tolerated before mirabegron was added. However, not all recommended drugs were tolerated and those prescribed could not be given in target doses. With incomplete reversal of  $Na^+$ - $K^+$

pump inhibition it is likely myocardial  $[Na^+]_i$  was raised. This is directly supported by an abnormally high  $[Na^+]_i$  measured in the myocardium from patients with end-stage HFrEF. Although use of ACE inhibitors,  $\beta_1$  AR- and aldosterone receptor antagonist was not reported<sup>4</sup>, it is reasonable to assume they were prescribed as tolerated. For raised  $[Na^+]_i$  as a pathophysiological determinant in HFrEF, those patients with WCHF most in need might be the least likely to tolerate optimised guideline-directed treatment.

While sharing a putative raised  $[Na^+]_i$  as a treatment target with mainstay conventional drugs,  $\beta_3$  AR agonists-induced  $Na^+-K^+$  pump stimulation actively reverses an increase in  $[Na^+]_i$  regardless of source<sup>7</sup> rather than incompletely countering inhibition of pump-mediated  $Na^+$  export that the conventional drugs do. Consistent with this, treatment of patients with mirabegron was associated with improved physical signs and symptoms on a time scale compatible with the rapid resolution of  $Na^+-K^+$  pump inhibition and congestive features in the rabbit model.

$\beta_3$  AR agonist-induced  $Na^+-K^+$  pump stimulation depends on activation of “soluble” guanylate cyclase (sGC) that is part of the classical NO-dependent signaling pathway<sup>6</sup>. A direct activator of sGC stimulates the pump in cardiac myocytes<sup>12</sup> and activators of sGC have been trialed in HFrEF. Intravenous treatment of acute cardiac decompensation with cinaciguat improved hemodynamic indices but frequently caused hypotension and a trend towards increased re-admission rate<sup>25</sup>. Oral treatment with vericiguat for 12 weeks of patients who were clinically stable after an episode of WCHF did not achieve the primary end point of a change in N-Terminal proBNP levels but was well tolerated. An exploratory secondary analysis suggested it might be beneficial<sup>26</sup> and an event-driven phase III trial is now in progress<sup>27</sup>.

While  $\beta_3$  AR agonists and sGC activators share downstream signaling pathways, they are not expected to have identical effects in HF. Expression of sGC is virtually ubiquitous and its

activation has a multitude of downstream effects <sup>28</sup>. However, the  $\beta_3$  AR is differently expressed and coupled to cellular signaling domains in different cells and organs <sup>23</sup>. In addition, myocardial expression of the  $\beta_3$  AR is up-regulated in severe human HFREF <sup>29</sup> and, in contrast to the other  $\beta$  ARs, does not desensitize to agonist activation <sup>30</sup>. These characteristics should allow myocardial targeting with  $\beta$  AR agonists in HF with more selectivity than activators of sGC and with fewer off-target effects.

Treatment of our patients with mirabegron was well tolerated. There was also a close temporal relationship between a favourable change in the clinical trajectory and the treatment. Our studies on rabbits showed mechanistic plausibility for treatment efficacy and there was coherence with evidence-based therapies for HFREF by the mechanistically shared stimulation of myocardial  $\text{Na}^+_i$  export <sup>5</sup>. These features suggest treatment of patients with mirabegron might be efficacious <sup>31</sup> but do not prove it.

There is a paucity of data describing outcomes for patients with advanced HF <sup>8</sup>. To our knowledge, only the REMATCH trial, that compared medical treatment with mechanical circulatory support (MCS) <sup>32</sup>, provides some insight. Sixty one medically managed patients had a 75% 1-year mortality and by 2 years almost all had died, more than 90% from heart failure. The mainstay of evidence-based treatments for the patients recruited to REMATCH trial ~20 years ago has not changed since then and the cardiovascular outcome of the patients we describe here seems to compare favourably with that in the trial. Nevertheless, efficacy of mirabegron of course cannot be implicated from the outcome of 9 patients.

Cardiac transplantation or MCS as destination therapy is recommend in the management of otherwise intractable advanced heart failure. However, many patients are not eligible for these treatments and few options beyond supportive care are available to them. While continuous infusion of inotropes at home may provide end-of-life symptomatic relief it probably also reduces survival <sup>8,9</sup>.

