Adrenergic Receptor Polymorphisms and Prevention of Ventricular Arrhythmias With Bucindolol in Patients With Chronic Heart Failure

Ryan G. Aleong, MD; William H. Sauer, MD; Alastair D. Robertson, PhD; Stephen B. Liggett, PhD; Michael R. Bristow, MD, PhD, FACC, FAHA

Background—β-blockers prevent cardiac arrhythmias in patients with chronic heart failure and reduced left ventricular ejection fraction, including ventricular tachycardia/ventricular fibrillation (VT/VF). We hypothesized that prevention of ventricular arrhythmias by the β-blocker/sympatholytic agent bucindolol is influenced by genetic variation in adrenergic receptors.

Methods and Results—From a substudy of the β-Blocker Evaluation of Survival Trial (n=1040), we identified those with the high functioning 389Arg versus the lower function 389Gly β1 adrenergic receptor variant, and the loss of function α2c 322-325 adrenergic receptor deletion versus the 322 to 325 wild-type (Wt)/deletion variant. VT/VF was recorded on case report forms as an adverse event. There were 493 Arg 389 β1 receptor homozygotes (β1389 Arg/Arg) versus 547 Gly389 carriers and 207 α2c 322-325 deletion carriers versus 833 homozygous Wts (α2c 322-325 Wt/Wt). In all genotypes bucindolol was associated with a lower incidence of VT/VF (subhazard ratio, 0.42 [0.27–0.64]; P=0.00006). Bucindolol reduced VT/VF in β1389 Arg homozygotes (subhazard ratio, 0.26 [0.14–0.50]; P=0.00005) but not in β389 Gly carriers (subhazard ratio, 0.60 [0.34–1.07]; P=0.09). For genotype combinations, the α2c 322-325 polymorphism altered the VT/VF bucindolol response in β1389 Gly carriers, with α2c deletion genotypes associated with complete efficacy loss. A test of interaction was statistically significant (P=0.028) for the treatment group and a β389/α2c 322-325 three genotype construct, effectively identifying patients who exhibited enhanced response, no substantial response modification and loss of response.

Conclusions—Bucindolol prevents VT/VF in subjects with heart failure and reduced left ventricular ejection fractions, and this effect is modulated by β389 Arg/Gly and α2c 322-325 Wt/deletion adrenergic receptor polymorphisms. (Circ Arrhythm Electrophysiol. 2013;6:137-143.)

Key Words: β-blockers • genetic polymorphisms • pharmacogenetics • ventricular arrhythmia

Ventricular arrhythmias are common in patients with chronic heart failure and reduced left ventricular ejection fractions (HFREF). β-blockers decrease the incidence of sudden death (SD)1-3 and ventricular arrhythmias4 in HFREF patients. For heart failure end points, there has been heterogeneity of response to any given β-blocker in multiple large clinical trials that have included >17 000 randomized patients.5 For the β-blocker/sympatholytic agent bucindolol,6 this response variability may be due, in part, to adrenergic receptor (AR) genetic polymorphisms,7-9 and it is possible that such pharmacogenetically based therapeutic heterogeneity is also present for antiarrhythmic effects.

Clinical Perspective on p 143

Two coding AR polymorphisms affect the therapeutic response to bucindolol in patients with HFREF. In the β1-AR primary drug target located on cardiac myocytes a position 1165 single nucleotide polymorphism (C→G) results in an amino acid substitution (Arg→Gly) at position 389.7 Compared with the Gly variant, the 389Arg variant of the β1-AR has a 3- to 4-fold higher signal transduction capacity,7 higher affinity for agonists including norepinephrine,8,10 and a larger proportion of constitutively active receptors.8,11 In α2c ARs, located on prejunctional nerve terminals and regulating cardiac norepinephrine release through a negative feedback mechanism,12,13 an amino acid position 322 to 325 insertion (wild type)/deletion (Wt/Del) polymorphism results from a 12-nucleotide 964→975 insertion/deletion.14 The Del allele results in a loss-of-function phenotype,14 and an exaggerated sympatholytic response to bucindolol that affects heart failure clinical responses.8

