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Bucindolol Decreases Atrial Fibrillation Burden in Patients with Heart Failure and the *ADRB1* Arg389Arg Genotype

Running title: *Piccini et al.; Bucindolol Decreases AF Burden*

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Abstract:

Background - Bucindolol is a genetically targeted β-blocker/mild vasodilator with the unique pharmacologic properties of sympatholysis and *ADRB1* Arg389 receptor inverse agonism. In the GENETIC-AF trial conducted in a genetically defined heart failure (HF) population at high risk for recurrent atrial fibrillation (AF), similar results were observed for bucindolol and metoprolol succinate for the primary endpoint of time to first atrial fibrillation (AF) event; however, AF burden and other rhythm control measures were not analyzed.

Methods - The prevalence of ECGs in normal sinus rhythm, AF interventions for rhythm control (cardioversion, ablation and antiarrhythmic drugs), and biomarkers were evaluated in the overall population entering efficacy follow-up (N=257). AF burden was evaluated for 24 weeks in the device substudy (N=67).

Results - In 257 patients with HF the mean age was 65.6 ± 10.0 years, 18% were female, mean left ventricular ejection fraction (LVEF) was 36%, and 51% had persistent AF. Cumulative 24-week AF burden was 24.4% (95% CI: 18.5, 30.2) for bucindolol and 36.7% (95% CI: 30.0, 43.5) for metoprolol (33% reduction, $p < 0.001$). Daily AF burden at the end of follow-up was 15.1% (95% CI: 3.2, 27.0) for bucindolol and 34.7% (95% CI: 17.9, 51.2) for metoprolol (55% reduction, $p < 0.001$). For the metoprolol and bucindolol respective groups the prevalence of ECGs in normal sinus rhythm was 4.20 and 3.03 events per patient (39% increase in the bucindolol group, $p < 0.001$), while the rate of AF interventions was 0.56 and 0.82 events per patient (32% reduction for bucindolol, $p = 0.011$). Reductions in plasma norepinephrine ($p = 0.038$) and NT-proBNP ($p = 0.009$) were also observed with bucindolol compared to metoprolol.

Conclusions - Compared with metoprolol, bucindolol reduced AF burden, improved maintenance of sinus rhythm, and lowered the need for additional rhythm control interventions in patients with HF and the *ADRB1* Arg389Arg genotype.

Clinical Trial Registration – www.clinicaltrials.gov; Unique Identifier: NCT01970501

Key words: atrial fibrillation; AF burden; bucindolol; heart failure; beta-blocker; pharmacogenetics

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Nonstandard Abbreviations and Acronyms
ACM = all-cause mortality
ADRB1 = b_1-adrenergic receptor gene
AF = atrial fibrillation
AFL = atrial flutter
Arg = arginine
AUC = area under the curve
DSMB = Data and Safety Monitoring Committee
DTRI = diagnosis to randomization index
DxT = time from initial diagnosis to randomization
ECG = electrocardiogram
ECV = electrical cardioversion
HF = heart failure
ICD = implantable cardioverter defibrillator
ICM = insertable cardiac monitor
LVEF = left ventricular ejection fraction
NE = norepinephrine
NSR = normal sinus rhythm
NT-proBNP = N-terminal pro B-type natriuretic peptide
NYHA = New York Heart Association
PRR = prevalence rate ratio

Introduction

Atrial fibrillation (AF) is common in patients with heart failure (HF), where it complicates disease management, and is associated with worse outcomes, including greater rates of HF hospitalization, stroke, and death.[1-3] Antiarrhythmic therapeutic efficacy has traditionally been evaluated using time to AF recurrence as the primary endpoint; however, AF burden is increasingly being recognized as a more sensitive measure of arrhythmia that is closely linked to key clinical outcomes.[4-6] In the case of patients with AF and HF, progression in AF burden has been associated with a 4-fold increased risk of HF hospitalization.[7]

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The GENETIC-AF (i.e., **Gen**otype-Directed Comparative **E**ffectiveness **Tri**al of Bu**c**indolol and Toprol-XL for the Prevention of Symptomatic **A**trial **F**ibrillation/Atrial Flutter in Patients with Heart Failure) was a multicenter, randomized, double-blind, comparative efficacy trial in a genotype-defined HF population with a history of paroxysmal or persistent AF.[8,9] Similar results were observed for bucindolol and metoprolol succinate for the primary endpoint of time to first event of AF, atrial flutter (AFL) or all-cause mortality (ACM) in 267 patients with HF and the *ADRB1* Arg389Arg genotype (hazard ratio 1.01 (0.71, 1.42)). Given the importance of AF burden, particularly in patients with HF, we sought to analyze cumulative AF burden, as well as other measures of rhythm control in the GENETIC-AF trial. We hypothesized that despite neutral effects on time to event of AF/AFL/ACM, genetically targeted bucindolol would result in greater overall rhythm control in patients with HF and the *ADRB1* Arg389Arg genotype.

Methods

Study Design

The data that support the findings of this study are available from the corresponding author upon reasonable request. The full rationale and design of the GENETIC-AF randomized clinical trial have been previously published.[8,9] Briefly, patients with HF and symptomatic AF episodes documented within the past 6 months were randomly assigned (1:1) to receive bucindolol or metoprolol succinate and were up-titrated weekly to obtain target doses of 100 mg bid (50 mg bid if < 75 kg) for bucindolol and 200 mg qd for metoprolol. Study drug was over-encapsulated to maintain blinding, with a placebo dose included for the metoprolol arm to allow twice-daily administration. Randomization was centralized and stratified by HF etiology (ischemic, non-ischemic), left ventricular ejection fraction (LVEF) (< 0.35, ≥ 0.35), device type (insertable

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cardiac monitor [ICM], pacemaker/defibrillator, no device), and rhythm at randomization (sinus rhythm, AF/AFL).

Following up-titration, electrical cardioversion (ECV) was performed if needed to establish sinus rhythm prior to the start of efficacy follow-up. During the 24-week efficacy follow-up period, continuous heart rhythm monitoring was conducted to assess AF burden in a subgroup of patients with a Medtronic Reveal LINQ ICM, pacemaker or defibrillator with an atrial lead. In the overall study population, a scheduled 12-lead electrocardiogram (ECG) was collected every 4 weeks and any clinically indicated ad hoc ECGs were collected as to assess the reciprocal of AF burden, i.e., the maintenance of normal sinus rhythm (NSR). Patients experiencing AF/AFL during follow-up remained on blinded study drug and could undergo ECV, ablation, or therapy with a guideline recommended antiarrhythmic drug (amiodarone or dofetilide) could be initiated.[10]

Patients had a diagnosis of HF with at least one LVEF ≤ 55% in the past 12 months, symptomatic paroxysmal or persistent AF in the past 180 days and were receiving optimal anticoagulation therapy for stroke prevention. Patients were genotyped at screening and those who were *ADRB1* Arg389Arg (52% of screened patients[9]) were eligible for randomization. Key exclusion criteria were New York Heart Association (NYHA) Class IV symptoms, clinically significant fluid overload, permanent AF (ongoing AF event >1 year), antiarrhythmic therapies in past 7 days, prior atrioventricular node ablation, high-grade atrioventricular block, catheter ablation for AF or AFL in past 30 days, and prior intolerance or contraindication to beta-blocker therapy.[8]

The current investigation is a *post hoc* analysis of AF burden measured by device continuous monitoring, time in SR as measured by ECG, and number of definitive AF

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interventions in the two treatment arms measured during the efficacy follow-up period. The reason for analyzing the endpoints during the 24 week efficacy follow-up period is that at the beginning of this interval all subjects were either in SR (N=236), had it attempted but had failed the scheduled ECV (N=19, including 9 of 69 bucindolol and 10 of 65 metoprolol patients) or were ineligible for or declined ECV (N =2). The cohort size is 257 vs. the original 267 randomized subjects, due to 10 randomized patients not entering efficacy follow-up.

Genotyping, neurohormone measurements

ADRB1 Arg389Gly genotype was determined by RT-PCR in DNA extracted from whole blood. Systemic venous plasma norepinephrine (NE) was assayed by high-pressure liquid chromatography with electrochemical detection and venous plasma N-terminal pro B-type natriuretic peptide (NT-proBNP) was measured by electrochemiluminescence immunoassay.

Trial oversight and ethics

Study design, conduct, and performance were overseen by a 11-member Steering Committee and was monitored by a 3-member Data and Safety Monitoring Committee (DSMB). The study was approved by the local ethics board at each institution and at the Institutional Review Board at Duke University, the site of the data coordinating center. All patients provided written informed consent.

