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REVIEW



Clinical pharmacology of β -3 adrenergic receptor agonists for cardiovascular diseases

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ABSTRACT

Introduction: Few agonists of the third isotype of beta-adrenergic receptors, the β 3-adrenoreceptor, are currently used clinically, and new agonists are under development for the treatment of overactive bladder disease. As the receptor is expressed in human cardiac and vascular tissues, it is important to understand their beneficial (or adverse) effect(s) on these targets.

Areas covered: We discuss the most recent results of clinical trials testing the benefit and safety of β 3-adrenoreceptor activation on cardiovascular outcomes in light of current knowledge on the receptor biology, genetic polymorphisms, and agonist pharmacology.

Expert opinion: While evidence from small clinical trials is limited so far, the β 3-agonist, mirabegron seems to be safe in patients at high cardiovascular risk but produces benefits on selected cardiovascular outcomes only at higher than standard doses. Activation of cardiovascular β 3-adrenoreceptors deserves to be tested with more potent agonists, such as vibegron.

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Adrenergic; beta; beta3-adrenergic; catecholamines; cardiovascular; clinical trial; heart failure

1. Introduction

Cardiovascular diseases represent a major public health burden with 2–3% of adults in developed countries suffering from heart failure [1], a debilitating condition with a high risk of mortality. Despite significant advances with the recent introduction of new drug treatments [2], a high residual risk mandates the development of new, complementary therapeutic approaches that should rapidly be made available to patients. Robust pre-clinical research over the last two decades suggested the third isotype of β -adrenergic receptors, the β 3-adrenoreceptor to be a worthy target. The parallel introduction in the clinic of specific agonists at this receptor for the treatment of urological conditions allowed to test the validity of pre-clinical paradigms through the ‘re-purposing’ of these new drugs for cardiovascular indications. In this Expert Review, we will examine the recent progresses with this approach as well as remaining challenges, in light of the particular pharmacology of the β 3-adrenoreceptor and newest agonists.

2. The β 3-adrenergic receptor: genetics, distribution, regulation, and coupling in adipose and cardiovascular tissues

The third isoform of human β -AR was cloned in 1989 from a human cDNA library [3]. The human gene contains two exons (and one intron) and codes for a 408-aa protein that shares approximately 40–50% amino-acid sequence identity with

beta1 and beta2-adrenoceptors. The main divergences are concentrated in the third intracellular loop and C-terminal tail, where the β 3 isotype lacks the serine and threonine residues that constitute the consensus sequence for phosphorylation by G-protein coupled receptor (GPCR) kinases and protein kinase A. This was assumed to confer resistance of the β 3 isotype to agonist-induced homologous desensitization [4,5]. This notion has subsequently been disputed on the basis of additional studies mostly done in rodent adipocytes or in heterologous expression systems [6]; reviewed in [7], taking into account receptor or effector(s) downregulation, without consideration of the specific context of β 3AR in human cardiovascular tissues. This is most saliently illustrated by the differential regulation of isotypes in the failing myocardium, where β 1 and β 2 are well-known to be desensitized and downregulated [8], whereas the expression of β 3 receptors is upregulated in humans and animal models of heart failure [9,10].

Another unique characteristic of the human β 3 receptor is its higher affinity for norepinephrine than for epinephrine, unlike other adrenoceptor isotypes [11,12]. This reinforces the role of the β 3 receptor in circumstances of high orthosympathetic stimulation, such as heart failure, a fortiori in light of its relative resistance to desensitization. In such circumstances, the balance between β 1-/ β 2- and β 3 receptor activation would be tilted toward a reinforcement of the β 3 receptor signaling in the myocardium, that may be viewed as a ‘salvage pathway’ to prevent the damage from overactivation of β 1/ β 2 adrenoceptors. By the same token, further activation of β 3

Article highlights

- β 3-adrenoreceptors were first cloned from a human adipocyte cDNA library, and subsequently found to be expressed in the bladder smooth muscle but also (at RNA and protein level) in human myocardium and vessels.
- In pre-clinical studies, β 3-adrenoreceptors mediate vasorelaxation and effects on human ventricular muscle that are antipathetic to the classical β 1- and β 2 isotypes; as such, β 3 receptors may protect from excessive catecholaminergic stimulation of β 1 receptors.
- These and additional properties, such as antioxidant effects and relative resistance to desensitization prompted pilot clinical trials of the first β 3-selective agonist, mirabegron (currently used to treat overactive bladder disease, OAB) as a 'repurposed' drug in cardiovascular diseases
- Small randomized, placebo-controlled trials demonstrated limited beneficial effects on selected cardiovascular outcomes in patients with mild to severe cardiac disease at doses higher than the standard therapeutic dose for OAB. Mirabegron was generally safe.
- The concept of β 3 receptor activation deserves further studies with more potent agonists in larger trials, taking into account indirect metabolic effects on cardiovascular endpoints resulting from the receptor's activation in adipose tissue.

receptors with pharmacologic agonists would be expected to confer cardiac protection, as will be detailed below.

Aside from brown and white adipocytes, human β 3AR are robustly expressed in the detrusor muscle of the bladder, the myometrium, in several brain neurons, endothelial cells of the retina and in the gallbladder epithelium. Previous detection in cardiovascular tissues has been questioned owing to the dubious specificity of radioligands or antibodies for protein immunodetection (with cross-reactivity to β 1/ β 2AR isoforms), and limitations regarding affinity and potency of selective agonists to dissect cellular signaling [13]. Nevertheless, using a proprietary antibody duly validated for its isotype specificity, Chamberlain et al. identified human β 3AR proteins in human atrial myocytes [14], and the same antibody was used to confirm the receptor's expression in human ventricular myocardium [9]. This confirmed previous detection of transcripts in similar human tissues [15], including by laser-capture microscopy in human ventricular myocytes [16]. Notably, β 3AR are also expressed in endothelial cells from human coronary resistance vessels [17] and, based on functional studies, probably in the endothelium of larger, conduit arteries of other vascular beds [18].

In human white adipose tissue, β 3AR mediate lipolysis through G- α -s coupling to adenylyl cyclase and increases in cAMP [19]. The role and coupling of β 3AR in human brown adipocytes to induce thermogenesis has recently been disputed, in favor of a predominant role of β 2AR [20]. However, β 3AR may induce the 'beiging' of human adipocytes [21] interspersed in white adipose tissue [22]. While these observations have raised hopes for beneficial effects of β 3AR activation to control metabolism and obesity (Figure 1), the results with different agonists have been mixed, at best, probably owing to poor specificity and/or potency of the agents used and inter-individual variability in receptor expression (see section 1.3).