The β-Blocker Evaluation of Survival Trial (BEST) was a placebo-controlled, randomized heart failure clinical trial of bucindolol conducted in HFREF patients with New York Heart Association (NYHA) Class III-IV heart failure and left
ventricular ejection fractions ≤0.35. In a genetic study of BEST, bucindolol had a differential effect on mortality and heart failure hospitalizations that was dependent on β1 Arg389Gly and α2c 322-325 Wt/Del genotypes. We hypothesized that there may also be AR genotype-specific responses to bucindolol for prevention of ventricular arrhythmias, and sought to characterize any potential pharmacogenetic interactions.

## Methods

### Study Sample

The BEST protocol, main outcomes, and DNA substudy have been previously described. This study uses the DNA substudy of BEST, a prospectively planned investigation designed to test the effects of AR polymorphisms on clinical responses. Median follow-up of BEST was 24 months, and background therapy was angiotensin-converting enzyme inhibition or type 1 angiotensin II receptor blockade (mandated, 97% at baseline), digoxin (optional, 90%), and diuretics (if needed, 93%). Although DNA analysis was performed after the trial ended, clinical data remained blinded from the investigators until the coded genetic results were submitted to the data coordinating center and analyzed by trial statisticians.

The prospectively defined primary clinical end points of the BEST DNA substudy were all-cause mortality (ACM) and mortality or cardiac arrest (CA) identified by the study Endpoints Committee as death due to unexpected cardiovascular collapse that was dependent on symptoms of worsening pump failure.

### Genetic Subgroups Analyzed

As previously reported, the 1040 patient BEST DNA bank includes 493 subjects who are Arg homozygotes (Arg/Arg) for the β1389 polymorphism, and 547 who are position 389 Gly carriers (at least 1 copy of the Gly minor allele). For the α1 322-325 polymorphism, 833 subjects are Wt homozygotes (Wt/Wt) and 207 are deletion carriers. In the current study, results for the mild efficacy modifying α1 322-325 polymorphism are not reported as monotypes, but are reported in genotype combinations with β1 389 Gly carriers. In a previous investigation of β1 389 Arg/Gly and α2c 322-325 Wt/Del polymorphisms on heart failure end points, genotype combinations were classified into 4 groups using the major allele homozygotes and minor allele carriers. In the current analysis of ventricular arrhythmia end points, there were too few events (eg, 4 for VT/VF) for analysis in the β1 389 major allele homozygote (Arg/Arg/α2c) minor allele (Del) carrier group, comprising only 73 subjects. For this reason and because α2c genotype does not obviously alter heart failure end point treatment effects in the presence of a β1 389 Arg/Arg genotype, we analyzed only β1389 Gly and α2c 322-325 Wt/Del genotype combinations, which are more evenly distributed and do differentiate bucindolol efficacy responses for heart failure end points.

### Statistical Analysis

The current inquiry is a post hoc analysis to investigate the effects of β1389 Arg/Gly polymorphisms and the impact of their therapeutic interaction with α2c 322-325 Wt/Del polymorphisms on ventricular arrhythmia end points in the BEST DNA substudy. A Cox Proportional Hazards model was used to calculate a hazard ratio (HR) or subhazard ratio (SHR) for time to event for bucindolol versus placebo, with associated probability values. To account for the competing risk of ACM, the model of Fine and Gray was an extension of the Cox model, as implemented in function crr of package crrsc at cran.r-project.org, used for each of the end points SD, CA, and VT/VF. Plots were made of cumulative incidence functions versus time, using the SAS macro %CUMINCID available in SAS V9.3 (SAS, Cary, NC). These plots are analogous to plots of survival functions versus time in the absence of competing risks. The assumption of proportional hazards in the Fine–Gray model was assessed by visual inspection of plots of log(−log[1−cumulative incidence functions]), analogous to the suggestion by Cantor of plots of log(−log[survival functions]). Results for competing risk regression were reported as SHR and for survival function (ACM) as the HR. Randomization in BEST was stratified by 4 binary covariates: sex, race (black, non-black), chronic heart failure cause (ischemic, nonischemic), and baseline left ventricular ejection fractions (≤0.20, >0.20). Analyses of all 4 end points are adjusted via stratification for these same 4 covariates, which is important because there were baseline differences in race and ischemic/nonischemic cause of cardiomyopathy.