The prevalence of advanced HF among all patients with HF is estimated at 1% to 10%. This prevalence is expected to increase<sup>9</sup> and treatment with mirabegron as an alternative to usual care of those not eligible for transplantation or MCS might be attractive. Mechanistically a  $\beta_3$  AR agonist addresses the need to target an identified myocardial abnormality in HF<sup>1,3</sup> as shown here. Clinically it integrates acute- with long-term management<sup>2,7</sup> and it can be used with renal function reduced to a level with a GFR of 15 ml/min/1.73 m<sup>2</sup>. In addition, as found here and previously<sup>7</sup>, the agonists do not cause the decrease blood pressure that often limits use of current guideline-directed treatments.

Our use of mirabegron off-label in doses recommended for the approved indication might not be optimal for treatment of HF and up to 6-fold higher doses have been used in studies on overactive bladder<sup>33,34</sup>. There were no effects expected to be adverse in HF and consistent with this, none were apparent when patients with mostly NYHA Class II HFrEF were given the 6-fold higher dose. The responses in a subset of patients with a particularly low baseline LVEF were consistent with benefit<sup>7</sup>. Mirabegron appears safe across the spectrum of severity of HFrEF and, if efficacious, might have a wide therapeutic index.

A study is now in progress on patients with HFrEF and NYHA Class III and IV. Effects of mirabegron on acute hemodynamics and remodelling after 3 months is being examined<sup>35</sup>. Relevant to heart failure with preserved ejection fraction (HFpEF) that also can progress to advanced HF<sup>9</sup> a study on the effect of mirabegron on cardiac hypertrophy is also in progress. Cardiac hypertrophy has a raised myocyte [Na<sup>+</sup><sub>i</sub>]<sup>36</sup> as a treatment target in common with HFrEF and the study is examining if mirabegron improves LV hypertrophy and diastolic function in patients at high risk of developing HFpEF<sup>37</sup>.

High event rate expected with advanced HF should allow a well powered randomized trial to be conducted with short follow-up time- and a modest number of patients. If efficacy were to be found, examining efficacy in patient in the broader cohort with worsening HFrEF should

be considered. These patients usually improve with adjustment of conventional treatment, allowing discharge from hospital, but nevertheless have a poor long term prognosis that no current treatment improves<sup>1 3</sup>

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### **Disclosures**

HB and HHR are named inventors on a patent owned by the Northern Sydney Local Health District and the University of Sydney for the use of  $\beta_3$  AR agonists in treatment of heart failure.

NAS performed procedures needed for the rabbit heart failure model, she performed in vitro experimental studies, participated in follow-up of patients and contributed to the overall design of the study, C-CL performed in vitro experimental studies, AG participated in procedures for the rabbit heart failure model and performed in vitro experimental studies, EJH performed in vitro experimental studies, KKG performed in vitro experimental studies and



contributed to clinical follow-up, YJK performed in vitro experimental studies, JLH, GICN, and DWW managed patients and were responsible for their follow-up. HB contributed to design of the study and consulted on inclusion of patients in the study, HHR participated in design of the study, managed patients and was responsible for their follow-up. All authors contributed to interpretation of data and writing of the manuscript and all authors have read and approve its final version.