In human coronary vessels, activation of β 3AR mediates an endothelium-dependent vasorelaxation that involves both the

nitric oxide and hyperpolarizing factor (EDHF) pathways [17]. Activation of the endothelial NO-dependent pathway has also been implicated in vasorelaxant effect in larger human conductance arteries [18] (Figure 1).

The dissection of coupling mechanism(s) in human cardiac muscle has generated a number of observations with some discrepancies depending on the model studied (atrial vs. ventricular myocardium; whole tissue vs. isolated cells) and pharmacologic agents (specificity of agonists/antagonists; concentrations) used. An exhaustive discussion would be out of scope here, but most elements can be found in [13]. Organ bath experiments with human atrial strips generated contradictory results, possibly due to a temperature artifact [24] and/or involving an indirect effect through pre-synaptic catecholamine release resulting in a β 1AR positive inotropic effect [25]. The evidence is more consensual at the ventricular level. In human ventricular biopsies, natural catecholamines (norepinephrine) evoked a dose-response increase in force of contraction, that was inversed to a decrease in contraction force when the preparation was pre-incubated with the β 1/ β 2 AR antagonist, nadolol. Such decrease in contraction force was reproduced upon incubation with the preferential β 3AR agonist, BRL37344 (at sub- μ M concentrations). This first indicated a coupling of the β 3AR to a contractile response that was antithetic to that of the classical β 1/ β 2AR tissues [9,15]. Further dissection identified β 3AR coupling to G- α -i proteins and downstream nitric oxide synthase (NOS) [9,15] and production of cGMP (Figure 1). Based on studies in pre-clinical models (e.g. genetic mouse model expressing human β 3AR in cardiac myocytes, at levels comparable to human myocardium) such coupling to NOS is expected to be protective against excessive catecholaminergic stimulation (of β 1AR); indeed, these mice exhibit a reduced myocardial hypertrophy and fibrosis in response to several stressors (i.p. or minipump infusion of catecholamines or angiotensin II; transaortic constriction [26]), in the absence of negative inotropic effect; this protection was attributed to an anti-oxidant effect of the β 3AR. A similar phenotype was recently confirmed in a second, transgenic mouse line with cardiac-specific expression of the human β 3AR generated independently [27].

Of interest, another group independently identified a similar anti-oxidant effect of β 3AR activation through NOS and cGMP production [28]. In their models, this resulted in the protection of the β -1 subunit of Na^+ - K^+ -ATPase from oxidation, that preserved the activity of the pump in the face of oxidant stress. The resulting extrusion of Na^+ would regulate the excitation-contraction coupling in an opposite way in healthy or diseased cardiac muscle; in the healthy muscle, it would reduce contractility, while in failing cardiac muscle, reducing the intracellular Na^+ overload would improve contractility [28]. The functional interaction between these signaling elements is reinforced from their confinement in membrane compartments such as caveolae, where β 3AR and NOS co-localize [26] with key regulators of excitation-contraction coupling. Using a pre-clinical model of diabetes in rabbits, the same group proposed a similar β 3AR-mediated antioxidant protection from glutathionylation of eNOS and of the β -1 subunit of the Na^+ - K^+ -ATPase in vessels, resulting in restoration of