### Table 1. Baseline Characteristics, by Genotype Group

<table>
<thead>
<tr>
<th>DNA Substudy</th>
<th>β1389 Arg/Arg (n=1040)</th>
<th>β1389 Gly Carrier (n=547)</th>
<th>β1389 Gly Carrier+α2c Wt/Wt (n=413)</th>
<th>β1389 Gly Carrier+α2c Del (n=134)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>60.3 (60.0–61.1)</td>
<td>60.5 (59.4–61.5)</td>
<td>61.0 (59.8–62.2)</td>
<td>58.9 (56.7–61.0)</td>
</tr>
<tr>
<td>Male, %</td>
<td>79</td>
<td>79</td>
<td>80</td>
<td>75</td>
</tr>
<tr>
<td>Black, %</td>
<td>20</td>
<td>26</td>
<td>&lt;0.001</td>
<td>12</td>
</tr>
<tr>
<td>BHR, bpm</td>
<td>81.4 (80.6–82.2)</td>
<td>81.6 (80.5–82.6)</td>
<td>81.3 (80.0–82.6)</td>
<td>82.4 (80.2–84.5)</td>
</tr>
<tr>
<td>HTN, %</td>
<td>56</td>
<td>59</td>
<td>55</td>
<td>72</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>35</td>
<td>34</td>
<td>32</td>
<td>38</td>
</tr>
<tr>
<td>Ischemic etiology, %</td>
<td>58</td>
<td>59</td>
<td>62</td>
<td>49</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>23.6 (23.2–24.0)</td>
<td>23.8 (23.2–24.4)</td>
<td>24.0 (23.3–24.7)</td>
<td>23.3 (22.1–24.5)</td>
</tr>
<tr>
<td>HF duration, mo</td>
<td>31 (10–60)</td>
<td>32 (10–60)</td>
<td>29 (9–60)</td>
<td>36 (11–72)</td>
</tr>
<tr>
<td>NYHA III, %</td>
<td>92.3</td>
<td>90.5</td>
<td>90.3</td>
<td>91.0</td>
</tr>
<tr>
<td>Digoxin, %</td>
<td>90</td>
<td>89</td>
<td>90</td>
<td>87</td>
</tr>
<tr>
<td>ICD present, %</td>
<td>2.9</td>
<td>2.4</td>
<td>2.3</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Values are mean (95% confidence interval) except HF duration, given as median (interquartile range). BHR indicates basal heart rate; bpm, beats per minute; Del, deletion; HF, heart failure; HTN, history of hypertension; ICD, internal cardioverter defibrillator; LVEF, left ventricular ejection fractions; NYHA, New York Heart Association; and Wt, wild-type.
in baseline characteristics (Table 1). For VT/VF within pharmacogenetic subgroups, results are also presented as unadjusted analyses. In all time-to-event analyses, subjects were censored at loss of follow-up, at end of study, or at time of cardiac transplantation.

Presentation of Cox/Fin–Gray model regression results included Effect Size, or the effect of bucindolol relative to placebo as a percent measured by (1-hour or SHR)x100, and Relative Effect Size (RES), a measure of pharmacogenetic modulation.8,9 RES transforms HR or SHR results into a continuous metric with 1.0 being equivalent to the respective outcome in the parent, all-genotype 1040 patient sample.8,9 Values >1.0 indicate a greater efficacy than in the parent sample, positive values <1.0 indicate a lesser efficacy than the parent sample but superior to that of placebo, and negative values indicate an efficacy inferior to placebo. RES was calculated as ln (HR or SHR for the specific genotype) divided by ln (HR or SHR for the all-genotype parent population). To identify the degree to which genetic subgrouping resulted in outliers for drug response,15 within each polymorphism construct differential efficacy in percent was calculated as the difference between the highest and lowest average RESx100.