Statistical Analyses

AF burden

Device-detected AF burden during the 24-week follow-up period was assessed in all protocol compliant substudy patients entering efficacy follow-up (N = 67). Two patients in the metoprolol group were excluded from the analysis; one patient died prior to the start of efficacy follow-up due to worsening HF precipitated by sepsis and one patient received an atrioventricular node

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ablation and implantation of a biventricular pacemaker at Week 0 (protocol deviation) that precluded AF burden device detection. AF burden, defined as the proportion (%) of time spent in AF per day as detected by continuous monitoring, was calculated for each patient entering efficacy follow-up. AF burden was calculated on a daily basis for each treatment group and plotted over time. Instantaneous estimates of average daily AF burden and its standard error were calculated from a 0-1 inflated beta regression model for each day of efficacy follow-up. A cubic B-spline effect of time was employed to test for temporal trends in daily AF burden, with comparison between groups expressed as the ratio of the estimates and tested for significance using a Wald test. Estimates of cumulative AF burden over the full efficacy follow-up period were expressed as the area under the curve (AUC) for each treatment group. The 95% confidence intervals for AUC were calculated using the log ratio of two truncated normal distributions for average daily % AFB on [0,1] with noninformative uniform [0,1] priors. Comparison between groups was expressed as the AUC ratio (i.e., AUC_{BUC}/AUC_{MET}), with significance expressed as a posterior p value. The methodologic rationale is given in the Supplemental Materials.

Time to First AF/AFL or All-Cause Mortality

Time to first event of device-detected AF/AFL or ACM was assessed in the substudy population (N = 69), with an AF/AFL event prospectively-defined as AF burden ≥ 6 hours per day as recorded by continuous monitoring.[8,9] Six hours of AF burden has previously been shown to be associated with an increased rate of hospitalization for HF.[11] Patients who died prior to start of follow-up and patients who failed to establish sinus rhythm post-ECV were assigned an event on day 1. Patients were censored on day 1 if they were in AF/AFL and the ECV procedure was not performed, if they withdrew from the study prior to start of follow-up, or if they had a disqualifying protocol deviation. Due to the small sample size in the substudy, treatment effect

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estimates were determined based on Cox proportional hazards models with no adjustment for randomization strata and testing for superiority was by the log-rank test using a 2-sided significance level of 0.05. Time to first event of symptomatic AF/AFL or ACM was assessed in the device substudy population and analyzed with the same methodology. A clinical events committee that was blinded to treatment assignment adjudicated the first occurrence of AF/AFL identified on ECG, as well as the association of new or worsening symptoms.[8]

ECG Measurements of Sinus Rhythm

Maintenance of normal sinus rhythm (NSR) was evaluated in the overall study population as the cumulative number of ECGs during the 24-week follow-up period assessed as NSR by the investigator with NSR defined as a sinus node driven ventricular rate ≥ 60 and ≤ 100 bpm. Sinus bradycardia and sinus tachycardia were excluded from the primary analysis. Prevalence rates, i.e., cumulative ECGs per patient, were generated for all patients entering efficacy follow-up. Comparisons between treatment groups were expressed by the prevalence rate ratio (PRR = PR_{BUC}/PR_{MET}) and modeled to test significance using Poisson regression.

The cumulative number of AF interventions for the maintenance of sinus rhythm (i.e., rhythm control) was analyzed for all patients entering efficacy follow-up. The composite AF interventions endpoint was defined as events of ECV, catheter ablation, or the use of guideline-directed antiarrhythmic drugs (amiodarone or dofetilide).[9] The initial protocol-defined ECV to establish sinus rhythm at the start of efficacy follow-up was not included as an event. Deaths were included as competing risk events. Prevalence rates (i.e., cumulative events per patient) were generated, with comparisons between treatment groups expressed by PRR and modeled to test significance using Poisson regression.

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Changes from baseline in plasma norepinephrine (NE) and N-terminal pro-b-type natriuretic peptide (NT-proBNP) were measured at weeks 4, 12 and 24. Median values and interquartile ranges are reported due to non-normal distribution in both groups. Comparisons within treatment group were tested with the Wilcoxon Sign Rank test and comparisons between treatment groups over the entire 24-week follow-up period were tested with the Wilcoxon Rank Sum test.

Results

Study Population, Baseline Characteristics and Clinical Events

A total of 267 patients were randomized to study drug and uptitrated to target doses, with 10 of these subjects not entering efficacy follow-up. Two bucindolol (BUC) and 7 metoprolol (MET) patients withdrew from the trial prior to the start of efficacy follow-up. Reasons for withdrawal for the bucindolol and metoprolol groups, respectively, were death (0/1), adverse event (0/3), withdrawal of consent (1/3), and investigator discretion (1/1). A patient who received an atrioventricular node ablation and implantation of a biventricular pacemaker at Week 0 (protocol deviation) was also excluded from the analysis as a major protocol deviation precluding interpretation of outcomes.

The device substudy included 69 patients from the U.S. (N=42), Canada (N=21), and Europe (N=6) who underwent continuous rhythm monitoring. ICMs were inserted in 43 patients for the trial, whereas 26 patients had a pre-existing pacemaker or implantable cardioverter defibrillator (ICD) with an atrial lead A total of 28 of the 32 patients in the metoprolol group and 33 of the 35 patients in the bucindolol group began efficacy follow-up in sinus rhythm and all patients included in the analysis (N=67) completed the 24-week observation period . Of these, 18

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of 22 metoprolol and 21 of 23 bucindolol patients were successfully cardioverted to sinus rhythm at the start of efficacy follow-up. Study drug compliance was greater than 98% in both groups (Table 1) and the mean number of days in follow-up was similar by treatment group for the overall population (BUC=142 days; MET=145 days) and for the device substudy (BUC=161 days; MET=158 days).

Baseline characteristics were well-balanced between the 257 patient entire cohort treatment groups (Table 1) and between the treatment groups and the device substudy. In the overall population (N=257), the mean age was 65.6 ± 10.0 years, 18% were female, the mean LVEF was 36%, 72% had NYHA II or III symptoms at baseline, 51% had persistent AF, and plasma NT-proBNP were elevated at baseline. ECV was performed in 52% of the population prior to follow-up start and 86% of these patients established SR. The baseline characteristics of the device substudy were well-balanced between the two groups and were generally similar to the overall population (Table 1), with a higher proportion of males, persistent AF, and AF at the time of randomization in the substudy cohort compared to the overall population.

There were 2 deaths in the metoprolol group, 1 of which was cardiovascular, and 1 noncardiovascular death in the bucindolol group. There were 8 cardiovascular hospitalizations in the bucindolol group, and 6 in the metoprolol arm. There were no strokes in either treatment group. In the 67 patient device study there were no deaths, and 3 cardiovascular hospitalizations (2 bucindolol, 1 metoprolol).

AF Burden by Continuous Monitoring

There were 35 patients in the bucindolol group who experienced a total of 1389 days in AF during the 24-week follow-up period (39.7 days in AF per patient), whereas 32 patients in the metoprolol group had a total of 1688 days in AF (52.7 days in AF per patient). As shown in

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Figure 1A, daily AF burden (i.e., percent time in AF per day) increased over the first 6 weeks of follow-up in both treatment groups and was maintained at this level in the metoprolol group for the remainder of follow-up. In contrast, AF burden decreased in the bucindolol group after week 6 and continued to decline throughout the remainder of the 24-week follow-up period. Estimates of daily AF burden at the end of the efficacy follow-up period based on data from the last 7 days were 15.1% (95% CI: 3.2, 27.0) in the bucindolol group and 34.7% (95% CI: 17.9, 51.2) in the metoprolol group. The Week 24 comparison between groups yielded a ratio of 0.45 (95% CI: 0.39, 0.50), indicating a 55% reduction in daily AF burden for the bucindolol group compared to metoprolol ($p < 0.001$). Cumulative AF burden over the 24-week follow-up period, as assessed by AUC, was 24.4% (95% CI: 18.5, 30.2) in the bucindolol group compared to 36.7% (95% CI: 30.0, 43.5) in the metoprolol group. The AUC ratio was 0.64 (95% CI: 0.46, 0.86), representing a 36% reduction for the bucindolol group compared to metoprolol ($p = 0.002$).

Figure 1B compares the treatment effect estimate for cumulative AF burden to two different analyses of time to first AF/AFL/ACM event in the device substudy population. When symptomatic AF/AFL events were determined by blinded adjudication of clinic-based 12-lead ECGs, the hazard ratio was 0.69 (95% CI: 0.38, 1.23; $p = 0.21$) for the comparison of bucindolol to metoprolol, whereas the hazard ratio was 0.75 (95% CI: 0.43, 1.32; $p = 0.32$) when an AF/AFL event was predefined as device-detected AF burden ≥ 6 hours per day.