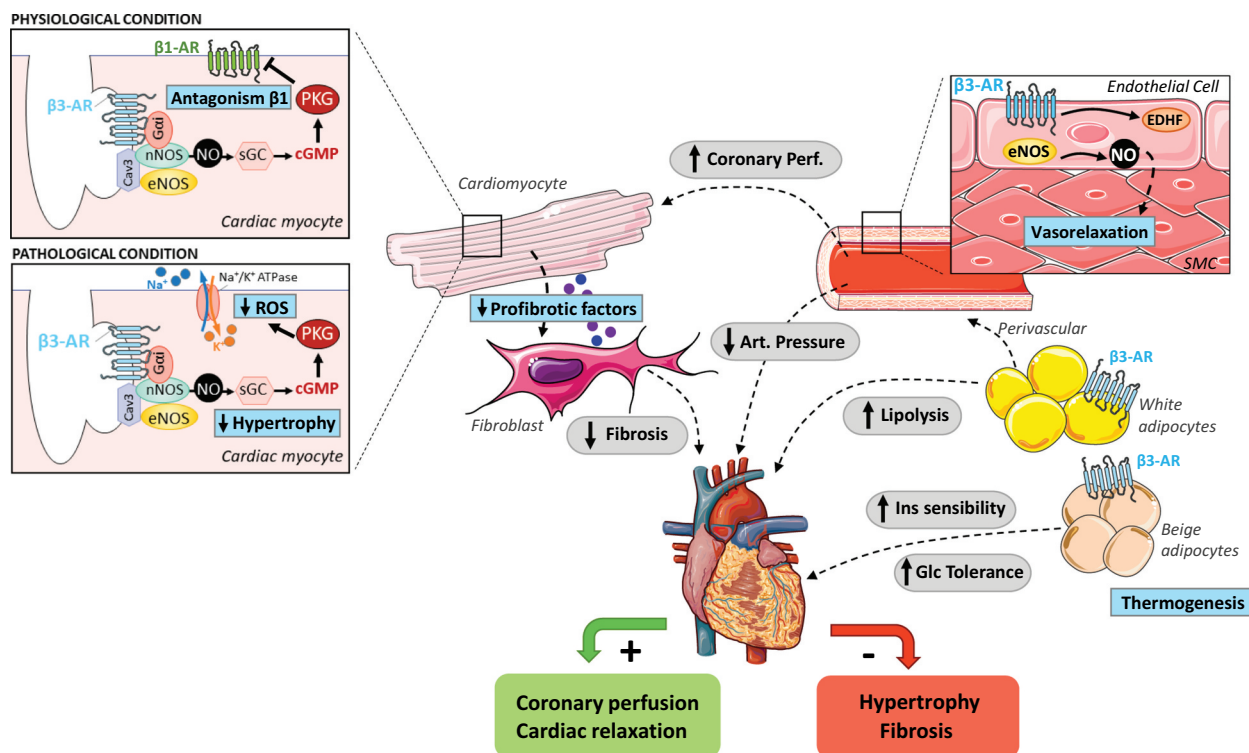


Figure 1. β3AR-mediated cardiovascular protection through paracrine and systemic signaling. β3AR are expressed in human white and beige adipocytes where their activation initiates lipolysis and thermogenesis processes, respectively. β3AR-mediated 'beiging' of (human) visceral adipocytes participates to improvements in peripheral metabolism, with improved insulin sensitivity and glucose tolerance [51]. In cardiac myocytes β3AR pathway limits β1AR signaling through NOS-mediated signaling [9,15]. In pathological situations, similar NOS-mediated signaling of β3AR leads to a localized pool of cGMP activating Protein Kinase G (PKG) and decreases intracellular oxidant stress [26] which among others protects the β1 subunit of Na⁺-K⁺-ATPase from oxidation preserving the activity of the pump. Similarly antioxidant capacity of β3AR results in a decrease in the synthesis of myocyte-derived profibrotic factors (e.g. CTGF, TGFβ2) acting on neighboring cardiac fibroblasts [23] and is implicated in the anti-hypertrophic effect of β3AR in the face of neurohormal and hemodynamic stress [26]. β3AR expression in coronary (and peripheral) endothelial cells results in NO and EDHF production which increase myocardial perfusion and reinforce the effect of myocyte β3-AR through paracrine effects [17].

endothelium NO-dependent relaxation as well as K⁺-dependent vasorelaxation in conditions of oxidant stress, such as diabetic vasculopathy [29,30] (Figure 1).

Based on these endothelium-mediated vasorelaxant effects, others have examined the potential of β3AR activation to reduce pulmonary vascular resistance and preserve right

ventricular function in models of pulmonary hypertension. Pre-clinical studies in a pig model with pulmonary artery banding indeed confirmed such protection, as well as β3AR-mediated attenuation of hypoxia-induced proliferation of human pulmonary vascular cells in vitro; however, the expression of transcripts of the human β3AR (by RT-qPCR) was low in

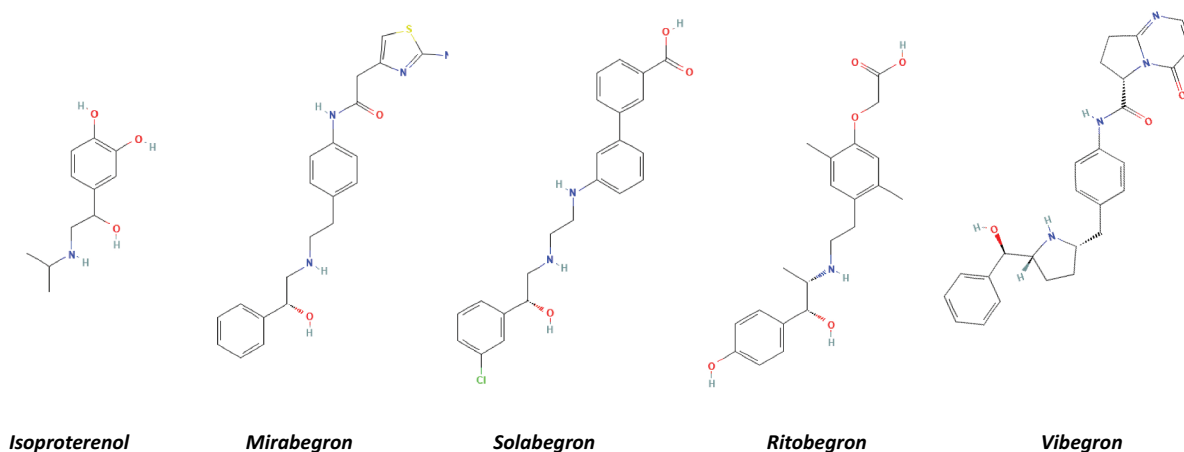


Figure 2. Chemical structures of the pan-β-adrenoreceptor agonist, isoproterenol and the following β3-adrenoreceptor agonists: mirabegron, solabegron, ritobegron and vibegron.

human pulmonary vessels (>30 Ct) and the expression of the protein was not documented [31].

All the above paradigms would favor beneficial effects of β_3 AR activation (instead of deleterious negative inotropic effects) and led to translational efforts to re-purpose clinically available β_3 AR agonist(s) in cardiovascular diseases, as will be developed in section 1.3. In most cases, this was based on evidence of the receptor's expression (at transcript or protein level) in target cardiac or vascular tissue, its persistence or upregulation in diseased tissue and putative resistance to desensitization, which would make it an attractive target for recent or new agonists specific for the human β_3 adrenergic receptor.

3. Pharmacology of β_3 -adrenoreceptor agonists currently studied or used for cardiovascular diseases

Agonists with high affinity for the human β_3 AR include the latest generation molecules, mirabegron, solabegron, ritobegron, and vibegron (Figure 2). All have been shown to produce a concentration-dependent relaxation of pre-contracted human bladder strips with EC₅₀ lower or close to 1 μ M and have been developed for the treatment of over-active bladder disease. Data on efficacy in human cardiovascular (and adipose) tissues are scarce and mostly available for mirabegron.

In human right atrial strips, mirabegron at 1–10 μ M produced an increase in contraction force that was less pronounced (approx. 10%) than the maximal effect of the pan- β AR-agonist, isoproterenol, and insensitive to the β_3 AR antagonist, L748337. Instead, this effect was reversed to a negative inotropic effect upon blockade of β_1 AR with CGP20712A; this reduction in contractile force was also insensitive to β_3 AR blockade. Based on the structure similarity of mirabegron with phenylethanolamine drugs (such as ephedrine) known to interact with neuronal transporters of noradrenaline and to be taken up by nerve terminals [32], one hypothetical explanation for the increase in contraction force proposes that mirabegron produces an indirect sympathomimetic effect through the release of noradrenaline and activation of β_1 AR. The mechanism(s) underlying the negative inotropic effect in the human atrial strips remains undetermined. Unfortunately, to our knowledge, similar experiments have not been repeated in human ventricular tissue with mirabegron (or any of the other agonists at human β_3 AR developed for clinical use).

The relevance of these observations to clinical effects may be disputed on the basis of pharmacokinetic properties of mirabegron. At therapeutic doses, the plasma concentrations of mirabegron were measured at 83–167 nM [33], well below those shown to produce the above effects in vitro. However, the lipophilicity of mirabegron and tissue penetration may still allow to approach active concentrations, particularly upon chronic treatment and in diseased hearts where target β_3 AR are upregulated [9].

