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Safety and tolerability of the M2 muscarinic acetylcholine receptor modulator BAY 2413555 in heart failure with reduced ejection fraction in the REMOTE-HF study

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BAY 2413555 is a novel selective and reversible positive allosteric modulator of the type 2 muscarinic acetylcholine (M2) receptor, aimed at enhancing parasympathetic signaling and restoring cardiac autonomic balance for the treatment of heart failure (HF). This study tested the safety, tolerability and pharmacokinetics of this novel therapeutic option. REMOTE-HF was a multicenter, double-blind, randomized, placebo-controlled, phase Ib dose-titration study with two active arms. Study participants had an established diagnosis of HF with NYHA Class I-III and LVEF ≤ 45%. Patients were required to have an implanted cardiac defibrillator (ICD) or cardiac resynchronization therapy (CRT) device because of the potential for bradycardia or AV conduction delay, which may be induced by BAY 2413555. The study period included a screening and run-in period, followed by a treatment period of over 28 days, consisting of two parts, A and B, comprising 14 days each. Participants were randomized into 1 of 3 arms: a placebo arm and two BAY 2413555 arms—one receiving 1.25 mg in both Part A and Part B (BAY 1.25 mg–1.25 mg) and the other receiving 1.25 mg in Part A followed by 5 mg in Part B (BAY 1.25 mg–5 mg). The primary safety endpoint was the number of participants with treatment-emergent adverse events (TEAEs). Secondary endpoints included number of participants with high degree AV block or symptomatic pauses/ bradycardia and changes from baseline in resting heart rate after 2 and 4 weeks of dosing with BAY 2413555. Changes from baseline in heart rate recovery (HRR) at 1 and 2 min after exercise testing and chronotropic reserve (CR) were also assessed. Of the anticipated 129 participants, 22 participants were randomized: 7 to placebo, 8 to BAY 1.25 mg–1.25 mg, and 7 to BAY 1.25 mg–5 mg. The study was terminated early based on new and unexpected preclinical findings from a chronic animal toxicology study in monkeys in which evidence of increased vascular inflammation was observed, leading to a no longer favorable risk-benefit balance for the intended long-term (i.e., life-long) treatment of heart failure patients. Comparable adverse events were not encountered in REMOTE-HF. Overall, until the termination of the study, BAY 2413555 was safe and well tolerated, with no deaths or TEAEs leading to discontinuation, and no symptomatic bradycardia or AV blocks observed. There was a larger change in the mean HRR at 60 s in the pooled BAY 2413555 treatment arms in Part A (1.25 mg) compared to the placebo (+ 7.3 vs. −6.7 bpm), indicating enhanced cardiac parasympathetic activity. Administration of 1.25 mg and 5 mg BAY 2413555 was safe and well tolerated in both active treatment arms, with no concerning safety findings observed. However, due to the limited number of subjects resulting from early termination, the results should be considered with caution and viewed as exploratory. There were promising signs of target engagement, providing grounds for further exploration of the mechanism

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Autonomic nervous system (ANS) imbalance, including increased sympathetic nervous system (SNS) activity and decreased parasympathetic nervous system (PNS) tone, is present in numerous cardiovascular diseases such as heart failure (HF)[1–3]. Specifically, the ANS becomes imbalanced in HF with withdrawal of parasympathetic tone and increased activation of SNS. Using heart rate variability (HRV) and baroreflex sensitivity analyses as surrogate measures, impaired parasympathetic tone has been associated with higher mortality in HF, independent of beta-blocker treatment[4]. Moreover, poor heart rate recovery (HRR), which is the difference between the peak heart rate during exercise and the heart rate soon afterwards is considered a more specific measure of parasympathetic withdrawal and is associated with increased mortality and adverse cardiovascular outcomes[5,6] Beta-blockers inhibit excess sympathetic stimulation and are one of the mainstays of the current treatment of HF with reduced ejection fraction. However, to date, there are no pharmacologic interventions that directly increase parasympathetic tone and thus potentially restore autonomic balance in HF[7,8].

The development of new treatments for HF remains a priority because of the high risk in these patients despite new therapies being developed in recent years[9] The ANS is a potential therapeutic target, as imbalances in ANS signaling are associated with worse outcomes and current drug therapies are limited to targeting the sympathetic branch of the autonomic dysfunction seen in HF[10]. The M2 receptor, an essential component of the PNS and highly expressed within the heart, plays an important role in the pathogenesis and progression of cardiovascular disorders[11].

BAY 2413555 is a positive allosteric modulator of the muscarinic M2 receptor. The compound is highly selective for the M2 over other muscarinic receptors and exerts no significant allosteric effects on other human muscarinic acetylcholine receptors[12]. As a positive allosteric modulator of the M2 receptor, BAY 2413555 has no intrinsic agonistic or antagonistic activity but increases the affinity of the endogenous ligand acetylcholine for the M2 receptor, resulting in a shift of the concentration response curve to the left. This signaling profile allows for the physiologic regulation of parasympathetic signaling while at the same time enhancing the effects of endogenous signaling at the M2 receptor. In healthy, conscious dogs BAY 2413555 and its metabolite M1a (BAY 2434967) showed equal effects on heart rate (HR) reduction and led to an increase in HRV.