To further describe the distribution of AF burden over time by AF subtype, a categorical analysis was conducted over the 24-week efficacy follow-up period (Figure 2). For patients with paroxysmal AF at baseline (upper panels) bucindolol delayed AF progression, with 77% of patients experiencing a weekly AF burden < 10% by the end of the 24-week follow-up period compared to 64% in the metoprolol group. The proportion of patients experiencing a weekly AF

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burden >90% at the end of follow-up was 15% in the bucindolol group and 27% in the metoprolol group. In patients with persistent AF at baseline (lower panels), 86% of patients in the bucindolol group had a weekly AF burden < 10% compared to 62% of patients in the metoprolol group. Only 14% of persistent AF patients receiving bucindolol had a weekly AF burden > 90% compared to 38% of those receiving metoprolol.

AF burden results by AF subtype are also given in Table 2. The AF burden AUC was reduced more in the bucindolol vs. the metoprolol group in the N=43 persistent AF subgroup, with an AUC ratio of 0.62 (P=0.018). The smaller, N=24 paroxysmal subgroup had a similar point estimate of 0.69 that was not statistically significant (P=0.24). As would be expected, the AF AUCs were higher in the persistent group, in both bucindolol and metoprolol treated subjects.

Maintenance of Normal Sinus Rhythm

Given the findings of reduced AF burden in the device substudy population, we conducted an ECG-based analysis of NSR in the overall study population as a supportive analysis, with NSR defined as sinus rhythm on ECG with a ventricular rate ≥ 60 and ≤ 100 bpm (Figure 3A). There were 132 patients in the bucindolol group who had a total of 555 ECGs demonstrating NSR during the 24-week follow-up period, leading to a prevalence rate of 4.20 events per patient. In contrast, 125 patients in the metoprolol group had a total of 379 ECGs in NSR, with a prevalence rate of 3.03 events per patient. Comparison between groups yielded a PRR of 1.39 (95% CI: 1.22, 1.58), indicating a 39% increase in the number of ECGs demonstrating NSR during the 24-week follow-up period for the bucindolol group compared to metoprolol (p <0.001).

Supplemental Table I gives the total number of ECGs with any rhythm per treatment group, demonstrating that on a per patient basis bucindolol patients received 8.06 recordings and

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metoprolol subjects 8.19 (P = 0.71). Thus the difference in NSR ECGs favoring bucindolol was not due to a larger number of recordings in the bucindolol group. Table I also gives the number of sinus bradycardia and tachycardia recordings in each treatment group, revealing an excess of each in the metoprolol group that achieved statistical significance for sinus bradycardia (prevalence rate ratios of 2.18 (metoprolol) vs. 0.86 (bucindolol), PRR = 0.40 (P <0.0001)). The average heart rates in the NSR, sinus bradycardia and sinus tachycardia groups were respectively 71.1±8.6, 51.9±5.9 and 112.3±16.2 bpm.

Rhythm Control Interventions

Patients experiencing AF/AFL during follow-up remained on blinded study drug and were eligible to undergo additional rhythm control strategies, including ECV, ablation, and treatment with guideline-recommended Class 3 antiarrhythmic drugs (amiodarone or dofetilide). Therefore, we examined the cumulative number of events during the 24-week follow-up period for a composite endpoint of these rhythm control or 'AF interventions' (Figure 3B). There were 132 patients in the bucindolol group who experienced a total of 74 events, leading to a prevalence rate of 0.56 events per patient during the 24-week follow-up period. In contrast, 125 patients in the metoprolol group had a total of 103 events, with a prevalence rate was 0.82 events per patient. The PRR was 0.68 (95% CI: 0.50, 0.91), indicating a 32% reduction in AF intervention events for the bucindolol group compared to metoprolol (p = 0.011). Interventions by AF type are given in Table 2. In Paroxysmal AF the interventions prevalence is reduced by 49% in the bucindolol vs. metoprolol groups (prevalence rate ratio 0.51 (0.30, 0.84), P = 0.009). In contrast, on persistent AF there is only a statistically nonsignificant 20% reduction in PRR. However, the interaction P value between the two AF types was not significant (P = 0.16).

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Supplemental Table II lists the AF interventions by type. Elective ECV was the most prevalent treatment modality deployed, occurring at a rate of 0.30/patient and 0.41/patient in the bucindolol and metoprolol groups respectively (P = 0.13). Treatment with Class III anti-arrhythmics was statistically less prevalent in the bucindolol group, 0.17/patient vs. 0.30/patient in the metoprolol group (P = 0.035). In contrast, catheter ablation did not differ in the 2 treatment groups.

Norepinephrine and NT-proBNP

In order to explore potential mechanisms for greater rhythm control with bucindolol, we compared plasma NE and natriuretic peptide levels between treatment arms in the overall cohort. Plasma NE at baseline was similar in the bucindolol (607 pg/ml, n = 128) and metoprolol (590 pg/ml, n = 134) groups (p = 0.672). In the bucindolol group, there was a significant decrease from baseline in plasma NE at all post-baseline timepoints (Figure 4A), whereas a significant decrease from baseline was only seen at week 12 in the metoprolol group. The decrease in plasma NE over the 24-week follow-up period was significantly greater in the bucindolol group compared to metoprolol in the overall population (p = 0.038) and in the device substudy population (p = 0.036; data not shown).

Median plasma NT-proBNP at baseline was similar in the bucindolol (777 pg/ml, n = 125) and metoprolol (861 pg/ml, n = 123) groups (p = 0.378). In the bucindolol group, there was a significant decrease from baseline in plasma NT-proBNP at all post-baseline timepoints (Figure 4B); whereas, a significant decrease from baseline was only seen at week 24 in the metoprolol group. In the overall population the decrease in plasma NT-proBNP over the 24-week follow-up period was significantly greater in the bucindolol group compared to metoprolol (p =

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0.009), with a nonsignificant trend in the device substudy population (p = 0.081; data not shown).

Discussion

In this analysis of the GENETIC-AF trial there are several important findings regarding the utility of genetically guided beta-blocker therapy with bucindolol versus metoprolol in individuals with AF, HF, and the *ADRB1* Arg389Arg genotype. First and foremost, treatment with bucindolol was associated with a 33% reduction in cumulative AF burden over 24 weeks of follow-up compared with metoprolol succinate, leading to a 55% reduction in daily AF burden by the end of the follow-up. Reductions in AF burden by continuous monitoring were observed with bucindolol in patients with persistent AF, as well as in patients with paroxysmal AF. Consistent with the results from the device substudy, there was a 39% increase in the prevalence of ECGs demonstrating normal sinus rhythm in the overall study population. This is even more notable considering that treatment with bucindolol also led to a 32% lower utilization of adjunctive rhythm control therapies during follow-up, including ECV, catheter ablation, and antiarrhythmic drug therapy. Lastly, the favorable treatment effects of bucindolol also appear to be mirrored by lower levels of NE and improvements in neurohormonal status as reflected by plasma NT-proBNP.

Clinical Implications of Effects on AF Burden

The results from this analysis demonstrate that bucindolol decreases cumulative AF burden substantially in a genetically predefined HF population when compared with metoprolol succinate. This finding has important clinical relevance as approximately half of patients with HF harbor the *ADRB1* Arg389Arg genotype.[12] Higher risks of stroke, heart failure and ACM

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have all been attributed to the more persistent forms of AF.[13-15] A recent Scientific Statement from the American Heart Association supports moving beyond single ECG-based assessments of AF or AF type to more continuous measures of AF that more closely predict the risk of adverse cardiovascular outcomes.[4] Moreover, there is now significant evidence that increasing AF burden is associated with adverse cardiovascular outcomes in patients with AF and HF. Prior investigation in a cohort of 1561 patients with HF and implanted CRT-D devices demonstrated that ≥ 6 hours of AF burden in 24 hours was associated with an increased risk of HF hospitalization.[11] Similarly, in the ASSERT trial, progression of AF burden to episodes greater than 24 hours in duration was associated with greater than a 4-fold increases risk of HF hospitalization.[7] Given the association between AF burden and HF events, the reductions in AF burden observed with bucindolol therapy are notable, especially without the risks and toxicities associated with antiarrhythmic medications.