Tests of interaction of treatment group and genotype were performed using the Cox/Fin–Gray model, with genotype as an interval-level variable (1 degree of freedom). The tests of interaction used 2 coding schemes for the 3-level genotype. The first coding scheme used 1, 2, and 3, respectively, for the hierarchical 3-group α, α/α, Arg/Gly pharmacogenetic construct identified as comprising of distinct response groups. The second coding scheme (RES) used RES results observed in the current study and normalized to 1.0. Interaction tests were first performed on the 3-group α, α/α, Arg/Gly pharmacogenetic construct, and then if results were not P<0.05 on the 2-group α, α/α construct. The estimated statistical power to detect an interaction of genotype and treatment group on VT/VF was approximated by a power calculation assuming no competing risks.20 Based on an n of 1040 and 96 events, the estimated power was 80% to detect an interaction SHR of 0.32 with a 2-sided β=0.20, >0.20). ACM indicates all-cause mortality; B, Bucindolol; CA, cardiac arrest; P, placebo; SD, sudden death; and VT/VF, ventricular tachycardia or ventricular fibrillation.

### Results

#### Baseline Clinical Characteristics

There were no clinically relevant differences in baseline characteristics between the 1040-subject DNA substudy (Table 1) and the 2708-subject full BEST cohort.6 There were significant differences in race and history of hypertension between β,389 Arg/Arg and Gly carrier (either Gly homozygotes or heterozygotes) groups, as well as between the 2 β,389 Gly carrier/αc, 322-325 groups. In addition, the percent ischemic/nonischemic cause of cardiomyopathy varied slightly (by 27%) in the β,389 Gly carrier/αc, 322-325 groups.

### Outcomes, Parent Population, and by β,389 Arg/Gly Genotypes

In the DNA substudies there were 96 VT/VF first-occurring events. Of these, 78 were VT and 18 were VF. In Table 2, results for the 4 efficacy end points are given as HR or SHR+95% confidence interval for the full DNA substudy, by β,389 Arg/Gly genotypes, and within the β,389 Gly carrier group by αc, 322 to 325 genotype combinations. In the DNA substudy parent population bucindolol treatment was associated with a lower incidence of VT/VF (SHR, 0.42 [0.27–0.64]; P=0.00006), but no significant treatment effects on the 3 other end points. In contrast, β,389 Arg homozygous (Arg/Arg) subjects had reductions in both ACM (by 38%; P=0.042) and VT/VF (by 74%; P=0.00005), and had lower SHRs than in the parent population for all 4 end points. Compared with β,389 Gly carriers, β,389 Arg homozygotes had substantially lower HRs or SHRs, and no end point was statistically significant in β,389 Gly carriers. The greater effect sizes in β,389 Arg/Arg subjects ranged from (38%) ACM to 74% (VT/VF), versus a range of 1% (SD) to 40% (VT/VF) in β,389 Gly carriers. Results by RES are given in Table 3. RES values were much higher in the β,389 Arg/Arg group (average of 1.88 compared with 0.30 in the Gly carrier group), representing respective changes in efficacy of an 88% enhancement versus a 70% decrease compared with the parent population. There was no overlap in the individual RES values of the 4 end points (β,389 Arg/Arg range of 1.55–2.19, Gly carrier 0.05–0.59). The differential efficacy between the 2 genotypes was 158%.