A first-in-man (FIM) study investigating single ascending doses of BAY 2413555 in healthy male participants was completed with no clinically relevant safety findings observed. The accumulated preclinical and clinical evidence was integrated in a translational approach (see Fig. 1) that allowed the planning and conduct of the here presented Phase Ib, study in HF patients (REMOTE-HF). This multicenter, double-blind, randomized, placebo-controlled dose titration study with 2 active arms aimed to evaluate the safety and tolerability of BAY 2413555 in participants with HF who are on beta-blocker. A secondary objective was to measure the effect of BAY 2413555 on cardiac conduction times. Additional exploratory endpoints sought to investigate the potential impact of PNS modulation on exercise function.

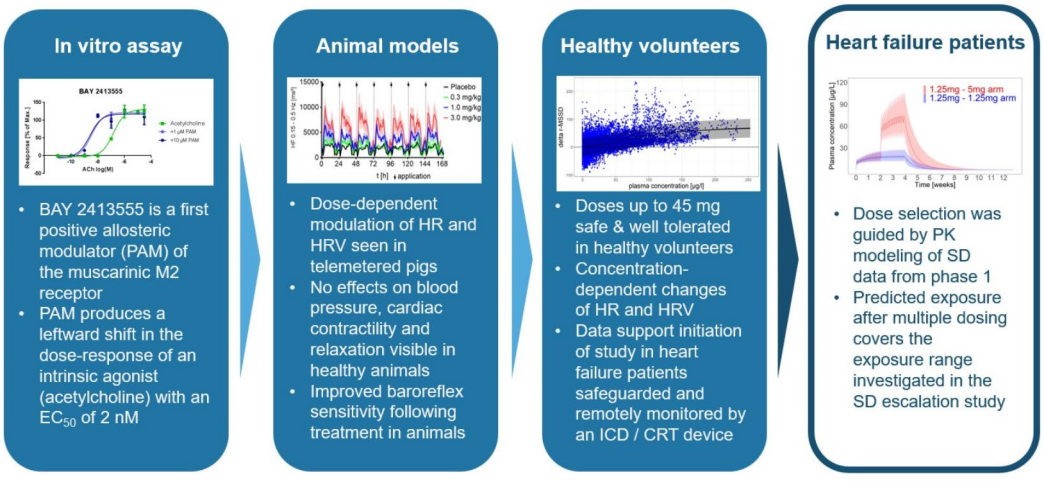

Fig. 1. Translational approach based on in vitro experiments, in vivo studies in animals and clinical data in healthy volunteers leading to the conduct of the remote-HF study in heart failure patients.

Methods

Ethical considerations

This study was approved by the institutional review board and independent ethics committee. This study was conducted in accordance with the Clinical Study Protocol and consensus ethical principles derived from international guidelines, ICH E6 (R2) Good clinical practice, declaration of Helsinki, applicable medical device guidelines and other applicable laws and regulations. All patients provided written informed consent.

Study design

The trial was registered with ClinicalTrials.gov number NCT05532046 and EudraCT number, 2021-005751-36 on 08/09/2022. This was a multicenter, double-blind, randomized, placebo-controlled dose titration study with 2 active arms. The study population consisted of participants with established diagnosis of chronic HF (NYHA I-III, LVEF $\leq$ 45%), on beta-blocker therapy and with ICD or CRT. A potential concern was HR lowering effects of the study agent, particularly in patients with HF and the fact that many patients with HF are additionally on HR lowering agents such as beta-blockers. To protect patients against potential bradycardia, only patients with preexisting implanted cardiac defibrillator (ICD) or cardiac resynchronization devices (CRT) were enrolled. Other inclusion criteria included age $\geq$ 18 years $<$ 80 years, body mass index within the range of 18–40 kg/ m^2 and patients on optimal drug therapy for HF treatment. Patients were excluded from the study if they had any rhythm abnormalities (i.e. anything except sinus rhythm), HR $>$ 100 bpm, history of higher degree AV block, a planned or recent cardiac surgery, procedure or recent myocardial infarction. A full list of inclusion and exclusion criteria, including other medical conditions and medications can be found in the Supplementary File.

Figure 2 shows the study design. Study periods included a screening and run-in period, treatment part A, treatment part B, and follow-up. The plan was that approximately 260 participants would be screened, and approximately 129 participants were to be randomized into 1 of 3 treatment arms: placebo for Part A and Part B, 1.25 mg BAY 2413555 for Part A and Part B, 1.25 mg BAY 2413555 for Part A and 5 mg BAY 2413555 for Part B. At least 29 participants per group were expected to complete the study. No formal statistical sample size estimation has been performed for this study due to its exploratory character. With the planned group sizes for Part B ($n \geq 29$ per group), the probability to observe a very common TEAE (i.e., an event with an expected incidence $\geq$ 0.10) is at least 0.95 with a confidence interval of 95%.