These pharmacokinetic properties (e.g. lipophilicity) may well subserve the efficacy of mirabegron in another tissue relevant for cardiovascular diseases, namely the adipose

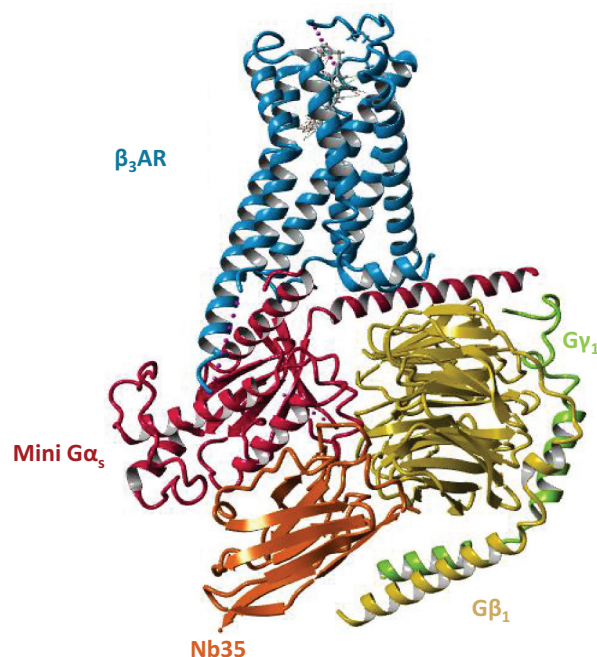


Figure 3. Ribbon representation of cryo-EM structure of the β_3 -adrenoreceptor- $G\alpha_5$ complex (β_3 AR, blue; mini $G\alpha_5$, purple; nb35, Orange; $G\beta_1$ yellow; $G\gamma_1$, green) (PDB: 7DH5).

tissue. Since the observation of brown/beige adipose tissue in human adults, and its proposed beneficial effects on metabolism based on pre-clinical evidence (and pilot human clinical studies; see section 1.3 below), the role of β_3 AR has recently been examined in human brown/beige adipocyte lipolysis and thermogenesis (Figure 1). Despite some controversy in the respective roles of β_2 - or β_3 AR (see above [20]), mirabegron was shown to increase lipolysis in human primary differentiated adipocytes by 1.6-, 1.9-, and 2.2-fold at 100 nM, 1 μ M and 10 μ M, respectively. The effect was reduced upon siRNA downregulation of *ADRB3* in these cells, and reproduced in a separate immortalized adipocyte cell line [34].

Among other agonists, vibegron exhibited a higher maximal response (cAMP production, relative to the full agonist, isoproterenol) than mirabegron in heterologous expression systems (HEK cells expressing the human β_3 AR), with mean EC₅₀ of 2.13 nM for vibegron and 10 nM for mirabegron [35]. While data on a similar comparison in human cardiac or vascular tissues are not available, this raises the possibility to more efficiently target β_3 AR in the heart, vessels, or adipose tissue with vibegron or even more potent agonists to be developed in the future.

Indeed, structural data underlying the subtype specificity and potency of β_3 AR agonists will probably help efforts to develop even better drug candidates. For example, the molecular determinants of sub-type selectivity of mirabegron for β_3 AR (vs. β_1 -2 AR) have been greatly revealed from recent cryo-EM studies of the dog β_3 AR complexed with heterotrimeric G- α -s and this agonist [36] (Figure 3). Interaction of the ligand at the orthosteric site involves 17 aa of the receptor (within transmembrane 2–3, and –5–7 domains and extracellular loop 2), where

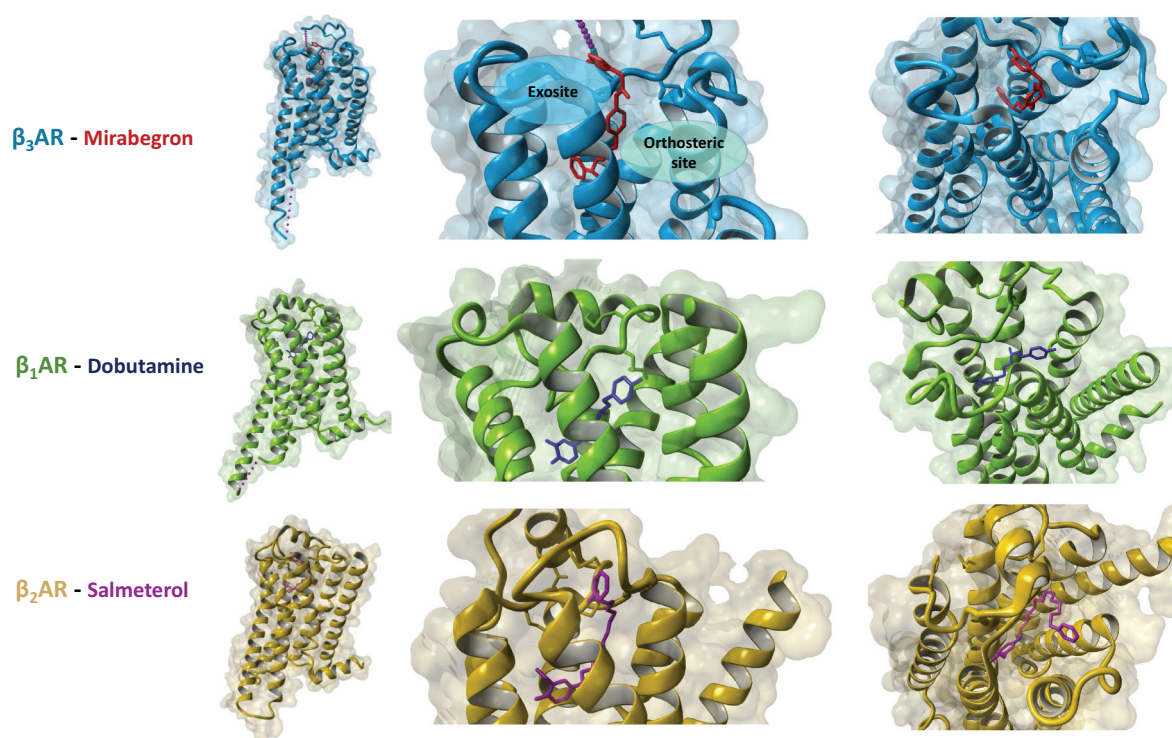


Figure 4. (Left) membrane plane views; (Middle) cross sections views; and (Right) views from the extracellular side of β -adrenergic receptors from cryo-EM-resolved structures. β_1 -AR (green) [38], β_2 -AR (yellow) [39] and β_3 -AR (blue) [36] and their respective agonists, dobutamine (dark blue), salmeterol (purple) and mirabegron (red) (with missing residues in the structure highlighted in pink)(PDB: 7DH5, 6MXT, 6H7L).