## References:

1. Gheorghide M, Larson CJ, Shah SJ, Greene SJ, Cleland JG, Colucci WS, Dunmon P, Epstein SE, Kim RJ, Parsey RV, Stockbridge N, Carr J, Dinh W, Krahn T, Kramer F, Wahlander K, Deckelbaum LI, Crandall D, Okada S, Senni M, Sikora S, Sabbah HN and Butler J. Developing New Treatments for Heart Failure: Focus on the Heart. *Circ Heart Fail.* 2016;9.
2. Cotter G, Cohen-Solal A, Davison BA and Mebazaa A. RELAX-AHF, BLAST-AHF, TRUE-AHF, and other important truths in acute heart failure research. *Eur J Heart Fail.* 2017;19:1355-1357.
3. Bayeva M, Sawicki KT, Butler J, Gheorghide M and Ardehali H. Molecular and cellular basis of viable dysfunctional myocardium. *Circ Heart Fail.* 2014;7:680-91.
4. Pieske B, Maier LS, Piacentino V, 3rd, Weisser J, Hasenfuss G and Houser S. Rate dependence of  $[Na^+]_i$  and contractility in nonfailing and failing human myocardium. *Circulation.* 2002;106:447-53.
5. Liu CC, Fry NA, Hamilton EJ, Chia KK, Garcia A, Karimi Galougahi K, Figtree GA, Clarke RJ, Bundgaard H and Rasmussen HH. Redox-dependent regulation of the  $Na^+K^+$  pump: new twists to an old target for treatment of heart failure. *J Mol Cell Cardiol.* 2013;61:94-101.
6. Bundgaard H, Liu CC, Garcia A, Hamilton EJ, Huang Y, Chia KK, Hunyor SN, Figtree GA and Rasmussen HH.  $\beta_3$  adrenergic stimulation of the cardiac  $Na^+K^+$  pump by reversal of an inhibitory oxidative modification. *Circulation.* 2010;122:2699-708.
7. Bundgaard H, Axelsson A, Hartvig Thomsen J, Sorgaard M, Kofoed KF, Hasselbalch R, Fry NA, Valeur N, Boesgaard S, Gustafsson F, Kober L, Iversen K and Rasmussen HH. The first-in-man randomized trial of a  $\beta_3$  adrenoceptor agonist in chronic heart failure: the BEAT-HF trial. *Eur J Heart Fail.* 2017;19:566-575.
8. Fang JC, Ewald GA, Allen LA, Butler J, Westlake Canary CA, Colvin-Adams M, Dickinson MG, Levy P, Stough WG, Sweitzer NK, Teerlink JR, Whellan DJ, Albert NM, Krishnamani R, Rich MW, Walsh MN, Bonnell MR, Carson PE, Chan MC, Dries DL, Hernandez AF, Hershberger RE, Katz SD, Moore S, Rodgers JE, Rogers JG, Vest AR, Givertz MM and Heart Failure Society of America Guidelines C. Advanced (stage D) heart failure: a statement from the Heart Failure Society of America Guidelines Committee. *J Card Fail.* 2015;21:519-34.
9. Crespo-Leiro MG, Metra M, Lund LH, Milicic D, Costanzo MR, Filippatos G, Gustafsson F, Tsui S, Barge-Caballero E, De Jonge N, Frigerio M, Hamdan R, Hasin T, Hulsmann M, Nalbantgil S, Potena L, Bauersachs J, Gkouziouta A, Ruhparwar A, Ristic AD, Straburzynska-Migaj E, McDonagh T, Seferovic P and Ruschitzka F. Advanced heart failure: a position statement of the Heart Failure Association of the European Society of Cardiology. *Eur J Heart Fail.* 2018;20:1505-1535.
10. Audigane L, Kerfant BG, El Harchi A, Lorenzen-Schmidt I, Toumaniantz G, Cantereau A, Potreau D, Charpentier F, Noireaud J and Gauthier C. Rabbit, a relevant model for the study of cardiac  $\beta_3$ -adrenoceptors. *Exp Physiol.* 2009;94:400-11.
11. Tagami T, Kushimoto S, Yamamoto Y, Atsumi T, Tosa R, Matsuda K, Oyama R, Kawaguchi T, Masuno T, Hiramata H and Yokota H. Validation of extravascular lung water measurement by single transpulmonary thermodilution: human autopsy study. *Crit Care.* 2010;14:R162.
12. William M, Vien J, Hamilton E, Garcia A, Bundgaard H, Clarke RJ and Rasmussen HH. The nitric oxide donor sodium nitroprusside stimulates the  $Na^+K^+$  pump in isolated rabbit cardiac myocytes. *J Physiol.* 2005;565:815-25.
13. Francis GS, Bartos JA and Adaty S. Inotropes. *J Am Coll Cardiol.* 2014;63:2069-78.