Agreement of AF Burden Substudy Data with ECG Rhythm Monitoring

While the reduction of cumulative AF burden was identified in the smaller device cohort, several observations in the overall trial population are consistent with the observed treatment effect. In the trial follow-up, use of rhythm control therapies was 32% lower in those treated with bucindolol compared with metoprolol succinate. Similarly, the prevalence of ECGs in normal sinus rhythm was also significantly greater in those randomized to bucindolol in the overall trial population, even with the higher use of additional rhythm control strategies that were employed in the metoprolol group. In contrast, the prevalence of sinus bradycardia was higher in the metoprolol group, and the few sinus tachycardia ECGs also demonstrated a numerical excess in metoprolol treated patients. Finally, it is notable that treatment effect estimate for cumulative AF burden was consistent with time to first AF event analyses. AF burden evaluates more

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information than time to first event methods, providing greater power to detect clinically meaningful differences between groups with limited sample size. The major difference between the time to event analyses and the cumulative AF burden analyses was the precision of the treatment effect estimate for AF burden, which was driven by a greater number of observations for continuous monitoring compared to a time to first event approach. Similar advantages have been leveraged in other trials of therapies to maintain sinus rhythm, including clinical investigations of dronedarone,[16] buidiodarone,[17] and ranolazine/dronedarone combination therapy.[18]

Potential Mechanism Responsible for the Differences between Bucindolol and Metoprolol

There are several potential mechanisms by which bucindolol led to greater reductions in AF burden when compared to metoprolol succinate. Importantly, in the overall GENETIC-AF trial population, analysis of plasma NE levels in the bucindolol group were consistently decreased compared to baseline and compared to the metoprolol group, in keeping with bucindolol known sympatholytic properties. This additional antiadrenergic effect may have contributed to bucindolol's efficacy superiority for AF burden and maintenance of NSR, particularly since the 389Arg variant of the $beta_1$-adrenergic receptor has much higher affinity for norepinephrine.[12] In addition, NT-proBNP, a biomarker of both HF and AF that is responsive to chamber filling pressures, was reduced to a greater degree in bucindolol treated patients compared with metoprolol succinate. This may reflect better control of chamber filling pressures, which would have a favorable impact on AF recurrence. Based upon these observations, we hypothesize that a larger sample size may ultimately identify lower rates of HF hospitalization and potentially lower rates of cardiovascular death in bucindolol treated patients compared with metoprolol succinate treated patients.

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Limitations

There are several limitations that should be considered when evaluating these results. First and foremost, the analyses of cumulative AF burden were conducted in the device substudy group, which is a portion of the overall cohort. However, it is important to note that the point estimate for cumulative AF burden was similar to the time to first AF event primary endpoint in the substudy population and was consistent with the findings in the overall population. Second, the analytical methods for AF burden were not a pre-specified prior to the start of the trial. However, the clinical evidence for the importance of AF burden evolved significantly during the conduct of the Phase 2 trial. Finally, we had limited power to investigate subgroup results in some subpopulations of interest, including HF sub-phenotypes.

Conclusion

In a pharmacogenetically-defined HF population at risk for AF recurrence, bucindolol significantly decreased cumulative AF burden compared to the active control metoprolol succinate. Treatment effect estimates for cumulative AF burden were consistent with time to first AF event analyses, but had greater precision as demonstrated by the lower variance of the estimate. Reductions in cumulative AF burden were mirrored by an increased prevalence of ECG-detected normal sinus rhythm, reductions in rhythm control interventions, lower plasma NE levels, and greater reductions in NT-ProBNP. Cumulative AF burden evaluates more information than time to first event methods, providing greater power to detect clinically meaningful differences between groups with limited sample size.

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Appendix: GENETIC-AF Investigators at Sites who Screened and/or Randomized Patients Canada: F Ayala-Paredes, A Bakbak, ML Bernier, DH Birnie, SJ Connolly, B Coutu, E Crystal, MW Deyell, KM Dyrda, MC Hartleib, Y Khaykin, ZW Laksman, P Leong-Sit, CA Morillo, AS Pandey, F Philippon, S Vizel, SB Wilton; Hungary: P Andréka, Z Csanadi, GZ Duray, T Forster, G Kerkovits, B Merkely, AC Nagy, T Simor; Poland: D Czarnecka, JD Kasprzak, WJ Musial, G Raczak, J Szachniewicz, JK Wranicz; Serbia: S Apostolović, S Hinić, V Miloradović, D Simić; The Netherlands: GJ Milhous, A Oomen, M Rienstra, TJ Romer, LM van Vijk; United States: PB Adamson, RG Aleong, JD Allred, N Amjadi, MM Bahu, AJ Bank, AE Berman, MA Bernabei, RS Bhagwat, L Borgatta, AJ Buda, RT Cole, JL Collier, SJ Compton, O Costantini, MR Costanzo, IM Dauber, MP Donahue, I Dor, GF Egnacyzk, EJ Eichhorn, CC Eiswirth, S Emani, GA Ewald, RC Forde-McLean, MD Gelernt, DE Haines, CA Henrikson, JM Herre, B Herweg, L Ilkhanoff, LR Jackson 2nd, SK Krueger, A Lala, R Lo, B London, BD Lowes, JA Mackall, V Malhotra, FA McGrew, S Murali, A Natale, KR Nilsson, J Okolo, MV Perez, RS

Phang, R Ranjan, MY Rashtian, MJ Ross, SM Samii, T Shinn, MB Shoemaker, SA Strickberger, VN Tholakanahalli, A Tzur, PJ Wang, LT Younis.

Supplemental Materials:
Supplemental Methods
Supplemental Tables I-II

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Table 1. Baseline Characteristics

	Entire Study		Device Substudy	
Parameter	**Bucindolol N = 132**	**Metoprolol N = 125**	**Bucindolol N = 35**	**Metoprolol N = 32**
Age, years	65.9 ± 10.3	65.3 ± 9.6	65.5 ± 11.5	66.2 ± 9.3
Male/Female, %	83/17	82/18	6/94	9/9
Race: W/B/A/O, %	96/2/1/2	96/2/1/1	94/0/3/3	97/3/0/0
LVEF	0.36 ± 0.10	0.36 ± 0.10	0.33 ± 0.08	0.35 ± 0.09
NYHA I/II/III, %	30/60/11	25/55/21	29/49/23†	19/63/19
Ischemic/Non-Ischemic HF, %	31/69	31/69	29/71	25/75
Randomized in AF/Not in AF, %	49/51	52/48	63/37	65/34
Persistent/Paroxysmal AF, %	49/51	50/50	63/37	66/34
HF DxT Duration, days	1264 ± 2082	1009 ± 1703	1208 ± 1880	1025 ± 1481
AF DxT Duration, days	1447 ±2 284	1164 ± 2235	1444 ± 1997	1247 ± 2051
Systolic blood pressure, mm Hg	124.7 ± 15.0	121.1 ± 15.6	122.4 ± 15.7	124.1 ± 14.6
Diastolic blood pressure, mmHg	75.6 ± 10.9	74.7 ± 10.7	73.7 ± 9.9	76.0 ± 9.6
Heart Rate, bpm	76.1 ± 17.4	76.7 ± 17.8	76.8 ± 16.4	80.6 ± 18.3
Previous ECV/AF Ablation/Type III AAD, %	48/20/49	49/19/45	57/17/57	53/9/50
Device Type: ICM/PM/ICD, %	17/9/18/6	16/10/11/10	66/20/14	59/25/16
Norepinephrine, pg/ml	679 ± 347	673 ± 354	710 ± 398	722 ± 337
NT-proBNP, pg/ml, median (IQR)	777 (349, 1316)	865 (404, 1592)	923 (365, 1506)	995 (531, 1844)
Study Drug Compliance %, median (IQR)	99.2 (97.2, 100.0)	99.2 (95.3, 99.9)	98.7 (94.7, 99.6)	98.5 (92.6, 99.8)

W/B/A/O=White/Black/Asian/Other. HF DxT Duration=time from HF diagnosis to randomization. AF DxT Duration=time from AF diagnosis to randomization. ECV=electrical cardioversion. AAD=antiarrhythmic drug. ICM=insertable cardiac monitor. ICD=implanted cardiac defibrillator. PM=pacemaker. IQR=interquartile range. Note: mean ± standard deviations are presented unless otherwise specified. *, P< 0 .05 for Bucindolol vs. Metoprolol group (either entire cohort or Device substudy; †, P <0.05 vs. entire cohort.

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Table 2. Interventions prevalence ratios (PRs) and PR ratios (PRRs); AF Burden (AFB) AUCs and Prevalence rate ratios (PRR) by AF type.