the phenylethanolamine central moiety of mirabegron forms hydrogen bonds with specific residues (i.e. D117 and N332) in a manner similar to other catecholamines in β_1 -2 AR (note that most of these 17 residues are conserved between the human and dog β_3 AR, except for S325, not critically involved) (Figure 4). However, the residues of the exosite are different between β AR isotypes; in the β_3 AR, they determine a conformation distinct from that of β_1 and β_2 AR, resulting in a narrower exosite and a ligand binding pocket of β_3 AR that is more perpendicular. This is thought to cause steric hindrance against the binding of β_2 or β_1 AR selective agonists, such as salmeterol and dobutamine, respectively. Conversely, mirabegron (and other β_3 AR selective agonists) may adopt a linear conformation allowing them to fit within the perpendicular binding pocket and narrower exosite. This was recently confirmed by the same approach for solabegron bound to the same dog β_3 AR [37]. In addition, interaction of the agonist moieties with residues of the extracellular loop 2 critically determines the potency of solabegron, as may be the case for other β_3 AR agonists. While the data will probably guide the design of novel agents targeting the β_3 AR, they do not completely explain the exclusive binding of mirabegron (or solabegron) to the β_3 isotype, and not to β_1 -2AR. This could be solved from future structural analyses of β_1 -2 AR bound to mirabegron and other β_3 AR selective agonists.

Nevertheless, the data provide a strong basis to explain the potency of selective β_3 AR agonists, such as mirabegron, solabegron, and vibegron that contain the basic pharmacophore

2-amino-1-phenylethanol (Figure 2), completed with appropriate residues at the two extremities.

4. Past and current clinical studies of β_3 AR agonists in cardiovascular diseases

Based on the paradigms described in 1.1, a number of clinical trials have tested the 'repurposing' of mirabegron, as the first β_3 AR selective agonist available for clinical use, in patients affected by several cardiovascular conditions (Table 1). A first randomized, placebo-controlled trial tested the effect of mirabegron (vs. placebo) in 70 patients suffering from heart failure with reduced ejection fraction (HFrEF). The rationale for this trial was based on the preclinical paradigm postulating that β_3 -AR stimulation protects the cardiac $\text{Na}^+\text{-K}^+$ -ATPase from oxidative inactivation, leading to a reduction of the detrimental increase in intracellular Na^+ levels in failing cardiac myocytes, as developed in section 1.1 [28]. Accordingly, the BEAT-HF trial (NCT01876433) enrolled patients with NYHA class II–III heart failure and left ventricular dysfunction (LVEF $\leq 40\%$) randomized to either 300 mg mirabegron per day (6 times the usual therapeutic dose in overactive bladder disease) or placebo up to 6 months. The trial missed the primary endpoint (increase in the LV ejection fraction) [40], but in an exploratory analysis of the data, mirabegron improved the ejection fraction in a (small) subgroup of subjects with more severe LV dysfunction at baseline (LVEF $< 40\%$). The same group recently performed a follow-up trial (BEAT HF II) (NCT03926754) investigating the same dose of mirabegron in patients selected on the basis of NYHA class III–IV heart failure

Table 1. β_3 -AR agonists in clinical trials in CVD.

Description	BEAT-HF I	BEAT-HF II	SPHERE-HF	BETA3_LVH	STAR-PAD
Patients	70	22	80	296	120
Pathology	HF with reduced ejection fraction ($\leq 40\%$) NYHA Class II–III	HF with reduced ejection fraction ($< 35\%$) NYHA Class III–IV	Chronic Pulmonary Hypertension Secondary to Heart Failure	Structural heart disease (stage B, AHA/ACC) at risk of development or worsening of HF max. NYHA Class II	Atherosclerotic Peripheral arterial disease with resting ankle-brachial index (ABI < 0.90)
Rationale from preclinical studies	- Reduction of oxidative stress - Reduction of myocyte Na^+ accumulation - Improvement of ejection fraction	- Reduction of oxidative stress - Reduction of myocyte Na^+ accumulation - Improvement of ejection fraction	Improvement of pulmonary hemodynamics, remodeling and RV performance	Improvement of LV diastolic relaxation, reduction of myocardial hypertrophy and fibrosis	Vasorelaxant effects of endothelial β_3 AR activation
Intervention	Mirabegron 25–300 mg 6 months	Mirabegron 300 mg 1 week	Mirabegron 50–200 mg 4 months	Mirabegron 50 mg 12 months	Mirabegron 50 mg 3 months
Parameters	LV ejection fraction	LV ejection fraction	Pulmonary vascular resistance	- Left ventricular mass index - Diastolic function (E/e')	Peak walk time
Secondary	- Diastolic function - Parameters assessing myocardial remodelling	- Diastolic function - Parameters assessing myocardial remodeling	- Parameters assessing myocardial remodeling - RV ejection fraction	- Parameters assessing myocardial remodeling and fibrosis - Systemic effect on endothelial function - Metabolic parameters	- Initial claudication - Walking impairment - ABI - Assessment of tissue perfusion - QOL
Results	- No improvement of LV ejection fraction - Beneficial effect in subgroup of patients with more severe LV dysfunction (LVEF $< 40\%$)	- Larger increase in cardiac index at rest (approx. 20%) - Decrease in pulmonary vascular resistance	- Primary endpoint not reached - small (3%) improvement in RV ejection fraction - No difference in heart rate or blood pressure	- Neutral effect on the co-primary endpoints, as well as on most of the secondary endpoints - Small decrease in hs-Troponin-I - Results sub-studies pending	Expected in 2024

and a more severely reduced ejection fraction (LVEF<35%). The setting was different, however, as 22 patients were randomized to placebo or mirabegron (300 mg), taken daily for only one week on top of recommended therapy for heart failure. Patients were evaluated for changes in cardiac index, pulmonary and systemic vascular resistance by invasive hemodynamic techniques at rest and during submaximal exercise at baseline, 3 hours after the first study dose and again after 1 week of treatment. Mirabegron did not induce any difference (vs. placebo) after 3 hours, but produced a larger increase in cardiac index (approx. 20%) after 1 week at rest (not during exercise), together with a decrease in pulmonary vascular resistance. Tolerance to the active drug was good, with no difference in heart rate, systemic vascular resistance, blood pressure, or renal function [41]. Note that, per protocol, all patients in the two trials were taking β -adrenergic blockers as part of their background treatment, which probably protected them from off-target effects of the high dose of mirabegron on β 1AR. While encouraging, the data need to be confirmed in larger phase II trials, with longer treatment periods to verify if the benefit is sustained, as should be done with all drug interventions that produce a positive inotropic effect in chronic heart failure. Nevertheless, despite these limitations, these observations again seem to highlight the antithetic influences between the deleterious β 1AR activation on the failing heart and β 3AR activation (as expected here with a high dose of mirabegron under β 1AR blockade), possibly due to alternative coupling of the β 3AR to NOS/cGMP.