The anticipated duration of study participation was approximately 90 days. The treatment period was 28 days (Part A: 14 days and Part B: 14 days). The study ended with a telephone follow-up visit on study Day 58.

Measures to minimize bias

Patients were randomized using interactive response technology (IRT) to one of the three groups in 1:1:1 ratio. Both the investigator and the participants were masked. The ICD/CRT Core Lab adjudicating defined events identified by retrospective readout of ICD/CRT devices was blinded to treatment intervention. An external, independent, unblinded Data Monitoring Committee (DMC) formally reviewed accumulating data from this study to facilitate the detection of any increased risk for harm to participants. The DMC consisted of 2

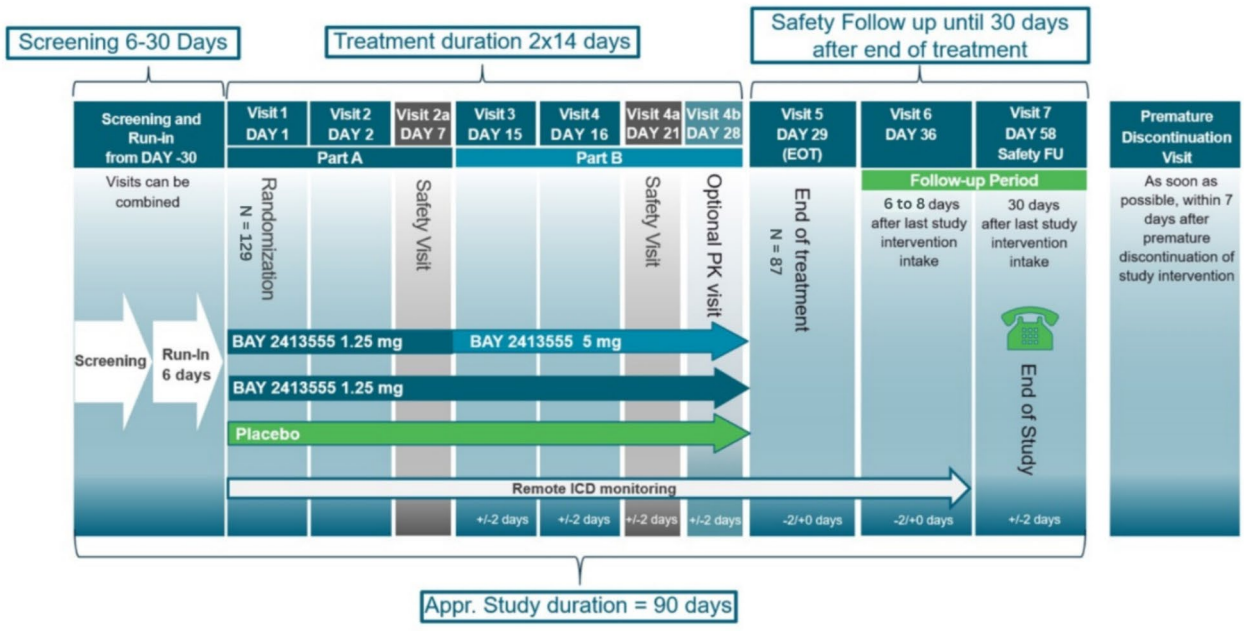

Fig. 2. Overview of study design.

cardiologists, a hematologist and a biostatistician. The DMC analyses were conducted by an external independent unblinded biostatistical group that was not involved with the conduct of this study.

Endpoints

The primary endpoint was number of participants with treatment-emergent adverse events (TEAEs). AEs were considered to be treatment-emergent if they have started or worsened after first application of study intervention up to 28 days after the last study intervention intake. The secondary endpoints included number of participants with high degree AV block or symptomatic pauses/bradycardia and changes from baseline in resting HR after 2 and 4 weeks of dosing with BAY 2413555. Further exploratory endpoints were change from baseline in heart rate recovery (HRR) at 1 and 2 min of recovery after exercise testing, chronotropic reserve (CR), and heart rate variability (HRV) after 2 and 4 weeks of dosing with BAY 2413555. Other exploratory endpoints included changes from baseline in exercise time (ET), N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitivity troponin T (hs-TNT) after 2 and 4 weeks. We also aimed to explore plasma concentrations of BAY 2413555 and its metabolite M1a (BAY 2434967). Various other biomarkers were also explored, including hematological, coagulation, general chemistry, liver function, and urinalysis parameters.

Statistical analysis

The analysis cohorts included safety analysis and per-protocol analysis. There was an overall safety analysis including all participants randomly assigned to study intervention who took at least 1 dose of study intervention. Pharmacodynamic parameters were analyzed for the per-protocol set including all participants without validity findings. An overall summary of the number of participants with any TEAEs was generated by the treatment group, including pooling of all BAY 2413555 doses and overall, as well as by treatment group and by part. The number of participants with TEAEs, treatment-emergent study intervention related AEs, TEAEs of special interest, drug related TEAEs resulting in discontinuation of study intervention, TEAEs by maximum intensity, treatment-emergent serious AEs were summarized by study intervention and overall using MedDRA preferred terms grouped by system organ class. TEAEs of special interest included any SA block associated with a pause of ≥ 4 s, any 2nd Mobitz type 2 or 3rd degree AV block, heart beat pauses ≥ 4 s, syncope and medically confirmed newly developed clinically relevant decrease in Hb, as judged by the investigator. MedDRA version 26.0 was used for analysis.