14. Mortara A. Inotropes and vasopressors in acute heart failure, when the devil dresses as an angel. *Eur J Heart Fail.* 2018;20:342-344.
15. Day R. Off-label prescribing. *Aust Prescr.* 2013;36:5-7.
16. Bers DM. Cardiac sarcoplasmic reticulum calcium leak: basis and roles in cardiac dysfunction. *Annu Rev Physiol.* 2014;76:107-27.
17. Bay J, Kohlhaas M and Maack C. Intracellular Na(+) and cardiac metabolism. *J Mol Cell Cardiol.* 2013;61:20-7.
18. Sossalla S, Wagner S, Rasenack EC, Ruff H, Weber SL, Schondube FA, Tirilomis T, Tenderich G, Hasenfuss G, Belardinelli L and Maier LS. Ranolazine improves diastolic dysfunction in isolated myocardium from failing human hearts--role of late sodium current and intracellular ion accumulation. *J Mol Cell Cardiol.* 2008;45:32-43.
19. Trenor B, Cardona K, Gomez JF, Rajamani S, Ferrero JM, Jr., Belardinelli L and Saiz J. Simulation and mechanistic investigation of the arrhythmogenic role of the late sodium current in human heart failure. *PLoS One.* 2012;7:e32659.
20. Kohlhaas M, Liu T, Knopp A, Zeller T, Ong MF, Bohm M, O'Rourke B and Maack C. Elevated cytosolic Na<sup>+</sup> increases mitochondrial formation of reactive oxygen species in failing cardiac myocytes. *Circulation.* 2010;121:1606-13.
21. Song Y, Shryock JC, Wagner S, Maier LS and Belardinelli L. Blocking late sodium current reduces hydrogen peroxide-induced arrhythmogenic activity and contractile dysfunction. *J Pharmacol Exp Ther.* 2006;318:214-22.
22. Chia KK, Liu CC, Hamilton EJ, Garcia A, Fry NA, Hannam W, Figtree GA and Rasmussen HH. Stimulation of the cardiac myocyte Na<sup>+</sup>-K<sup>+</sup> pump due to reversal of its constitutive oxidative inhibition. *Am J Physiol Cell Physiol.* 2015;309:C239-50.
23. Balligand JL. Cardiac Salvage by Tweaking with Beta3-adrenergic Receptors. *Cardiovasc Res.* 2016;111:128-33.
24. Mihailidou AS, Bundgaard H, Mardini M, Hansen PS, Kjeldsen K and Rasmussen HH. Hyperaldosteronemia in rabbits inhibits the cardiac sarcolemmal Na<sup>+</sup>-K<sup>+</sup> pump. *Circ Res.* 2000;86:37-42.
25. Erdmann E, Semigran MJ, Nieminen MS, Gheorghide M, Agrawal R, Mitrovic V and Mebazaa A. Cinaciguat, a soluble guanylate cyclase activator, unloads the heart but also causes hypotension in acute decompensated heart failure. *Eur Heart J.* 2013;34:57-67.
26. Gheorghide M, Greene SJ, Butler J, Filippatos G, Lam CS, Maggioni AP, Ponikowski P, Shah SJ, Solomon SD, Kraigher-Krainer E, Samano ET, Muller K, Roessig L, Pieske B, Investigators S-R and Coordinators. Effect of Vericiguat, a Soluble Guanylate Cyclase Stimulator, on Natriuretic Peptide Levels in Patients With Worsening Chronic Heart Failure and Reduced Ejection Fraction: The SOCRATES-REDUCED Randomized Trial. *JAMA.* 2015;314:2251-62.
27. Armstrong PW, Roessig L, Patel MJ, Anstrom KJ, Butler J, Voors AA, Lam CSP, Ponikowski P, Temple T, Pieske B, Ezekowitz J, Hernandez AF, Koglin J and O'Connor CM. A Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial of the Efficacy and Safety of the Oral Soluble Guanylate Cyclase Stimulator: The VICTORIA Trial. *JACC Heart Fail.* 2018;6:96-104.
28. Derbyshire ER and Marletta MA. Structure and regulation of soluble guanylate cyclase. *Annu Rev Biochem.* 2012;81:533-59.
29. Moniotte S, Kobzik L, Feron O, Trochu JN, Gauthier C and Balligand JL. Upregulation of beta(3)-adrenoceptors and altered contractile response to inotropic amines in human failing myocardium. *Circulation.* 2001;103:1649-55.
30. Liggett SB, Freedman NJ, Schwinn DA and Lefkowitz RJ. Structural basis for receptor subtype-specific regulation revealed by a chimeric beta 3/beta 2-adrenergic receptor. *Proc Natl Acad Sci U S A.* 1993;90:3665-9.