Measure	Persistent AF				Paroxysmal AF			
	Interventions (Entire Cohort, N = 257)		AFB (Substudy, N =43)		Interventions (Entire Cohort, N N= 257)		AFB (Substudy, N =24)	
	B N=67	M N=63	B N=22	M N=21	B N=65	M N=62	B N=13	M N=11
PR or AUC* →	0.76	0.95	0.27	0.41	0.35	0.69	0.19	0.28
PRR or AUC ratio (CI)→	0.80 (0.55, 1.16		0.62 (0.39, 0.95)		0.51 (0.30, 0.84)		0.69 (0.23, 1.80)	
P value →	0.24		0.018		0.009		0.24	
Interaction P value vs. Persistent →					0.16		–	

B = Bucindolol, M = Metoprolol; *proportion of time over 180 days spent in AF

Figure Legends:

Figure 1. AF Burden. **A.** Daily AF burden as a proportion of time spent in AF in the two treatment groups. The vertical lines in each curve are the 95% confidence intervals of predicted values. The respective curves have been smoothed using a cubic B-spline method. **B.** Comparison of AF Burden to Time to First Event Endpoints. SxAF/AFL = symptomatic atrial fibrillation/atrial flutter. ACM = all-cause mortality. AFB = atrial fibrillation burden. AUC = Area under the AFB-time curve. Treatment effect = hazard ratio for time to first event analyses and AUC ratio for AF burden (AUC_{BUC}/AUC_{MET}).

Figure 2: AF Burden by AF Subtype**.** AF burden weekly running average in bucindolol (left panels) and metoprolol (right panels) groups for patients with paroxysmal (upper panels) or

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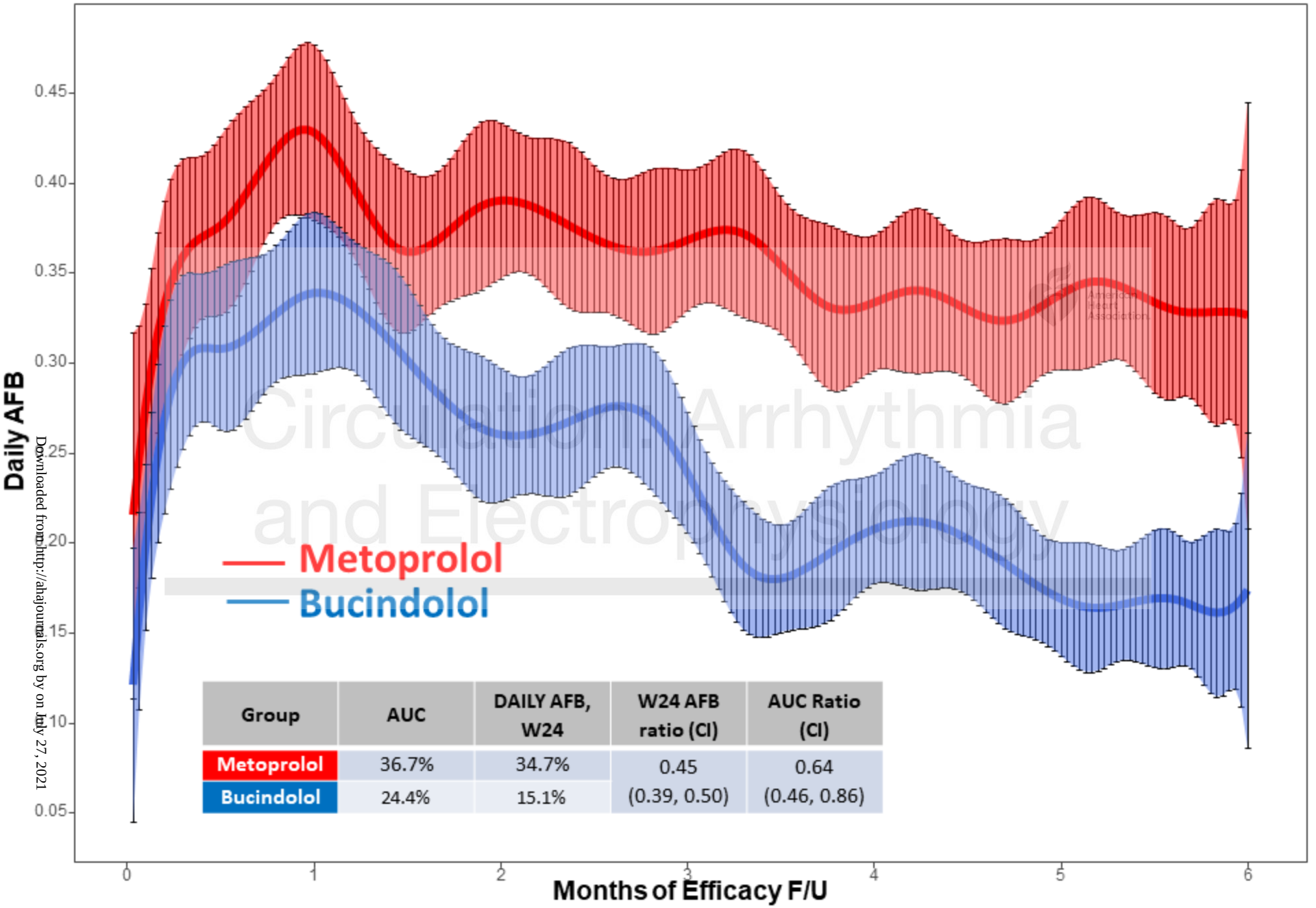
persistent (lower panels) AF at randomization. Proportion of patients in each category is shown on the y-axis.

Figure 3: Cumulative Clinical Events. **A.** ECGs in normal sinus rhythm. **B.** AF interventions. Normal sinus rhythm = sinus rhythm on ECG with ventricular rate ≥ 60 and ≤ 100 bpm. AF Interventions = electrical cardioversion, ablation, or use of guideline-directed antiarrhythmic drugs after start of follow-up.

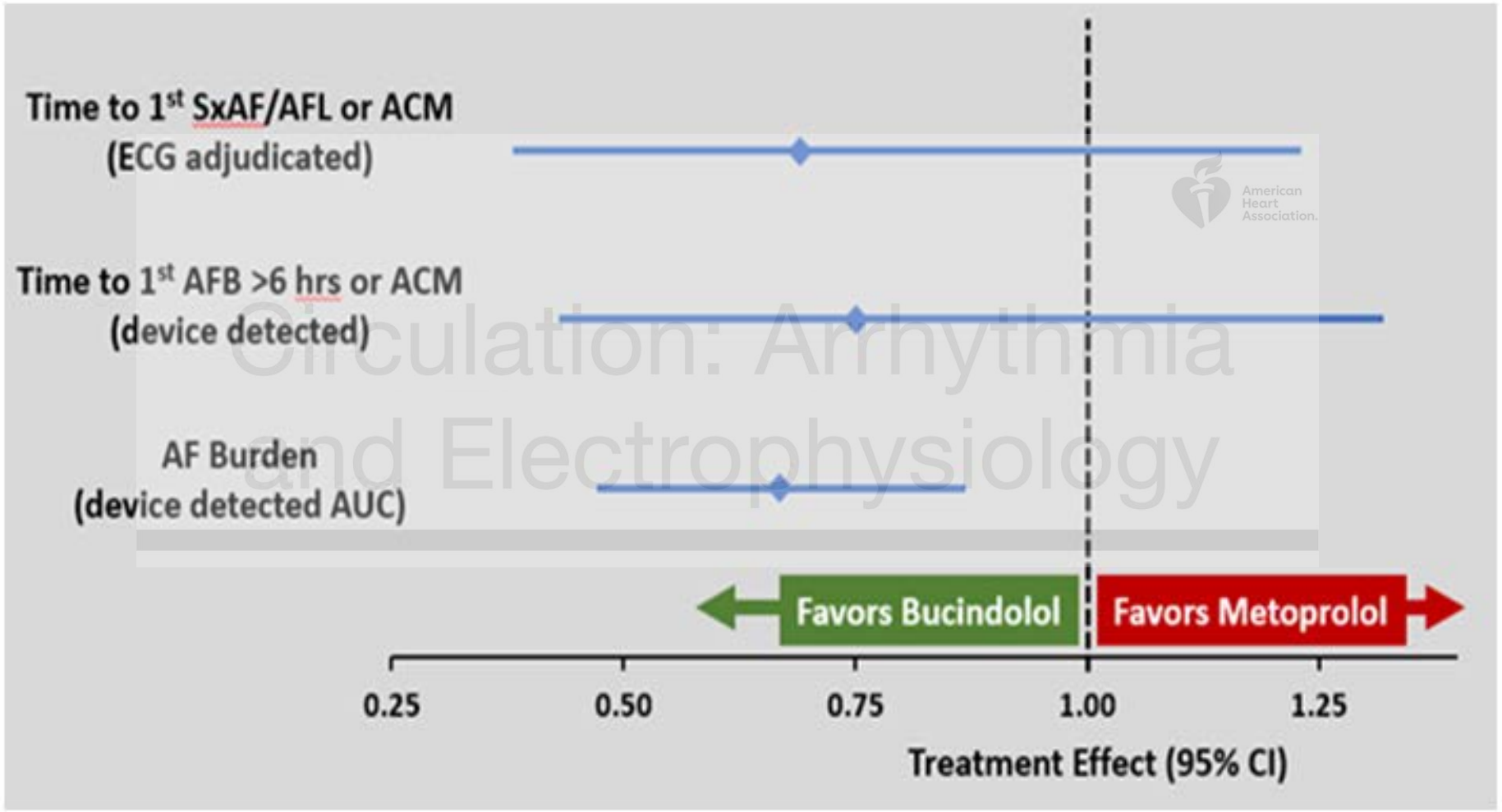
Figure 4: Biomarkers. **A.** Plasma norepinephrine change from baseline; **B.** Plasma NT-proBNP change from baseline. Baseline norepinephrine: MET (590 pg/ml); BUC (607 pg/ml). Baseline NT-proBNP: MET (861 pg/ml); BUC (777 pg/ml). Median (bars) and interquartile range (inside bars). † Wilcoxon Sign Rank test within group at each timepoint; *Wilcoxon Rank Sum test between groups over 24-week follow-up.