Individual listings of the AEs of participants with higher degree AV blocks and symptomatic pauses/bradycardia were noted. The number of participants with higher degree AV block and symptomatic pauses/bradycardia was summarized by treatment group, including pooling of all BAY 2413555 doses and overall, as well as by treatment group and part, including pooling of BAY 2413555 doses, and overall. The changes from baseline in resting HR were summarized by treatment group and sampling time points using descriptive statistics. Descriptive statistics were presented for Part A pooling the data from 1.25 mg BAY 2413555 from both active treatment groups, i.e., comparing placebo with 1.25 mg BAY 2413555. The mean changes from baseline in resting HR after 2 and 4 weeks of study intervention in the placebo and active arms were compared using the Bayesian approach. The Bayesian posterior probability was derived using non-informative Jeffreys priors for single parameters and corresponding 95% credible intervals were calculated.

Results

Early termination

The study was terminated early based on new and unexpected preclinical findings from a chronic animal toxicology study in monkeys in which evidence of increased vascular inflammation was observed in several organs after 39 weeks of treatment. Notably, this type of finding was not observed in shorter studies of 4 and 13 weeks, which supports the safety of short-term treatment up to 13 weeks in humans.

Thorough evaluation of available data at present led to no indication of acute or long-term risk for patients who have received a maximum of four weeks of treatment. However, given the new and unexpected findings from the preclinical study, the pathomechanism of which is unclear, the assessment of the risk-benefit balance for the intended long-term (i.e., life-long) treatment of heart failure patients was re-evaluated and was no longer favorable. All ongoing clinical studies with BAY 2413555 were terminated.

Participant disposition

Of the anticipated 129 participants, we enrolled and screened 37 participants by the time of study termination. Study recruitment was underway in 18 study centers in 6 countries (3 in the US, 3 in Denmark, 6 in Spain, 3 in Israel, 2 in Italy and 1 in Germany). Overall, 22 participants (1 in the US, and 21 in EU) were randomized to 1 of 3 treatment arms: 7 to placebo, 8 to BAY 2413555 1.25 mg in both Part A and B (BAY 1.25 mg–1.25 mg), and 7 to BAY 2413555 1.25 mg in Part A and 5 mg in Part B (BAY 1.25 mg–5 mg). All 22 randomized participants were treated with at least 1 dose of study drug.

Of 22 participants starting Part A, 19 participants (86.4%) completed the treatment phase. Altogether 3 participants (13.6%), 1 in each treatment arm, did not complete Part A due to early study termination. Out of 19 participants starting Part B, 10 participants (45.5%) completed the treatment phase. Altogether 9 participants (40.9%) (4 in 1.25 mg–1.25 mg, 2 in 1.25 mg–5 mg, and 3 in placebo) did not complete Part B. 8 participants did not complete Part B due to early study termination, and 1 participant discontinued due to own decision. All 22 treated participants completed follow-up.

Baseline characteristics

All participants were white (100%), and 19 participants were male (86%). Across all arms, mean age was 66.4 ± 7.2 years, mean BMI was 26.8 ± 4.7 kg/m^2, mean eGFR was 71.4 ± 19.2 mL/min/1.73 m^2, and mean LVEF was 34 ± 8%. Demographic and baseline characteristics were mostly well balanced between the treatment arms. These results are summarized in Table 1.

Overall safety summary

Ten participants (45.5%) reported at least one TEAE (8/15 [53.3%] in the active treatment arms; 2/7 [28.6%] in the placebo). All but one TEAE were mild. There was one severe TEAE, which was also a serious adverse event (SAE) and was unrelated to the study medication. The participant developed an 'infrarenal aorta aneurysm' on Day 28 and was treated via a transcatheterwe implantation of an aorta-bi-iliac endo-prosthesis. Three TEAEs in 2 participants were considered related to BAY 2413555 ('change of bowel habit', 'salivary hypersecretion', and 'asthenia'). There were no deaths in the study, no TEAEs led to discontinuation of the study and no adverse events of special interest occurred. There were no participants with high degree AV blocks or symptomatic pauses/bradycardia. The mean changes in clinical laboratory values, vital signs, and ECG measurements were mostly related to an underlying health condition. None of these changes were clinically significant as assessed by the investigator. An overall summary of the number of participants with TEAEs, as well as an overall summary grouping TEAEs is presented in Table 2 [Table S1–S5].

Non-treatment-emergent adverse events

Overall, 2 participants, both in the BAY 1.25 mg–1.25 mg arm, had post-treatment and non-treatment emergent AEs. One had 'aortic aneurysm' of moderate intensity, 29 days after last dose of study drug. The second patient had dizziness of mild intensity, 32 days after last dose of study drug.