31. Glasziou P, Chalmers I, Rawlins M and McCulloch P. When are randomised trials unnecessary? Picking signal from noise. *BMJ*. 2007;334:349-51.
32. Rose EA, Gelijns AC, Moskowitz AJ, Heitjan DF, Stevenson LW, Dembitsky W, Long JW, Ascheim DD, Tierney AR, Levitan RG, Watson JT, Meier P, Ronan NS, Shapiro PA, Lazar RM, Miller LW, Gupta L, Frazier OH, Desvigne-Nickens P, Oz MC, Poirier VL and Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure Study G. Long-term use of a left ventricular assist device for end-stage heart failure. *N Engl J Med*. 2001;345:1435-43.
33. Vij M and Drake MJ. Clinical use of the beta3 adrenoceptor agonist mirabegron in patients with overactive bladder syndrome. *Ther Adv Urol*. 2015;7:241-8.
34. Chapple CR, Dvorak V, Radziszewski P, Van Kerrebroeck P, Wyndaele JJ, Bosman B, Boerrigter P, Drogendijk T, Ridder A, Van Der Putten-Slob I, Yamaguchi O and Dragon Investigator G. A phase II dose-ranging study of mirabegron in patients with overactive bladder. *Int Urogynecol J*. 2013;24:1447-58.
35. ClinicalTrials.gov [Internet] Bethesda (MD): National Library of Medicine (US) 2019. Identifier: NCT03926754. Beta 3 Agonist Treatment in Heart Failure-2 (BEAT-HF II). Available at: <https://ClinicalTrials.gov/show/NCT03926754>
36. Aksentijevic D, O'Brien BA, Eykyn TR and Shattock MJ. Is there a causal link between intracellular Na elevation and metabolic remodelling in cardiac hypertrophy? *Biochem Soc Trans*. 2018;46:817-827.
37. Pouleur AC, Anker S, Brito D, Brosteanu O, Hasenclever D, Casadei B, Edelmann F, Filippatos G, Gruson D, Ikonomidis I, Lhommel R, Mahmood M, Neubauer S, Persu A, Gerber BL, Piechnik S, Pieske B, Pieske-Kraigher E, Pinto F, Ponikowski P, Senni M, Trochu JN, Van Overstraeten N, Wachter R and Balligand JL. Rationale and design of a multicentre, randomized, placebo-controlled trial of mirabegron, a Beta3-adrenergic receptor agonist on left ventricular mass and diastolic function in patients with structural heart disease Beta3-left ventricular hypertrophy (Beta3-LVH). *ESC Heart Fail*. 2018;5:830-841.

TABLE 1. Haemodynamic variables and serum creatinine in CL-316,243 and ASP9531 treated sham- and congestive heart failure rabbits.

	Sham	Sham+CL	CHF	CHF+CL	CHF	CHF+ASP
Rabbits	10	8	13	8	6	6
HR (beats min <sup>-1</sup> )	233 ± 2	230 ± 5	295 ± 15	268 ± 6	294 ± 22	316 ± 11
MAP (mmHg)	79 ± 7	73 ± 4	70 ± 6	72 ± 9	72 ± 4	74 ± 7
Creatinine (μmol/L)	76 ± 5	71 ± 4	90 ± 10	76 ± 9	94 ± 18	83 ± 15

Hemodynamic data and serum creatinine levels from sham, CL-316,243 (CL) treated sham, congestive heart failure (CHF), CL and ASP9531 (ASP) treated CHF groups. Result are presented as mean ± SEM.

Table 2. Summary of Patient Characteristics and Outcomes

Patient #	1	2	3	4	5	6	7	8	9
Age (years)	84	70	73	68	84	76	80	68	64
Gender	Male	Male	Female	Male	Male	Male	Male	Male	Male
Diabetes	-	-	-	+	-	-	-	+	+
Ischaemic Cardiomyopathy	+	-	-	+	+	+	-	+	+
Valvular disease	-	+	+	-	-	-	-	-	-
LVEF (%)	35-40	28	20-25	25	25-30	25-30	20	30	20
BNP (ng/L, < 159)	N/A	1553	7005	882	991	1033	N/A	2642	2591
NYHA Class	IV	III	IV	IV	III	IV	IV	III	IV
Hyponatremia	-	-	-	-	+	-	-	-	-
Previous inotropes	-	-	+	-	-	-	+	-	+
Bi-V pacing/ICD	-	+/+	+/+	+/+	-/+	-	+/+	-	-/+
Creatinine (µmol/L)	135	89	85	70	131	95	221	240	310
Follow-up (months)	30‡	27‡	37	2‡	33	32	16†	26	10‡
Furosemide before¶	120	160*	80	480*	100*	160*	120	40	240*
Furosemide after¶	40	100	40	240	40	80	80	60	160
Spironolactone before¶	12.5	25	25	50	25	12.5	25	-	25
Spironolactone after¶	12.5	25	25	50	25	12.5	25	-	25
Thiazide diuretic	-	-	-	+	-	-	-	-	+
Beta blocker	-	+	+	+	-	+	+	+	+
ACE inhibitor before	+	-	-	+	-	-	+	-	-
ACE inhibitor after	-	-	-	+	-	-	-	-	-