#### Outcomes by β,389 Gly Carrier/αc, 322-325 Genotype Combinations

HR or SHR results by β,389 Gly carrier/αc, 322-325 genotype combination for the 4 efficacy end points are given in Table 2. Compared with the β,389 Gly carrier/αc, 322-325

<table>
<thead>
<tr>
<th>Table 2. Time-to-Event Results, by Genotype Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>End Point</strong></td>
</tr>
<tr>
<td>ACM</td>
</tr>
<tr>
<td>SD</td>
</tr>
<tr>
<td>CA</td>
</tr>
<tr>
<td>VT/VF</td>
</tr>
</tbody>
</table>

Each cell contains hazard ratio (ACM) or subhazard ratios (SD, CA, VT/VF) (95% confidence interval). Analysis is stratified for the following covariates: sex, race (black, non-black), CHF cause (ischemic, nonischemic), and baseline left ventricular ejection fractions (≤0.20, >0.20). ACM indicates all-cause mortality; B, Bucindolol; CA, cardiac arrest; P, placebo; SD, sudden death; and VT/VF, ventricular tachycardia or ventricular fibrillation.

*Adjusted for competing risk of ACM.
Table 3. Relative Effect Sizes, by Individual Genotype

<table>
<thead>
<tr>
<th>End Point</th>
<th>β,389Arg/Arg (P=236, B=257)</th>
<th>β,389Gly Carrier (P=289, B=258)</th>
<th>β,389Gly Carrier+α,322-325 WT (n=413; P=214, B=199)</th>
<th>β,389Gly Carrier+α,322-325Del Carrier (n=134; P=75, B=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACM</td>
<td>1.83</td>
<td>0.32</td>
<td>1.10</td>
<td>−0.15</td>
</tr>
<tr>
<td>SD</td>
<td>2.19</td>
<td>0.05</td>
<td>1.90</td>
<td>−2.95</td>
</tr>
<tr>
<td>CA</td>
<td>1.89</td>
<td>0.26</td>
<td>1.30</td>
<td>−2.94</td>
</tr>
<tr>
<td>VT/VF</td>
<td>1.55</td>
<td>0.59</td>
<td>0.78</td>
<td>0.32</td>
</tr>
<tr>
<td>Average</td>
<td>1.88</td>
<td>0.30</td>
<td>1.33</td>
<td>−1.86</td>
</tr>
<tr>
<td>Range</td>
<td>(1.55–2.19)</td>
<td>(0.05–0.59)</td>
<td>(0.78–1.90)</td>
<td>(−2.95–0.32)</td>
</tr>
<tr>
<td>Differential efficacy (%)</td>
<td>NA</td>
<td>319 vs [β,389Gly carrier+α,322-325Del carrier]</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

Average, Range, and Differential Efficacy statistics apply to SD, CA, VT/VF only. ACM indicates all-cause mortality; B, bucindolol; CA, cardiac arrest; Del, deletion; NA not applicable; P, placebo; RES, relative effect size; and SHR, subhazard ratio.

Wt/Wt) combination genotype the [β, Gly carrier+α, Del carrier] group had a smaller effect size for VT/VF, and SHRs >1.0 for ACM, SD, CA, as well as nonoverlap of SHRs between the 2 genetic groups. Although none of the end points in the [β,389 Gly carrier+α,322-325 Wt/Wt] group were statistically significant, the VT/VF SHR was 0.51 (0.26–1.01).

The [β, Gly carrier+α, Wt/Wt] group average RES of 1.33 (Table 3) indicates a mild enhancement of efficacy compared with the parent population. The [β, Gly carrier+α, Del carrier] group had a negative mean RES (−1.86), indicating that the average event rate hazard in the bucindolol group was 186% worse than in the placebo group. There was no overlap in the various end point RES values, which ranged from 0.78 to 1.90 in the [β, Gly carrier+α, Wt/Wt] group to −2.95 to 0.32 in the [β, Gly carrier+α, Del carrier] group. The differential efficacy between the [β, Arg/Arg] group and the [β, Gly carrier+α, Del carrier] group or the [β, Gly carrier+] group was, respectively, 374% or 319%, meaning that categorizing patients by combination genotypes further differentiated drug response.