Evaluation of clinical laboratory tests

Overall, there were no clinically meaningful changes in laboratory results and biomarkers. One patient had an 'increased creatinine' and another had an 'abnormal urine sample'. Neither was judged to be related to BAY 2413555. There were no relevant differences in the proportion of participants with laboratory abnormalities between the active treatment arms and placebo arm. Individual laboratory profiles for participants with values above 1.5 x the upper limit of normal (ULN) in multiples of ULN are shown in Table S6. No participant had laboratory values below 0.5 x the lower limit of normal (LLN). Treatment-emergent high and low abnormalities in hematology parameters are summarized in Tables S7 and S8. Overall, the number of participants with high or low abnormalities was low, and there were no relevant differences in the proportion of participants with abnormalities in hematology parameters between the active treatment arms and placebo.

Electrocardiograms and adjudicated and device-derived data

The time courses of ECG parameter mean values did not differ between the BAY 1.25 mg-1.25 mg and placebo arms. In the BAY 1.25 mg-5 mg arm, the time courses of some ECG parameters differed compared to the other two arms. Mean VR was already higher at Visit 1 in the BAY 1.25 mg-5 mg arm compared to the other two arms, and it declined over the course of the study. Mean PR interval, as well as PR interval corrected according to Soliman and Rautaharju were also higher at Visit 1 in the BAY 1.25 mg-5 mg arm compared to the other two arms, and remained higher for the rest of the study[13]. The mean QRS duration, QT and QTcB interval were similar in all treatment arms. In the BAY 1.25 mg–5 mg arm, mean QTcF declined until Visit 4, after which it increased until Visit 5. In the other two arms, mean QTcF increased until Visit 4, after which it slightly decreased at Visit 5. All participants were in sinus rhythm at all times.

In the ICD device readouts, there was no relevant increase in right ventricular pacing (RVP), indicative of significant bradycardia, observed in the treatment groups compared to the placebo group (Table S9). There were 3 participants (2 in active arms and 1 in placebo) with TEAEs related to electrogram findings documented in the ICD/CRT device data. One participant randomized to the BAY 1.25 mg–1.25 mg arm, had the TEAE 'ventricular tachycardia' on Day 8 of the treatment period. Another participant, randomized to the BAY 1.25 mg–5 mg arm, had the TEAEs 'ventricular tachycardia' on Day 14 and 'atrial fibrillation' on Day 23 of the treatment period. Both were singular events recorded by the participant's ICD. The third participant, randomized to the placebo arm, had the TEAE 'AT/AF burden' on Day 1 of the treatment period. All of these were singular events recorded by the participants' ICDs and were recovered/resolved on the same day. None was considered related to BAY 2413555.

Heart rate recovery

Exercise stress testing, performed on all patients, showed a posterior probability of 99.9% of larger HRR at 60 s for the pooled active treatment arms compared to the placebo arm with a posterior mean change from baseline of 14.4 (95% credible interval, 6.20–22.47) for BAY 2413555 pooled vs. placebo during Part A. The arithmetic mean change of HRR at 60 s after BAY 2413555 treatments pooled was + 7.3 bpm in Part A compared to −6.7 bpm after placebo.

The data are summarized in Tables 3 and 4.

Pharmacokinetics

Measured plasma concentrations of BAY 2413555 and its metabolite M1a (BAY 2434967) were in the range as expected from model-based PK predictions based on data from the previous, Phase I study. Simulated versus observed time profiles of sum concentrations of BAY 2413555 and M1a after multiple dosing for the BAY 2413555