\* Oral equivalent of iv administration assuming 50% bioavailability with oral dosing

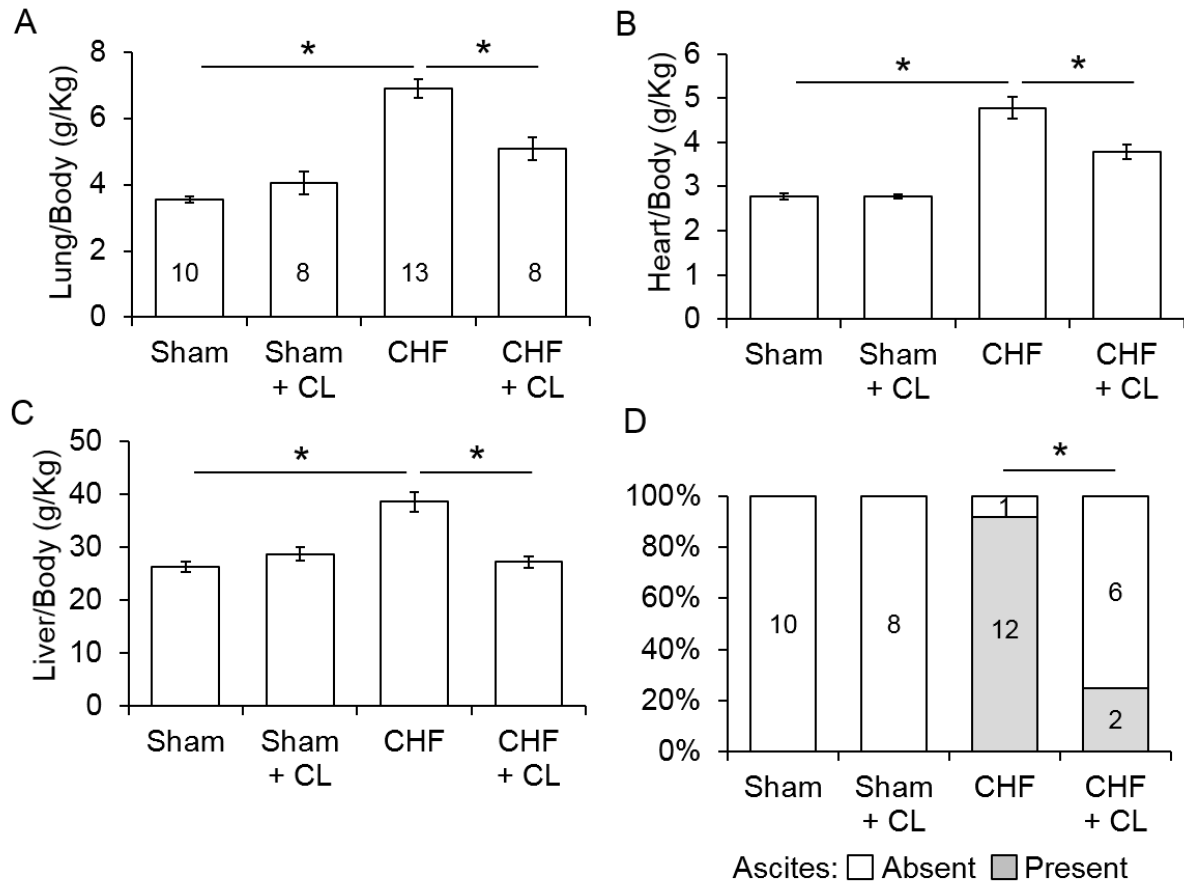
‡ Cumulative daily dose in mg

† Deceased from progressive cardiac failure

‡ Deceased

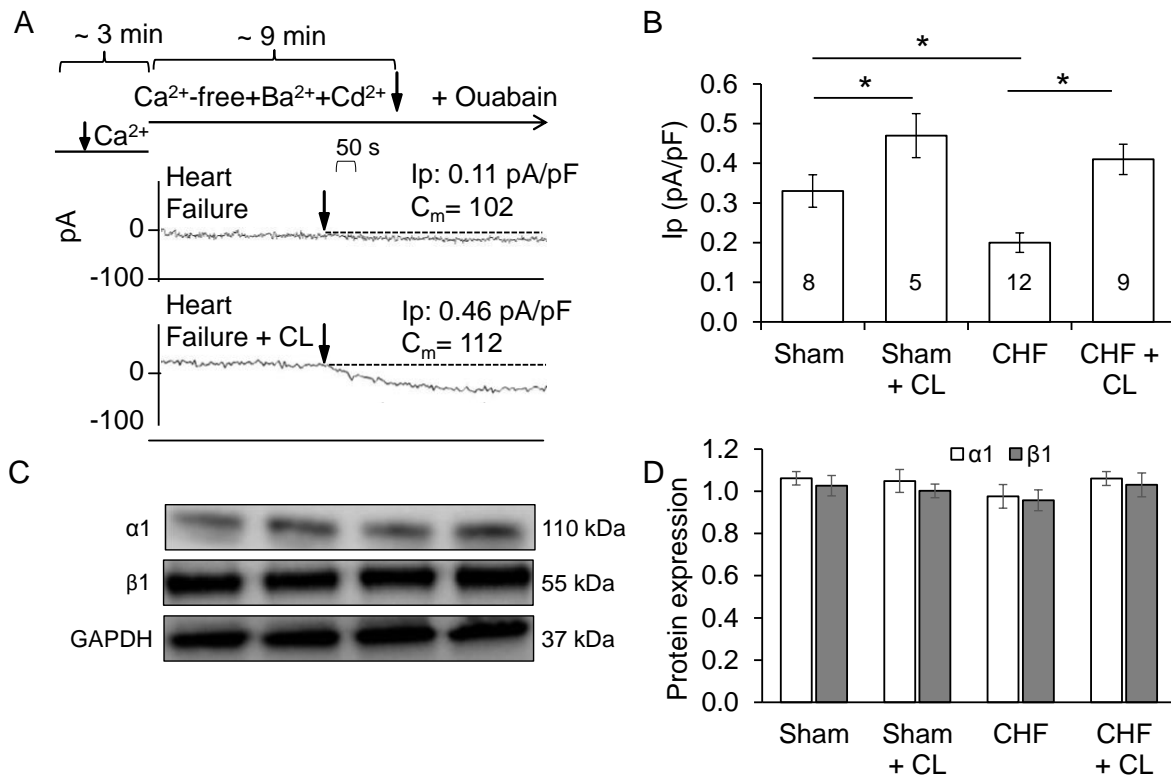
## Figures

### Figure 1



**Figure 1. Indices of heart failure in coronary ligation model and effects of treatment with CL316,243. A.** Lung: body weight ratio, **B.** Heart: body weight ratio, **C.** Liver: body weight ratio. **D.** Ascites in sham-operated rabbits and rabbits with congestive heart failure (CHF), with or without treatment with CL316,243 (CL). Numbers of rabbits are indicated in columns. \* indicates  $P < 0.05$ .