Interaction Between Genotype and Treatment

As shown for all races in Table 4, for the VT/VF end point there was a statistical interaction between treatment and the 3-group [β,389/α,322-325 Del carrier] group and 13% and 12% in the [β,389 Arg/Arg] and [β,389 Gly carrier+α,322-325 Wt/Wt] groups, respectively. Consequently, we performed an analysis of the VT/VF end point by race in these 3 genotype groups. As seen in Table 4, results in nonblacks were similar to results for all races, and the interaction tests remain statistically significant (P=0.018, 0.035). Although the interaction P values in blacks are not significant, there are very few events, and the event rates in the bucindolol group exhibit the same pattern as in nonblacks, that

Table 4. Effect of Race on the VT/VF End Point Across the 3 Genotype Construct

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Black/Nonblack (%)</th>
<th>VT/VF Blacks No. Events/No. Pts (%)</th>
<th>VT/VF Nonblacks No. Events/No. Pts (%)</th>
<th>All Races No. Events/No. Pts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B 42/215 (16/84)</td>
<td>B 1/42 (2.4)</td>
<td>B 11/215 (5.1)</td>
<td>B 12/257 (4.7)</td>
<td></td>
</tr>
<tr>
<td>[β, Gly C+α, Wt/Wt] n=413</td>
<td>P 26/188 (12/88)</td>
<td>P 0/26 (0)</td>
<td>SHR 0.13 (0.01–2.54)*</td>
<td>SHR 0.28 (0.14–0.53)*</td>
</tr>
<tr>
<td>B 24/175 (12/88)</td>
<td>B 1/24 (4.2)</td>
<td>B 11/175 (6.3)</td>
<td>B 12/199 (6.0)</td>
<td></td>
</tr>
<tr>
<td>[β, Gly C+α, Del C] n=134</td>
<td>P 48/27 (14/36)</td>
<td>P 7/48 (14.6)</td>
<td>P 1/27 (3.7)</td>
<td>P 8/67 (10.7)</td>
</tr>
<tr>
<td>B 43/16 (73/27)</td>
<td>B 3/43 (7.4)</td>
<td>B 3/16 (18.8)</td>
<td>B 6/59 (10.2)</td>
<td></td>
</tr>
<tr>
<td>Interaction tests, P value</td>
<td>0.61 (1.0 interval)</td>
<td>0.018 (1.0 interval)</td>
<td>0.028 (1.0 interval)</td>
<td>0.032 (RES interval)</td>
</tr>
</tbody>
</table>

B indicates Bucindolol; C, carrier; NC, not calculable; P, placebo; RES, relative effect size; and SHR, subhazard ratio.

*Subhazard ratio (95% confidence interval).
†(1.0–2.4–4.8).
is, increasing from the $\{\beta_1, 389\text{ Arg/Arg}\}$ group progressing through the $\{\beta_1, 389\text{ Gly carrier}+\alpha_2c 322-325\text{ Del carrier}\}$ group.

**Cumulative Incidence Curves**

For the 3-group combination genotype construct, cumulative incidence curves for time to VT/VF adjusted for ACM competing risk are shown in the Figure. $\beta_1$ Arg/Arg subjects had a large relative benefit of bucindolol (Figure A; SHR unadjusted 0.29, $P=0.0002$; covariate adjusted 0.26, $P=0.00005$) that was greater than for the $\{\beta_1, 389\text{ Gly carrier}+\alpha_2c 322-325\text{ Wt/Wt}\}$ group (Figure B; SHR unadjusted or covariate adjusted 0.51, $P=0.054$). The $\{\beta_1\text{ Gly carrier}+\alpha_2c\text{ Del carrier}\}$ subjects exhibited no evidence of therapeutic benefit (Figure C; SHR unadjusted 1.00, $P=1.00$; covariate adjusted HR 0.76, $P=0.58$). The interaction $P$ value for the unadjusted analyses (interval 1.0) was 0.045.