β 3AR activation, in this regard, may be viewed as equivalent to the effect of a β 1AR blocker. In the setting of heart failure, such effect may produce negative inotropism, with initially impaired cardiac pump function. One may ask, then, whether there may be some utility for β 3AR antagonists in this condition. While some pre-clinical data may suggest so [42], the results of the recent BEAT-HF II trial support the opposite, i.e. benefit from activating β 3AR, as detailed above.

Aside from effects on cardiac hemodynamics, the same β 3AR-mediated signaling pathway would be expected to attenuate the maladaptive myocardial remodeling leading to worsening heart failure, as exemplified in pre-clinical models (see section 1.1 above). To test this hypothesis, the BETA3LVH trial (NCT02599480), a phase IIb, multicentric, randomized, placebo-controlled trial enrolled 296 patients at risk of developing or worsening heart failure with preserved or mildly reduced ejection fraction based on the presence of structural heart disease (stage B, AHA/ACC). They were assigned to receive placebo or mirabegron 50 mg/day and followed up to 12 months. As co-primary endpoints, changes in left ventricular mass index (by cardiac MRI) and in left ventricular diastolic function (E/e' , by Doppler echocardiography) were recorded between baseline and 12 months; changes in cardiac fibrosis, left atrial volume index, exercise capacity, and specific biomarkers reflective of myocardial remodeling were measured as secondary endpoints. In two additional sub-studies, the effects of mirabegron were measured (i) on peripheral endothelial function (by pulse amplitude tonometry; ENDO-PAT) together with NO bioavailability in erythrocytes by EPR spectroscopy [43]; and (ii) on the activity of brown adipose tissue (BAT) by ^{18}F FDG-PET and on BAT abundance by CT-scan

together with additional metabolic parameters (serum lipids, glucose, HbA1c, insulin sensitivity by HOMA test). The results of the primary study were recently disclosed at the AHA Scientific Sessions (Chicago, November 2022 [44]). At the usual therapeutic dose of 50 mg/day, mirabegron had a neutral effect on the co-primary endpoints, as well as on most of the secondary endpoints (except a small decrease in serum hs-Troponin-I). This dose of mirabegron may not have been sufficient to potentially activate cardiac β 3AR known to be less abundant in normal versus failing hearts [9]. Compared with the BEAT-HF trials, the patients in BETA3LVH were much 'healthier,' i.e. with minimal cardiac remodeling or symptoms (maximum NYHA II symptoms) at baseline, leaving little room for improvement under mirabegron, particularly on top of an already protective background therapy (with most patients under ACEi and/or ARB; and about half under β -blockers) and over a relatively short time frame. On the other hand, BETA3LVH is the first prospective, randomized study to closely monitor potential side-effects of mirabegron in a patient population with high cardiovascular risk (>90% hypertensive, 20% diabetics, 9% with atrial fibrillation); in that regard, the results confirm that mirabegron is safe, both in terms of the incidence of cardiovascular (AF/Flutter; blood pressure by ambulatory blood pressure monitoring) or other adverse effects (e.g. worsening renal or liver function). The complete results of the main and sub-studies are due to be reported shortly.

The vasodilatory effects of β 3AR activation in several human vascular beds [17,18] also justified additional clinical trials of mirabegron, particularly in pulmonary hypertension. Robust pre-clinical data in pigs with pulmonary aortic banding indeed suggested beneficial effects, as mentioned in section 1.1. The multicenter, randomized, placebo-controlled SPHERE-HF trial randomized 80 patients with combined pre- and post-capillary pulmonary hypertension associated with HFrEF or HFpEF to placebo or mirabegron that was up-titrated from 50 to 200 mg daily (with a median dose of 150 mg/day) for 4 months [45]. The primary outcome was the change in pulmonary vascular resistance (PVR), measured at right heart catheterization. Secondary endpoints included changes in right ventricular ejection fraction (by cardiac MRI or cardiac CT), additional hemodynamic parameters, as well as in functional class and quality of life. The primary endpoint was not reached, but mirabegron did produce a (small, 3%) improvement in RV ejection fraction compared with placebo. There was no difference in heart rate or blood pressure. Mirabegron had a neutral effect on other secondary endpoints. The discrepancy between improved RV ejection fraction and unchanged PVR in the same study may not be unusual, but the absence of effect on PVR in this trial contrasts with the positive effect of mirabegron on the same parameter in BEAT-HF-II (see above). Aside from the mixed population of HFrEF (1/3) and HFpEF (2/3 of patients) with approx. 50% with NYHA class III in SPHERE, the dose of mirabegron was also lower (median: 150 mg/day; with >70% under beta-blockade) than in the BEAT-HF trials. Assuming that the effect on PVR would derive from an improvement in left ventricular function, mirabegron at this dose may not have been sufficient to target cardiac β 3AR in this less sick population. Alternatively, there

may be too little or no expression of β 3AR in the human pulmonary vasculature (see above, section 1.1) to produce significant effects on PVR.

Another trial of mirabegron in patients with peripheral artery disease, the STAR-PAD trial (registered in Australia and New Zealand under ACTRN12619000423112) is still ongoing. The rationale again stems from pre-clinical [17,18,28] and human ex vivo [17,18,30] observations of vasorelaxant effects of endothelial β 3AR activation; in addition, the antioxidant effect of β 3AR and preservation of endothelial eNOS activity would be expected to prevent or reverse endothelial dysfunction, improve angiogenesis and, perhaps, peripheral limb perfusion. Accordingly, STAR-PAD is enrolling 120 patients with stable peripheral artery disease (PAD) and intermittent claudication who randomly receive either mirabegron at the usual therapeutic dose (50 mg daily) or placebo for 3 months [46]. The primary endpoint focuses on the change in peak walking distance (graded treadmill test), a clinically relevant parameter commonly used to evaluate the efficacy of treatments for PAD. Secondary endpoints evaluate additional parameters of functional status and quality of life. Additional sub-studies include mechanistic evaluations of mirabegron's effects on flow-mediated dilatation, peripheral limb perfusion, parameters of vascular oxidant stress as well as the angiogenic potential of endothelial progenitor cells. The results are expected to be disclosed in the course of 2024.