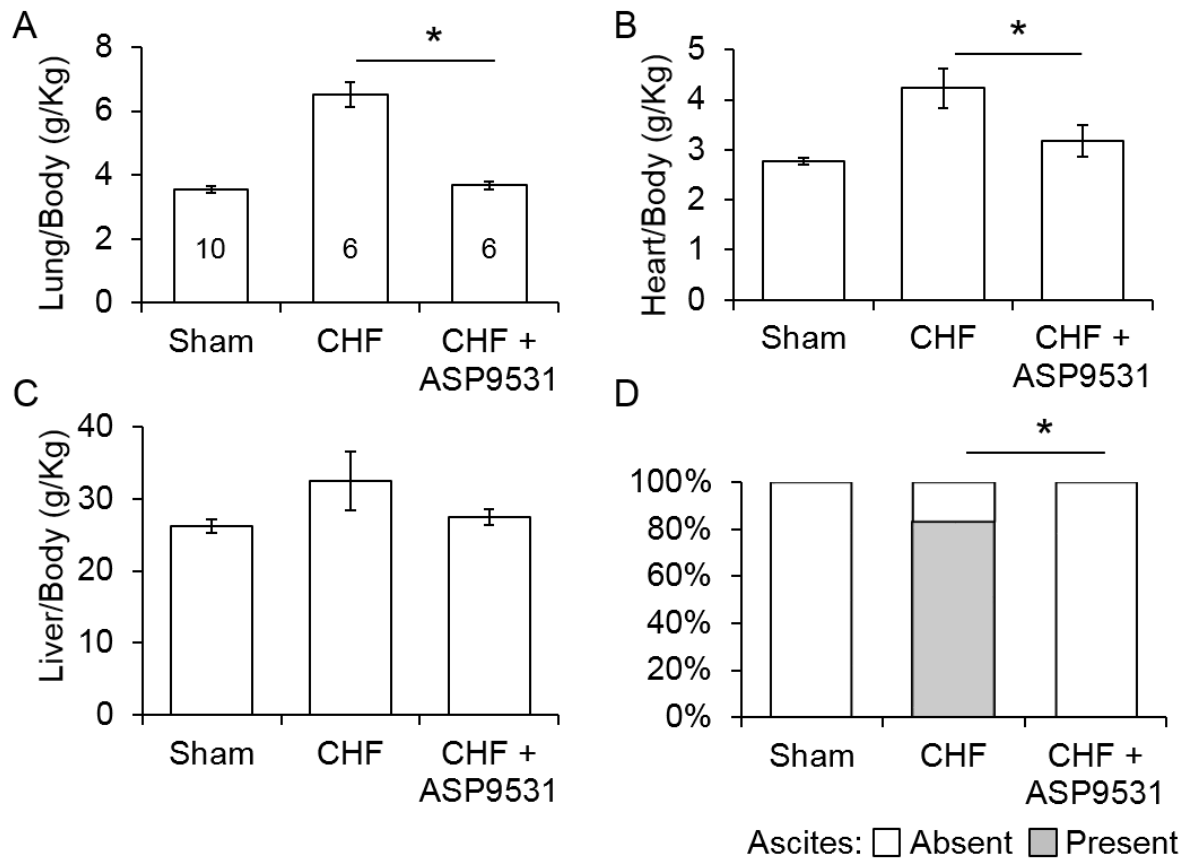
Figure 2



**Figure 2. Effects of heart failure and treatment with CL316,243 on Na<sup>+</sup>-K<sup>+</sup> pump function and expression of its subunits. A.** Membrane currents of myocytes isolated from rabbits with CHF treated or not treated with CL. I<sub>p</sub> was identified by the shift in the holding current induced by ouabain. C<sub>m</sub> indicates membrane capacitance. **B.** Effects of CHF and treatment with CL on mean I<sub>p</sub>. Number of myocytes are indicated in the columns (isolated from ≥ 5 rabbits for each treatment group). \* indicates P < 0.05. **C.** Representative blots of the effects of CHF and treatment with CL on expression of the α<sub>1</sub> and β<sub>1</sub> Na<sup>+</sup>-K<sup>+</sup> pump subunit. GAPDH was used as the loading control. **D.** Effects of CHF and treatment with CL on expression of the α<sub>1</sub> and β<sub>1</sub> pump subunit. Expressions of the α<sub>1</sub>- and β<sub>1</sub> pump subunits were measured for 6 rabbits in each treatment group.



Figure 3



**Figure 3. Indices of congestive heart failure with and without treatment with ASP9531.** **A.** Effect of treatment with ASP9531 on lung: body weight ratio. Ratios for sham-operated rabbits in hatched columns are the same as those in Figures 1 A-C and are not used for statistical comparisons here. **B.** Heart: body weight ratios. **C.** Liver: body weight ratios. One rabbit in the CHF group had a congenitally small liver, reducing the mean ratio and increasing variance of ratios. **D.** Prevalence of ascites at autopsy. Numbers of rabbits are indicated in columns. \* indicates  $P < 0.05$ .