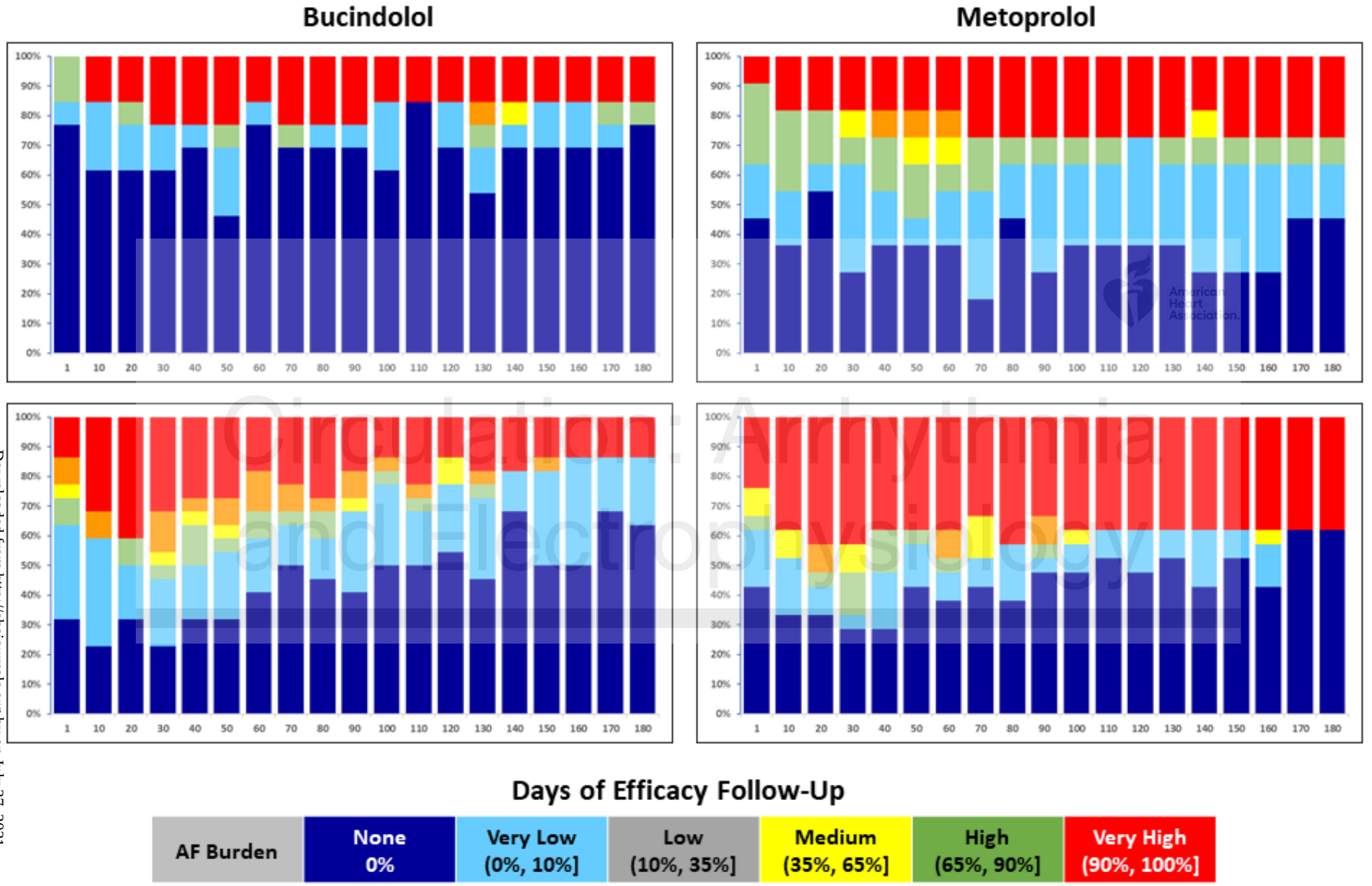
A.



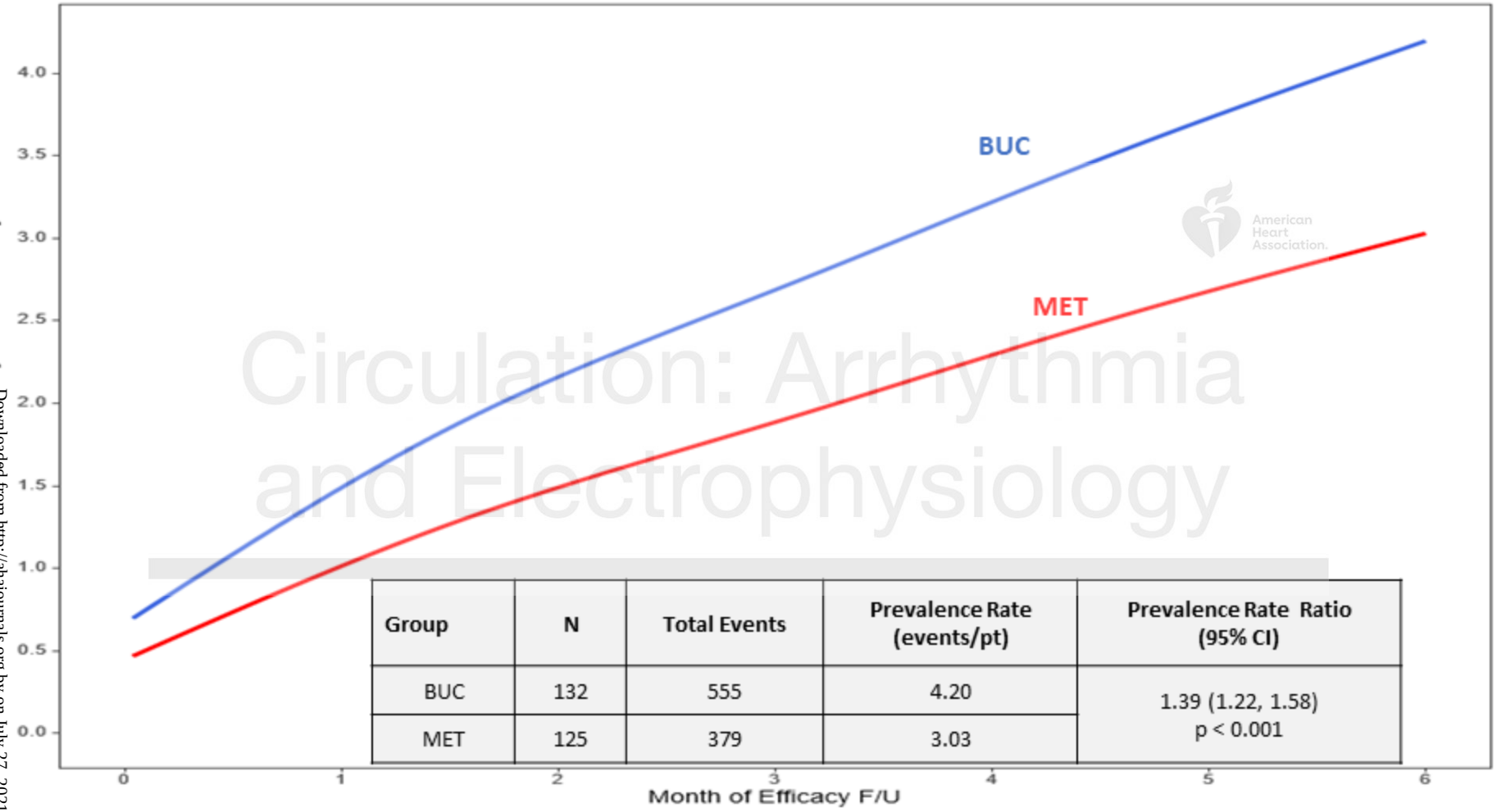
Group	AUC	DAILY AFB, W24	W24 AFB ratio (CI)	AUC Ratio (CI)
Metoprolol	36.7%	34.7%	0.45 (0.39, 0.50)	0.64 (0.46, 0.86)
Bucindolol	24.4%	15.1%		

B.