**Discussion**

This study tested the hypothesis that AR polymorphisms influence the effects of bucindolol on serious ventricular arrhythmia end points in patients with HFREF. The basis for the hypothesis was that for heart failure end points 2 AR polymorphisms do affect the therapeutic response to bucindolol, and the results of the current study provide support that similar pharmacogenetic interactions occur for serious ventricular arrhythmias.

The $\beta_1$-389 AR polymorphism creates 2 different functional versions of the $\beta_1$-AR, with respective allele frequencies of 0.70 (Arg) and 0.30 (Gly) in the general US population. In cardiac-restricted receptor overexpression studies in transgenic mice, compared with the $\beta_1\text{ Gly389 AR}$ the $\beta_1\text{ Arg389 AR}$ has greater adverse downstream signaling effects, including a more rapid development of a dilated cardiomyopathy. Uniquely, among $\beta$-blockers bucindolol acts as an inverse agonist on $\beta_1$389 Arg receptors in isolated human heart preparations, and lowers systemic levels of norepinephrine, the cardiac neurotransmitter that selectively signals through 389 Arg versus Gly $\beta_1$-ARs. For heart failure end points, bucindolol but not other $\beta$-blockers produces greater therapeutic responses in patients who are $\beta_1$389 Arg homozygous, compared with 389 Gly carriers. For heart failure end points, inverse agonism and norepinephrine affinity the $\beta_1$389 Gly receptor variant is dominant negative.

Bucindolol is a sympatholytic agent in addition to being a $\beta$-blocker, and the degree of norepinephrine lowering by bucindolol in HFREF patients is regulated genetically, with the $\alpha_2c$ Del polymorphism associated with large (levels reduced by $>200$ pg/mL) amounts of sympatholysis and the Wt $\alpha_2c$ AR associated with mild (<100 pg/mL) norepinephrine lowering. For heart failure end points bucindolol is associated with a complete loss of efficacy in $\{\beta_1, 389\text{ Gly carrier}+\alpha_2c\text{ Del carriers}\}$. This is likely due to exaggerated sympatholysis combined with $\beta$-AR blockade adversely affecting signaling through the low norepinephrine affinity, low signal transduction Gly receptor, resulting in inadequate adrenergic support to the failing heart.

In the current study, for VT/VF and the 3 other ventricular arrhythmia-related end points there was a benefit of bucindolol in $\beta_1$389 Arg/Arg patients not present to the same extent in $\beta_1$389Gly carriers. The VT/VF hazard was substantially (by 74%) reduced ($P=0.00005$) in the $\beta_1$389 Arg/Arg group, but not in 389 Gly carriers. As previously reported, ACM was also reduced only in the $\beta_1$389 Arg/Arg group. Although in the $\beta_1$389 Arg/Arg group the lower event rate end points SD and CA did not achieve statistical significance, their respective RES values of 2.19 and 1.89 were higher than the VT/VF RES (1.55). No RES value in the $\beta_1$389 Gly carrier group was $>0.59$, with an average of 0.30 compared with 1.88 in the 389 Arg/Arg group. RES is an observational method on which standard
statistics cannot be performed because of uncertain degrees of relation among the multiple end points measured, but there was no overlap in individual RES values between the 2 β389 genotypic groups. The degree of differential efficacy conferred by subdividing groups by β389 genotypes was 158%. When β389 Gly carrier+α2,322-325 genotype combinations were analyzed, there was evidence of substantial (by 319%) differential efficacy between the [β389 Gly carrier+α2,322-325 Wt/Wt] and [β389 Gly carrier+α2,322-325 Del carrier] groups, which exceeds by 3-fold the differential efficacy observed for heart failure end points in these same genotypes. Although no end point HR or SHR in the [β389 Gly carrier+α2,322-325 Wt/Wt] was statistically significant, there was no overlap in HRs/SHRs or RES values compared with the [β389 Gly carrier+α2,322-325 Del carrier] group. RES values averaged 1.33 (mild efficacy enhancement) in the [β389 Gly carrier+α2,322-325 Wt/Wt] group, and −1.86 (event rate hazard in the bucindolol group worse than placebo) for patients with a [β389 Gly carrier+α2,322-325 Del carrier] genotype. For SD and CA the SHRs converted from <0.70 in the [β389 Gly carrier+α2,322-325 Wt/Wt] group to, respectively, 1.86 and 2.85 in the [β389 Gly carrier+α2,322-325 Del carrier] group, suggesting an increased risk for SD on an arrhythmic basis conferred by the addition of α2,322-325 Del alleles to β389 Gly genotypes. The nonoverlap of [β389 Gly carrier+α2,322-325 Wt/Wt] and [β389 Gly carrier+α2,322-325 Del carrier] HRs/SHRs and RES values, plus the very pronounced differential efficacy between the 2 groups provided a rational for subdividing β389 Gly carriers for pharmacogenetic testing against the β389 Arg/Arg genotype. Furthermore, the significant interaction for VT/VF across the 3-group genetic construct was not confounded by race, because nonblacks (80% of the study population but only 32% of the [β389 Gly carrier+α2,322-325 Del carrier] group) exhibited the same behavior for bucindolol treatment group event rates and HRs as did all races, and the interaction test remained statistically significant. In addition, in blacks the bucindolol event rates are the same as those that affect heart failure outcomes, either in parallel or secondary to changes in heart failure.