	BAY 2413555 1.25 mg–1.25 mg N = 8 (100%)	BAY 2413555 1.25 mg–5 mg N = 7 (100%)	Placebo N = 7 (100%)	Total N = 22 (100%)
Sex				
Female	3 (37.5%)	0	0	3 (13.6%)
Male	5 (62.5%)	7 (100.0%)	7 (100.0%)	19 (86.4%)
Race				
White	8 (100.0%)	7 (100.0%)	7 (100.0%)	22 (100.0%)
Ethnicity				
Hispanic or Latino	0	0	1 (14.3%)	1 (4.5%)
Not Hispanic or Latino	8 (100.0%)	7 (100.0%)	6 (85.7%)	21 (95.5%)
Age (years)				
n	8	7	7	22
Mean (SD)	67.9 (6.2)	68.4 (9.2)	62.6 (5.3)	66.4 (7.2)
Median	66.5	71.0	62.0	65.5
Min, Max	59, 75	51, 79	56, 71	51, 79
Age group acc. EudraCT				
Adults (between 18 and 64 years)	2 (25.0%)	2 (28.6%)	5 (71.4%)	9 (40.9%)
From 65 to 84 years	6 (75.0%)	5 (71.4%)	2 (28.6%)	13 (59.1%)
Age group				
< 65 years	2 (25.0%)	2 (28.6%)	5 (71.4%)	9 (40.9%)
≥ 65 years	6 (75.0%)	5 (71.4%)	2 (28.6%)	13 (59.1%)
Weight (kg)				
n	8	7	7	22
Mean (SD)	70.6274 (11.8688)	86.0714 (18.9591)	82.3143 (15.4355)	79.2600 (16.2694)
Median	69.8000	87.0000	92.0000	79.2500
Min, Max	53.300, 90.000	55.000, 113.700	59.200, 98.300	53.300, 113.700
Height (cm)				
n	8	7	7	22
Mean (SD)	164.243 (9.385)	175.000 (8.602)	177.571 (8.142)	171.906 (10.281)
Median	160.000	175.000	179.000	174.000
Min, Max	154.94, 180.00	160.00, 185.00	162.00, 186.00	154.94, 186.00
Body mass index (kg/m²)				
n	8	7	7	22
Mean (SD)	26.38 (5.29)	27.90 (4.89)	26.06 (4.38)	26.76 (4.72)
Median	24.60	26.00	26.90	25.85
Min, Max	20.7, 35.6	21.5, 34.7	19.0, 30.7	19.0, 35.6
eGFR (mL/min/1.73 m²)				
n	8	7	7	22
Mean (SD)	76.1 (18.9)	67.6 (22.1)	69.7 (18.3)	71.4 (19.2)
Median	79.5	75.0	80.0	76.5
Min, Max	46, 97	32, 93	45, 86	32, 97
NYHA class				
NYHA class I	3 (37.5%)	2 (28.6%)	1 (14.3%)	6 (27.3%)
NYHA class II	5 (62.5%)	4 (57.1%)	5 (71.4%)	14 (63.6%)
NYHA class III	0	1 (14.3%)	1 (14.3%)	2 (9.1%)
LVEF (%)				
n	8	7	7	22
Mean (SD)	37.3750 (6.4794)	34.8571 (10.0238)	29.0000 (7.3258)	33.9091 (8.4228)
Median	37.5000	37.0000	30.0000	35.0000
Min, Max	25.000, 45.000	15.000, 45.000	20.000, 40.000	15.000, 45.000
Category of date of last hospitalization				
> 1 year	1 (12.5%)	1 (14.3%)	0	2 (9.1%)
> 5 years	0	0	1 (14.3%)	1 (4.5%)
Device type				
Biventricular pacemaker	4 (50.0%)	1 (14.3%)	1 (14.3%)	6 (27.3%)
Implantable cardioverter-defibrillator, dual	1 (12.5%)	2 (28.6%)	2 (28.6%)	5 (22.7%)
Implantable cardioverter-defibrillator, singular	3 (37.5%)	3 (42.9%)	4 (57.1%)	10 (45.5%)

Continued

	BAY 2413555 1.25 mg–1.25 mg *N* = 8 (100%)	BAY 2413555 1.25 mg–5 mg *N* = 7 (100%)	Placebo *N* = 7 (100%)	Total *N* = 22 (100%)
Other	0	1 (14.3%)	0	1 (4.5%)
Atrially paced beats (%)				
n	5	4	3	12
Mean (SD)	3.8 (2.6)	0.8 (1.5)	3.3 (5.8)	2.7 (3.3)
Median	3.0	0.0	0.0	2.0
Min, Max	1, 8	0, 3	0, 10	0, 10
Right ventricularly paced beats (%)				
n	7	7	6	20
Mean (SD)	56.9 (53.2)	19.0 (36.2)	32.3 (50.1)	36.3 (47.3)
Median	99.0	0.0	0.0	0.0
Min, Max	0, 100	0, 99	0, 100	0, 100
Biventricularly paced beats (%)				
n	4	2	2	8
Mean (SD)	99.5 (0.6)	99.5 (0.7)	97.0 (4.2)	98.9 (2.0)
Median	99.5	99.5	97.0	99.5
Min, Max	99, 100	99, 100	94, 100	94, 100

Table 1. Demographics (safety analysis set). Abbreviations: eGFR = estimated glomerular filtration rate, EudraCT = European Union Drug Regulating Authorities Clinical Trials, LVEF = left ventricular ejection fraction, NYHA = New York Heart Association, SD = standard deviation NYHA class at visit 1 Pacing frequency at visit 1 LVEF: most recent measurement within 12 months recorded by any imaging modality prior to Visit 1

1.25 mg–1.25 mg arm and the BAY 2413555 1.25 mg–5 mg arm are presented in Figure S1 of Supplement B. Other exploratory results and biomarkers are not reported due to early study termination.

Discussion

BAY 2413555 is a novel, selective, and reversible positive allosteric modulator of the type 2 muscarinic acetylcholine (M2) receptor that was being developed for the treatment of HF. As a positive allosteric modulator of the M2 receptor enhancing intrinsic parasympathetic activity, the M2PAM BAY 2413555 is a novel therapeutic option in HF directly augmenting parasympathetic signaling and rebalancing the autonomic nervous system (ANS). This study evaluated safety and tolerability of 4 weeks' treatment of 2 doses of BAY 2413555 versus placebo in participants with HF who were on beta-blockers. As stated above, the study was terminated early based on new and unexpected preclinical findings from a chronic animal toxicology study in monkeys in which evidence of increased vascular inflammation was observed, leading to a no longer favorable risk-benefit balance for the intended long-term (i.e., life-long) treatment of heart failure patients. However, no safety concerns emerged in humans and BAY 2413555 had no significant effect on markers of vascular inflammation, such as C reactive protein. Moreover, there were promising signs of target engagement as demonstrated by an improved HRR.