Of note, the vasodilatory and pro-angiogenic effects of β 3AR activation may not be uniformly beneficial in all clinical conditions. In diabetic retinopathy, local hypoxia drives the upregulation of the pro-angiogenic Vascular Endothelial Growth Factor (VEGF) that aggravates retinal lesions with ensuing vision loss. Strikingly, hypoxia also drives the local expression of beta3 adrenoceptors that promote angiogenesis, in part by mediating VEGF release [47]. This raises the interest for β 3-adrenoceptor antagonists as adjuvant treatment for hypoxic retinopathy, but also calls for cautionary use of agonists in patients with advanced diabetic retinopathy.

Given recent evidence on the role of β 3-adrenergic activation of beige/brown fat and its proposed favorable effect on peripheral metabolism (see section 1.1 above), a few pilot studies have tested mirabegron for its ability to modulate the glycemic and lipid profile in healthy subjects, as well as in patients with obesity and insulin resistance. While few of these studies were placebo-controlled and only included small numbers of subjects, they deserve interest not only in the search of improved treatments for the metabolic syndrome, but also for the indirect impact of any metabolic effect of mirabegron on the development and progression of cardiac and vascular diseases [48]. After the (re)discovery of brown fat in adult humans [49], a pilot trial showed that a single, high dose of mirabegron could activate brown adipose tissue activity (^{18} F-DG-PET uptake) in lean, healthy adults [50]. Aside from dubious selectivity for β 3AR activation at this supra-therapeutic dose (in absence of β 1AR blockade), questions remained on the efficacy to reproduce such activation in older and obese subjects, known to bear little remaining

brown tissue. A subsequent study [51] suggested this to be possible, not only through stimulation of residual (if any) brown adipocytes, but also by 'beiging' of white adipose tissue. In lean and obese subjects, a 10-day treatment with 50 mg/day of mirabegron induced 'beiging' of white adipose tissue more potently than cold exposure [21,51]. This contrasts with the absence of lipolytic effect of a single dose of the drug in human white adipose tissue (despite their demonstrated expression of β 3AR [19], and points to the need for chronic activation [21,51,52], in line with the upregulation of β 3AR expression by chronic adrenergic stimulation [53]). In 13 obese, insulin-resistant subjects, a daily intake of 50 mg mirabegron for 3 months induced the expression of 'beiging' genes (*UCP1*, *TMEM26*, *CIDEA*) in subcutaneous white adipose tissue, together with some improvement of the oral glucose tolerance and HbA1c level. The brown adipose tissue mass and activity by PET-CT was unchanged, as was the homeostatic model assessment of insulin resistance (HOMA-IR). However, other measures of insulin sensitivity (from euglycemic clamps) and pancreatic β -cell function were improved [51]. Together with ex vivo experiments demonstrating the expression of β 3AR and effect of mirabegron on primary human adipocytes to regulate lipolysis and thermogenesis [34], this would argue in favor of clinically relevant metabolic benefits of β 3AR agonists. However, recent disclosure of results from the BETA3LVH clinical trial, randomly testing mirabegron (50 mg/day) versus placebo on 296 patients (mostly obese) over 12 months did not show any improvement on glycemic levels or HbA1c. A more detailed analysis of metabolic parameters as well as brown fat by PET-CT is due to be reported later.

5. Cardiovascular adverse events of clinically-available β 3AR agonists

Abundant evidence has been collected from phase III/IV studies for mirabegron and the more recent agonist, vibegron used for the treatment of overactive bladder disease (OAB). A systematic review of the literature on cardiovascular effects of mirabegron taken for OAB shows a dose-dependent, small increase in heart rate compared with placebo, which is reversible upon treatment discontinuation [54,55]. At the dose of 50 mg/day, the increase in heart rate (vs. placebo) did not exceed 1 beat per minute [56]. In a cohort of 279 patients with OAB, symptomatic palpitations under mirabegron occurred in 2.9% of patients [57], consistent with 3.8% reported from a systematic review of the literature [55]. A small increase in QTc interval on ECG was also observed in phase III trials with mirabegron. While the underlying mechanism is unclear, toxicity studies in dogs with plasma levels of mirabegron well in excess of therapeutic concentrations in humans showed no effect on ion channel conductance or arrhythmogenesis [58]. Changes in blood pressure from baseline or compared to placebo also did not indicate a risk for developing hypertension with mirabegron [59]. This was rigorously tested by ambulatory

blood pressure monitoring (ABPM) in 715 patients from the SYNERGY trial, including patients randomly taking mirabegron with or without solifenacin or placebo for 3 months. ABPM showed no increase from baseline in the mean 24-h systolic or diastolic blood pressure in combination or monotherapy (including mirabegron at 50 mg/day) versus placebo. A similar neutral effect was seen by analyzing 1-h blood pressure averages in the timeframe that included the Tmax value of mirabegron. No difference was seen in the number of switches between normotension/hypertension stages. Likewise, the 24-h heart rate signals were unchanged [60]. While these data seem reassuring, it should be noted that in most studies recruiting patients with OAB, subjects with serious cardiovascular comorbidities, including hypertension, arrhythmia, or heart failure, were excluded. In contrast, the BETA3LVH trial included a safety analysis in the 296 patients specifically selected for their high cardiovascular risk and randomly taking mirabegron (50 mg/day) or placebo for 12 months. More than 90% had hypertension (controlled under stable treatment), 20% were diabetic, 9% had atrial fibrillation at inclusion, together with symptoms (up to NYHA stage II) and evidence of structural heart disease (left ventricular hypertrophy, by echocardiography) at baseline. Blood pressure was measured clinically and by ABPM at baseline and sequentially until the end of treatment. The initial analysis showed that the number of new episodes of hypertension or arrhythmia was not different between the two treatment arms. No death was recorded during the trial. These placebo-controlled data in a high cardiovascular risk population are reassuring in the face of pre-clinical data that suggested potential adverse effects on atherosclerosis [61], adipose tissue inflammation [62–64] or indirect β 1-adrenergic system activation [65]. Regarding the latter, additional protection can be afforded by concomitant use of a β 1AR blocker, as indicated for many cardiovascular conditions and systematically used in all patients taking high doses of mirabegron in the smaller BEAT-HF and SPHERE trials, and in 50% of patients in the BETA3LVH trial.