A second limitation is the small sample size or number of events for some genotypes. Another limitation is the post hoc nature of the arrhythmia analysis, as BEST was primarily designed to investigate total mortality and other heart failure end points. Another limitation is that VT and VF events included both sustained and nonsustained episodes. Therefore, the results of this study are only hypothesis generating, and it should be emphasized that a larger, prospectively designed trial will be necessary to confirm these findings.

**Conclusions**

The current study demonstrates therapeutic interactions between bucindolol and the β389 Arg/Gly and α2 Del322–325 Wt/Del AR polymorphisms for treatment effects on ventricular arrhythmias. The data suggest that β389 Arg/Gly and α2,322-325 Wt/Del AR genotypes can be used to better define patient cohorts that would or would not benefit from treatment with bucindolol. The existence of efficacy modifying common genetic polymorphisms in the target(s) of a rhythm modifying agent has not been previously reported, but should be considered in antiarrhythmic drug development.

**Disclosures**

Dr Aleong has speaking honoraria and consulting from St. Jude Medical. Dr Bristow is an employee, director, and stockholder of ARCA biopharma. Dr Robertson consults with ARCA biopharma. Dr Liggett is a co-founder of ARCA biopharma and stockholder. Dr Sauer consults with ARCA biopharma.

**References**


**CLINICAL PERSPECTIVE**

Ventricular Arrhythmias are common in heart failure patients and lead to increased mortality. Although β-blockers have been shown to decrease the incidence of ventricular arrhythmias in heart failure, there is variability in response. Bucindolol is a β-blocker with sympatholytic properties that has been shown to have differential effects on mortality and heart failure admissions via an interaction with 2 adrenergic receptor polymorphism, β389 Arg/Gly and α2C 322–325 Wt/Del. The current analysis demonstrates that these same adrenergic polymorphisms modulate the effect of bucindolol on ventricular arrhythmias in heart failure.
Adrenergic Receptor Polymorphisms and Prevention of Ventricular Arrhythmias With Bucindolol in Patients With Chronic Heart Failure
Ryan G. Aleong, William H. Sauer, Alastair D. Robertson, Stephen B. Liggett and Michael R. Bristow

Circ Arrhythm Electrophysiol. 2013;6:137-143; originally published online December 30, 2012; doi: 10.1161/CIRCEP.111.969618
Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circep.ahajournals.org/content/6/1/137

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Arrhythmia and Electrophysiology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Arrhythmia and Electrophysiology is online at:
http://circep.ahajournals.org//subscriptions/