These results show that the administration of 1.25 mg and 5 mg BAY 2413555 as immediate release (IR) tablets were overall well tolerated in both active treatment arms compared to placebo, and no relevant safety findings were observed. Although around half of all patients experienced TEAEs, most were mild and resolved without treatment spontaneously. No AEs of special interest were identified. In the ICD device readouts, there was no relevant increase in right ventricular pacing, indicative of significant bradycardia, observed in the treatment groups compared to the placebo group. There were no participants with high degree AV block or symptomatic pauses/bradycardia. There were no meaningful and consistent differences observed between 1.25 mg or 5 mg BAY 2413555 and placebo in the changes from baseline in resting HR after 2 and 4 weeks of dosing with BAY 2413555. Our results also display improved HRR at 60 s (HRR_{60}) in patients on BAY 2413555 compared to placebo. HRR during the first minute after exercise reflects parasympathetic activity[14] Moreover, results from the MyoVasc study show HRR_{60} is a strong predictor of outcomes in heart failure patients, regardless of NT-proBNP levels[15] Hence, our study not only provides promising signs of target engagement and a probability of positive proof-of-mechanism, but it also provides a potential efficacy outcome that could have been used for further exploration in a phase II trial.

For several years, it has been demonstrated that autonomic dysfunction has adverse impacts on cardiac function, including association with sudden cardiac death[16] Cardiac autonomic dysfunction also plays a role in exercise intolerance in patients with HF, which consequently affects both quality of life and morbidity[17,18]. Although beta-blockers have been used to target neurohormonal activation including the sympathetic nervous system, no drugs are currently being used to treat HF by targeting the PNS. There is a need to develop drugs that can upregulate parasympathetic control without causing cardiac depression, especially in the presence of concomitant beta-blocker therapy. This trial aimed to demonstrate the safety and to generate first signals indicating possible efficacy of M2PAM, an oral drug which was theorized for this purpose. There have been several attempts to identify and study suitable therapeutic options to enhance parasympathetic activity on the

(a) Overall summary of number of participants (safety analysis set)					
	BAY 2413555 1.25 mg–1.25 mg *N* = 8 (100%)	**BAY 2413555 1.25 mg–5 mg *N* = 7 (100%)**	**Placebo *N* = 7 (100%)**	**BAY 2413555 pooled *N* = 15 (100%)**	**Total *N* = 22 (100%)**
Number (%) of participants with the specified treatment-emergent adverse events					
Any AE	3 (37.5%)	5 (71.4%)	2 (28.6%)	8 (53.3%)	10 (45.5%)
Maximum intensity for any AE					
Mild	3 (37.5%)	4 (57.1%)	2 (28.6%)	7 (46.7%)	9 (40.9%)
Severe	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Any BAY 2413555-related AE	0	1 (14.3%)	1 (14.3%)	1 (6.7%)	2 (9.1%)
Maximum intensity for BAY 2413555-related AEs					
Mild	0	1 (14.3%)	1 (14.3%)	1 (6.7%)	2 (9.1%)
Any AE related to procedures required by the protocol	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Any AE leading to discontinuation of BAY 2413555	0	0	0	0	0
Any SAE	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
BAY 2413555-related SAEs	0	0	0	0	0
SAE related to procedures required by the protocol	0	0	0	0	0
SAE leading to discontinuation of BAY 2413555	0	0	0	0	0
AE with the outcome death	0	0	0	0	0
Any AE of special interest	0	0	0	0	0
(b) Number of participants by primary system organ class, preferred term (safety analysis set)					
Primary system organ class Preferred term MedDRA version 26.0	**BAY 2413555 1.25 mg–1.25 mg N = 8 (100%)**	**BAY 2413555 1.25 mg–5 mg N = 7 (100%)**	**Placebo N = 7 (100%)**	**BAY 2413555 pooled N = 15 (100%)**	**Total N = 22 (100%)**
Number (%) of participants with at least one such adverse event	3 (37.5%)	5 (71.4%)	2 (28.6%)	8 (53.3%)	10 (45.5%)
Cardiac disorders	1 (12.5%)	1 (14.3%)	2 (28.6%)	2 (13.3%)	4 (18.2%)
Atrial fibrillation	0	1 (14.3%)	1 (14.3%)	1 (6.7%)	2 (9.1%)
Cardiac failure	0	0	1 (14.3%)	0	1 (4.5%)
Ventricular tachycardia	1 (12.5%)	1 (14.3%)	0	2 (13.3%)	2 (9.1%)
Gastrointestinal disorders	0	2 (28.6%)	1 (14.3%)	2 (13.3%)	3 (13.6%)
Change of bowel habit	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Diarrhoea	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Salivary hypersecretion	0	0	1 (14.3%)	0	1 (4.5%)
General disorders and administration site conditions	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Asthenia	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Infections and infestations	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Influenza	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Investigations	1 (12.5%)	0	1 (14.3%)	1 (6.7%)	2 (9.1%)
Blood creatinine increased	0	0	1 (14.3%)	0	1 (4.5%)
Urine analysis abnormal	1 (12.5%)	0	0	1 (6.7%)	1 (4.5%)
Musculoskeletal and connective tissue disorders	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Back pain	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Nervous system disorders	1 (12.5%)	2 (28.6%)	0	3 (20.0%)	3 (13.6%)
Dizziness	1 (12.5%)	1 (14.3%)	0	2 (13.3%)	2 (9.1%)
Headache	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Paraesthesia	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Somnolence	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Skin and subcutaneous tissue disorders	1 (12.5%)	0	0	1 (6.7%)	1 (4.5%)
Rash	1 (12.5%)	0	0	1 (6.7%)	1 (4.5%)
Vascular disorders	1 (12.5%)	1 (14.3%)	0	2 (13.3%)	2 (9.1%)
Aortic aneurysm	0	1 (14.3%)	0	1 (6.7%)	1 (4.5%)
Raynaud's phenomenon	1 (12.5%)	0	0	1 (6.7%)	1 (4.5%)