The more potent β 3AR agonist, vibegron seems to have a similar safety profile, including in older patients with more prevalent cardiovascular conditions. Patients >65 or >75 years from the EMPOWUR study who received vibegron (75 mg/day) experienced hypertension or increased blood pressure at similar or lower rates than those receiving placebo. The incidence of other CV side-effects (e.g. atrial fibrillation) in the vibegron arm was low and similar to that in the placebo group [66]. The data are consistent with observations in a post-hoc study of a placebo-controlled phase III Japanese trial including older patients with cardiovascular diseases, who received vibegron at 50 or 100 mg/day for 3 months. There was no difference in blood pressure or pulse rate among the 2 treatment groups and placebo [67].

6. ADRB3 polymorphism

A number of polymorphisms have been identified in the *ADRB1*, -2 and -3 genes with much attention to rs4994 in *ADRB3* (resulting in a substitution of Trp for Arg at position 64, on the intracellular side of transmembrane domain 1), given

early evidence of a potential impact on weight gain in a French population [68] and earlier onset of non-insulin dependent diabetes melitus (NIDDM) in Pima Indians [69]. Since then, subsequent association studies provided contradictory evidence for any physiologically relevant effects on the associated phenotype. This is perhaps due to the mixing of heterogeneous populations in meta-analyses and the interference of poorly controlled confounding factors. The prevalence of the Trp64Arg polymorphism varies among different ethnic groups and is more prevalent in the Asian population with, for example, a frequency up to 35% of combined heterozygous and homozygous variants in a Japanese cohort. In the latter cohort of more than 700 subjects aged 40 and older, the Trp64Arg polymorphism was associated with insulin resistance and the risk of developing hypertension [70]. Despite the caveat regarding meta-analyses, in one such study of 9555 subjects, this polymorphism was only marginally associated with the risk of essential hypertension in the whole population, but significantly so in the specific subgroups of Chinese and Caucasians [71]. Another meta-analysis of more than 11000 subjects from 15 independent studies concluded that the risk of coronary heart disease was significantly associated with the polymorphism in Chinese, but not in Caucasians, again emphasizing an ethnicity-specific effect [72]. More confusion in the field came from experiments in heterologous expression systems showing the absence of functional alteration in the signaling of the Trp64Arg mutant receptor [73]. Nevertheless, human adipocytes bearing the native mutant receptor were shown to exhibit a 10-fold lower sensitivity to a β 3AR agonist [74]. This would reinforce the pathogenic significance of this mutation (or possibly of additional-yet unidentified- portions of the gene in linkage disequilibrium with rs4994).

7. Conclusion

Mirabegron is the only selective β 3-AR agonist 'repurposed' for cardiovascular indications so far. In small RCT using doses higher than the standard therapeutic dose for OAB (50 mg/day), mirabegron produces an improvement of left ventricular function in patients with severe heart failure in a hospital setting. In another RCT, it also slightly improves right ventricular function in patients with pulmonary hypertension. In both instances, mirabegron seemed to be safe despite the high doses used (with concomitant use of a β 1/2 blocker), but studies with longer treatments are desirable to confirm both the benefits and innocuity. The efficacy and safety of long-term use of the standard dose (50 mg/day) is tested on cardiovascular outcomes in the BETA3LVH trial and on peripheral artery disease in the STAR-PAD trial, the full results of which should be disclosed soon. These and future studies should also clarify whether metabolic effects resulting from β 3-mediated activation of the adipose tissue with mirabegron result in clinically relevant benefit on cardiovascular outcomes.

8. Expert opinion

Since its first molecular identification almost 40 years ago, the β 3AR has raised hopes and disappointment as a

regulator of metabolism, energy expenditure, and weight control in humans. This can be explained, in part, by unrealistic expectations to find a rapid cure for human obesity with poorly selective agonists that produced prohibitive cardiovascular side-effects through off-target activation of β 1-2 AR. Admittedly, the pharmacology of the β 3 isotype is complex; this is reflected by sometimes contradictory reports using agonists/antagonists with dubious specificity; the detection of the receptor itself (at protein level) in human tissues remains a challenge with poorly validated commercial reagents. Nevertheless, robust pre-clinical studies established its expression and function beyond the adipose tissue, e.g. in the bladder muscle and cardiovascular tissues. While much less abundant than in the bladder, and than β 1/2AR in the normal heart, the cardiac β 3AR remains an interesting target because of its resistance to desensitization and upregulation in the diseased human myocardium. Together with the recent development of new agonists, this opens the possibility of 'repurposing' some of these new drugs for cardiovascular diseases. Clinical trials testing such repurposing of mirabegron have not shown efficacy so far, at least at standard therapeutic doses; at higher doses, the positive effects on left and right ventricular function (in BEAT-HF and SPHERE, respectively) should be confirmed in larger studies and for longer periods of treatment; in any case, they must be tested on a background treatment of β 1-blockade, which is part of the standard in heart failure patients anyway. So, this approach is viable, but the challenge will be to demonstrate an additional benefit beyond standard-of-care, which will now include not only ARNi's (Angiotensin type I Receptor and Neprilysin inhibitor, i.e. valsartan/sacubitril) but also SGLT2 inhibitors, that demonstrated significant benefit across the spectrum of heart failure patients. In the acute setting, however, as in BEAT-HF, mirabegron, and probably GLP-1 agonists may still have a role to help the failing patient transition to a more stable condition.

Based on initial disclosure of primary outcomes, mirabegron has a neutral effect on myocardial remodeling, at least in the specific conditions of the BETA3LVH trial. The baseline characteristics of the randomized patients show that they suffered a milder cardiac pathology (compared to BEAT-HF), with little structural heart disease (left ventricular hypertrophy) that did not progress over the 1-year period of observation in the placebo arm. This left little room for the active treatment to either prevent or improve the outcome. The neutral effect on some metabolic endpoints, i.e. glycemic control, contrasts with recent pilot trials in obese adults with comparable clinical profiles (which, however, were not placebo-controlled). In both cases, at 50 mg/day, mirabegron was probably not sufficient to activate the lowly expressed cardiac β 3AR in relatively healthy hearts and in the few dispersed 'beige' adipocytes in elderly, mostly overweight patients. The discovery of more potent molecules guided by recent structural data by cryo-EM should allow to hit more efficiently the relatively low abundant β 3AR in cardiovascular and adipose tissues of such patients. The already available

newer molecule, vibegron could already be used to test the ' β 3AR paradigm' in future RCT that should give us more definitive answers in the next 5 years.

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Declaration of interest

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A reviewer of this manuscript has disclosed that their institution has a patent on the use of β 3AR in pulmonary hypertension, of which they are a co-inventor. Peer reviewers in this manuscript have no other relevant financial relationships or otherwise to disclose.

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