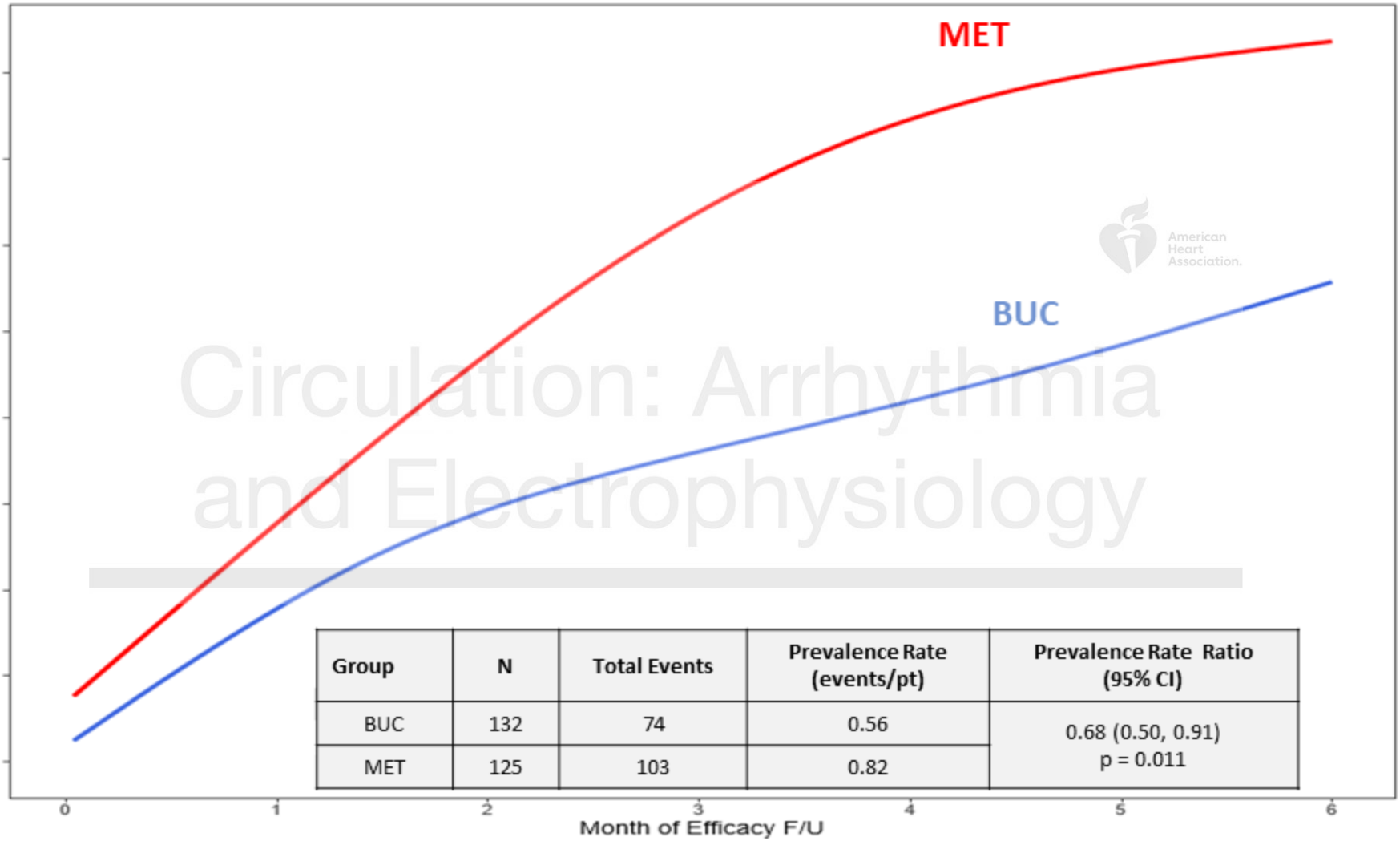


A.



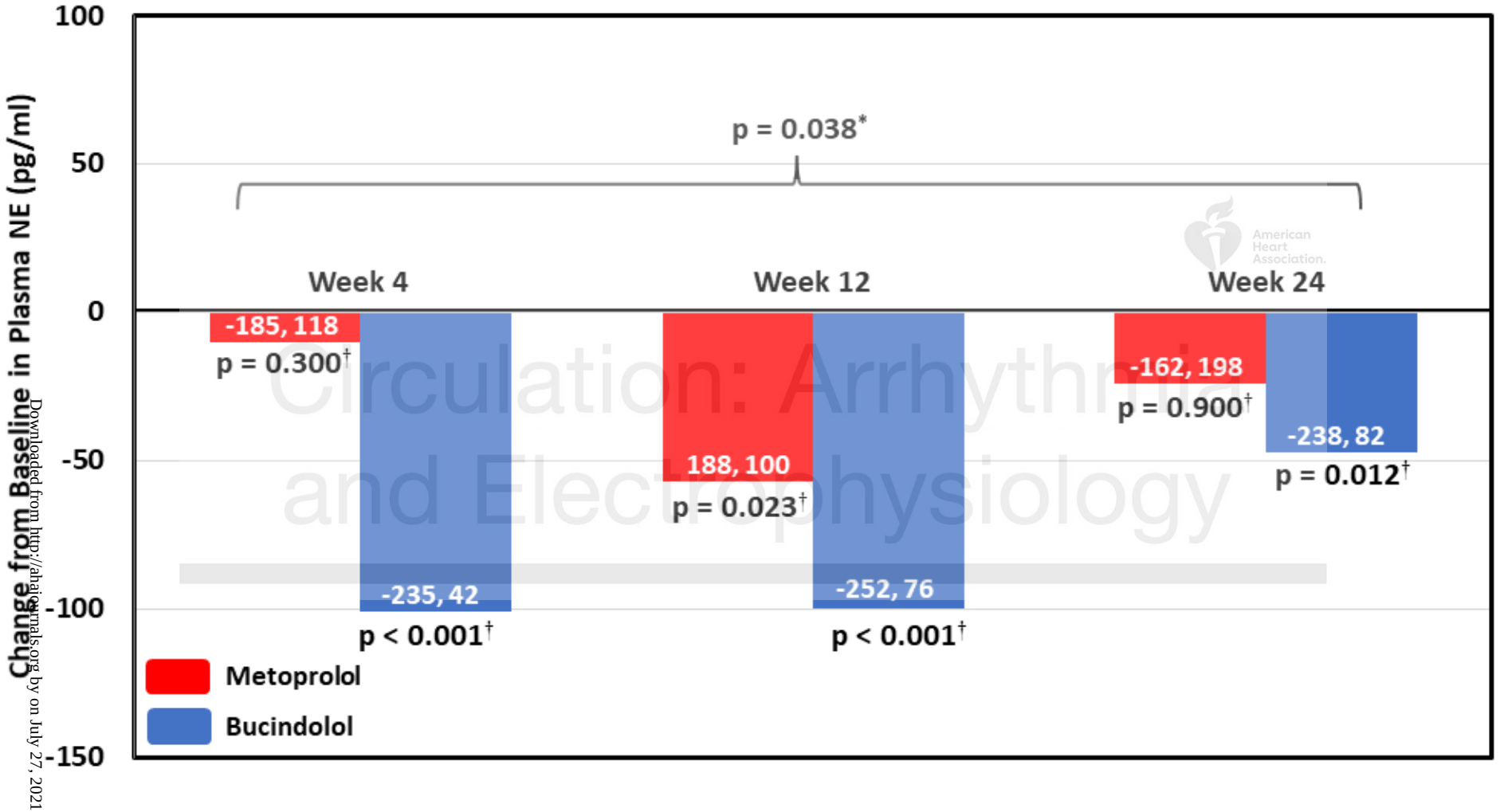
Group	N	Total Events	Prevalence Rate (events/pt)	Prevalence Rate Ratio (95% CI)
BUC	132	555	4.20	1.39 (1.22, 1.58) p < 0.001
MET	125	379	3.03	

B.