Table 2. Treatment-emergent adverse events. The column 'Total' counts the participants over all treatment periods. For cross-over designs, this is not the sum of the other columns.

Treatment group	Visit	Value at visit				Change from baseline by visit			
		n	Mean (SD)	Median	Min, Max	n	Mean (SD)	Median	Min, Max
BAY 2413555 1.25 mg (pooled Part A)	Visit 1	11	20.8 (11.2)	17.0	10, 43				
	Visit 3	12	27.1 (13.3)	22.0	13, 51	10	7.3 (7.3)	4.5	-1, 22
Placebo	Visit 1	6	20.3 (8.2)	23.0	5, 28				
	Visit 3	6	13.7 (7.2)	16.0	3, 22	6	-6.7 (3.7)	-7.0	-11, -2

Table 3. Exercise stress test – heart rate recovery 60 s (HRR) (per protocol set A).

Bayesian analysis of the mean changes from baseline in HRR after 2 weeks of study intervention in the placebo and active arms- Part A (per protocol set A)						
Effect	*n*	Posterior probability	Mean	2.5 Percentile	50 Percentile	97.5 Percentile
BAY 2413555 pooled vs. Placebo - Part A, 120 s	18	0.4479	-0.524382757	-8.670821419	-0.507153394	7.6660982094
BAY 2413555 pooled vs. Placebo - Part A, 60&120 s	18	0.9705	6.9426088584	-0.256151326	6.9680825462	14.067358265
BAY 2413555 pooled vs. Placebo - Part A, 60 s	18	0.9992	14.409600473	6.197636284	14.415083225	22.471295156

Table 4. Exercise stress test – bayesian analysis of Heart Rate Recovery (HRR). Posterior probability P(u0 < uv | data), u0 and uv being the means after placebo and active treatment.

heart. Devices and procedures targeting vagal nerve stimulation, renal sympathetic denervation, baroreceptor activation therapy and carotid body removal have been studied[19] Although the preclinical data have been promising, clinical trials have been small and limited.

Pharmacologically, acetylcholinesterase inhibitors have been shown to improve hemodynamics in HF patients and ameliorate the autonomic balance[20] This is due to their direct effect increasing acetylcholine in the synaptic junction. These drugs include donepezil, used for treating Alzheimer's Disease and pyridostigmine[19] Digoxin, another well-established HF drug, has vagal effects that may play a role in correcting the parasympathetic withdrawal affecting HF patients[21]. The neurohormonal modulation provided by these drugs is limited due to their side-effect profile and there is a lack of demonstrated widespread efficacy in patients with HF specifically. Lastly, supervised exercise is another noninvasive intervention which has been shown to offset the deterioration of cardiac autonomic control and improve outcomes[22].

Keeping in mind the current therapeutic landscape, there seems to be great potential for the exploration and development of appropriate agents with vagomimetic effects in the heart. This study suggests that BAY 2413555 administered for 28 days may be safe and tolerable in humans and does not appear to cause overt conduction abnormalities. Although our study is small and thus should be interpreted with caution, especially in the context of its early termination due to unforeseen inflammatory effects in a single long-term preclinical study, it is the first of its kind in providing Phase Ib trial data on targeting M2 receptors for neuro-hormonal modulation in the treatment of HF.

Data availability

Data is provided within the manuscript or supplementary information files.

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Author contributions

N.K.K., M.M., T.M., H.T., and W.D. were involved in the conception, design and supervision of the work. D.L., J.L., C.M. were involved in the creation of tables and figures and data procurement. M.F., M.S.K. and R.J.M. were responsible for the data synthesis, analysis, manuscript writing and results. All authors have reviewed the manuscript and are responsible for the accuracy and integrity of the work.

Declarations

Competing interests

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Additional information

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