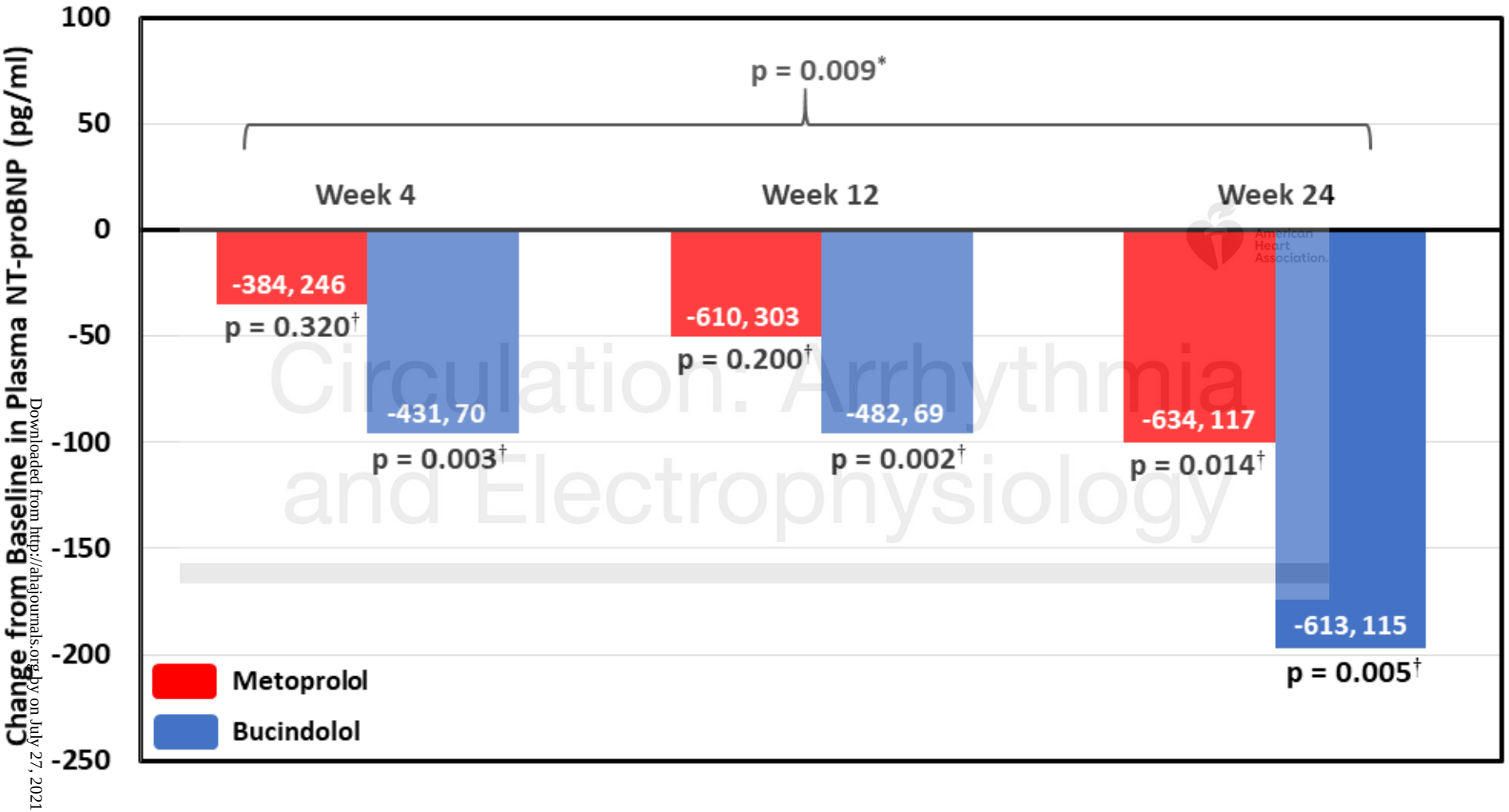


Group	N	Total Events	Prevalence Rate (events/pt)	Prevalence Rate Ratio (95% CI)
BUC	132	74	0.56	0.68 (0.50, 0.91) p = 0.011
MET	125	103	0.82	

A

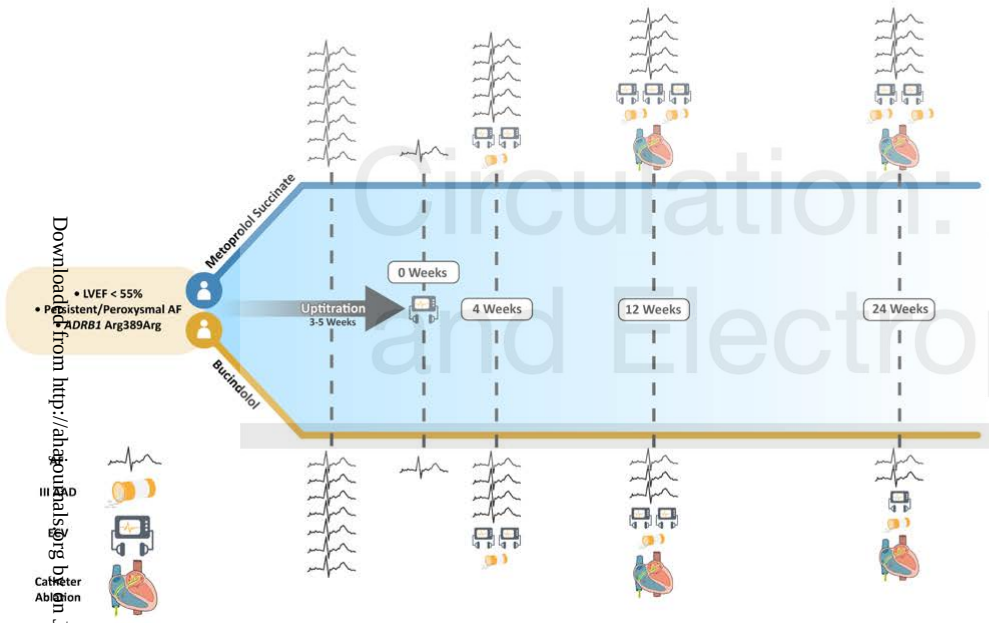


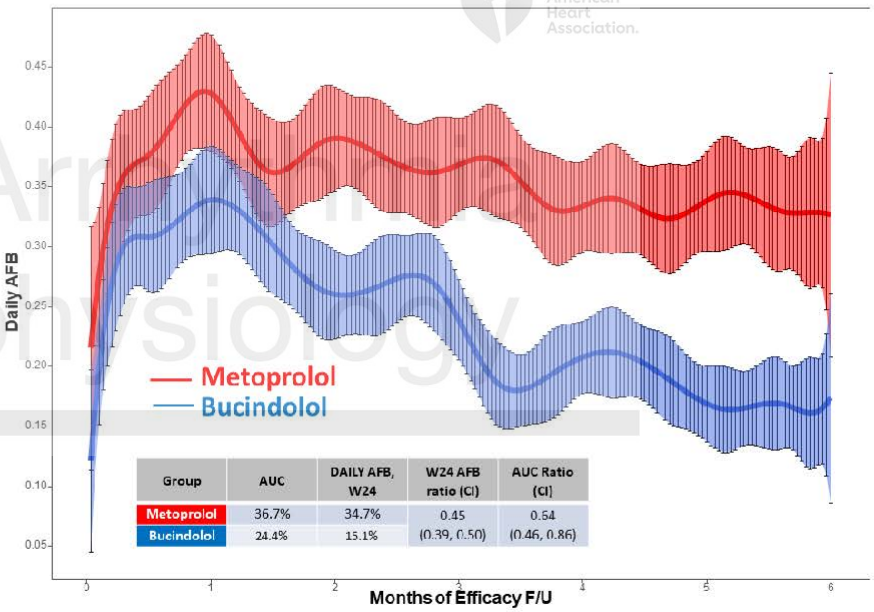
B



Graphic Abstract

Reduction in Atrial Fibrillation Burden in Heart Failure Patients Treated with Bucindolol in GENETIC-AF





Group	AUC	DAILY AFB, W24	W24 AFB ratio (CI)	AUC Ratio (CI)
Metoprolol	36.7%	34.7%	0.45	0.54
Bucindolol	24.4%	15.1%	(0.39, 0.50)	(0.46, 0.86)