

- 1 This supplement contains the following items:
- 2 1. Original protocol, final protocol, summary of changes.
- 3 2. Original statistical analysis plan, final statistical analysis plan, summary of changes.
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A Prospective Randomized Crossover trial of Oral Flecainide for Catecholaminergic Polymorphic Ventricular Tachycardia

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29 1.0 Background

30
31 Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a genetic arrhythmia
32 syndrome characterized by frequent ventricular ectopy and polymorphic, classically
33 bidirectional ventricular tachycardia with physical or emotional stress, which also carries
34 a risk of ventricular fibrillation and sudden death, despite no structural heart
35 abnormality.(Liu et al.) The disease is caused by mutations in the cardiac ryanodine
36 receptor gene (RYR2) or cardiac calsequestrin gene (CASQ2).(Priori et al.;Postma et
37 al.) Exercise can elicit ectopy or VT in the majority of CPVT patients. Treatment
38 consists of beta-blockers and/or calcium channel blockers, but up to 30% of patients
39 require implantable cardioverter-defibrillators (ICDs) due to recurrent symptoms on
40 medical therapy(Priori et al.).

41 42 2.0 Rationale and Specific Aims

43
44 Additional forms of therapy for CPVT are needed, as current medications are not
45 completely effective in all patients. By studying mouse models of CPVT (CASQ null
46 mice), we have observed beneficial effects of flecainide, a class IC sodium channel
47 blocker. In a retrospective clinical study in patients with CPVT we have also shown
48 improvement of ventricular ectopy on exercise tests when flecainide is added to
49 standard therapy.

50
51 The Specific Aims of this protocol are:

- 52
53 1: to test the hypothesis that the addition of oral flecainide to standard therapy will
54 reduce cardiac events, defined as either VT treated by ICD or cardiac death, compared
55 to placebo plus standard therapy, in patients with CPVT.
56
57 2. to test the hypothesis that ventricular ectopy and/or VT on treadmill exercise test in
58 patients with CPVT on standard therapy is reduced by flecainide, but not by placebo.
59
60 3. to test the hypothesis that reduction in ventricular ectopy on exercise test is
61 associated with reduction in cardiac events.
62

63 This will be a single-blind (blinded subjects) randomized cross-over study, in which each
64 patient will receive treatment A (flecainide or placebo) for 18 months and, after a 1 week
65 wash-out, treatment B (placebo or flecainide). The event rate and time to event will be
66 assessed during each treatment period. Any events that occur during treatment A will
67 result in early crossover to treatment B after 1 week of wash-out. Any events during
68 treatment B will result in the end of the study for that subject.

69 70 3.0 Animal Studies and Previous Human Studies

71

72 Since the causal association between stress and arrhythmic symptoms of CPVT was
 73 recognized, beta-blockers have been the mainstay of therapy. Moreover, since
 74 polymorphic VT is reproducibly induced with exercise in the majority of patients with
 75 CPVT, it is common practice to use repeated exercise testing to evaluate the efficacy of
 76 β -blocker therapy. While beta-blockers are very effective for preventing exercise-
 77 induced sustained polymorphic VT, the majority of patients with CPVT continue to have
 78 different degrees of ventricular ectopy during exercise despite maximally tolerated
 79 dosages. In addition, studies report high mortality rates and a high incidence of
 80 recurrent polymorphic VT despite beta-blocker therapy.

81 The addition of calcium channel blockers to beta-blockers was reported in 6 children
 82 with CPVT, with an improvement in exercise-induced ectopy in all, and a marked clinical
 83 improvement in 1 (reduction in ICD shocks). (Rosso et al.) Left cardiac sympathetic
 84 denervation has also been reported as an effective alternative in 5 patients when
 85 symptoms persist despite the maximum tolerable dose of β -blocker. (Wilde et al.,
 86 Collura et al.)

87 Oral flecainide has also been reported to reduce ventricular tachycardia in another
 88 genetic arrhythmia syndrome, Andersen-Tawil syndrome (ATS). (Bokenkamp et al.)
 89 Two siblings with ATS who failed therapy with beta-blockers and calcium channel
 90 blockers had marked improvement with oral flecainide. ATS shares some features with
 91 CPVT, in that the characteristic bidirectional ventricular tachycardia (only seen in CPVT,
 92 ATS, and digoxin toxicity) is frequently observed, again with minimal symptoms, yet
 93 patients are at risk for ventricular fibrillation.

94 Recently, we discovered that the class 1C antiarrhythmic agent flecainide directly
 95 blocked *RyR2* channels, prevented *RyR2*-mediated premature Ca^{2+} release, and
 96 suppressed triggered beats in myocytes isolated from mouse hearts lacking

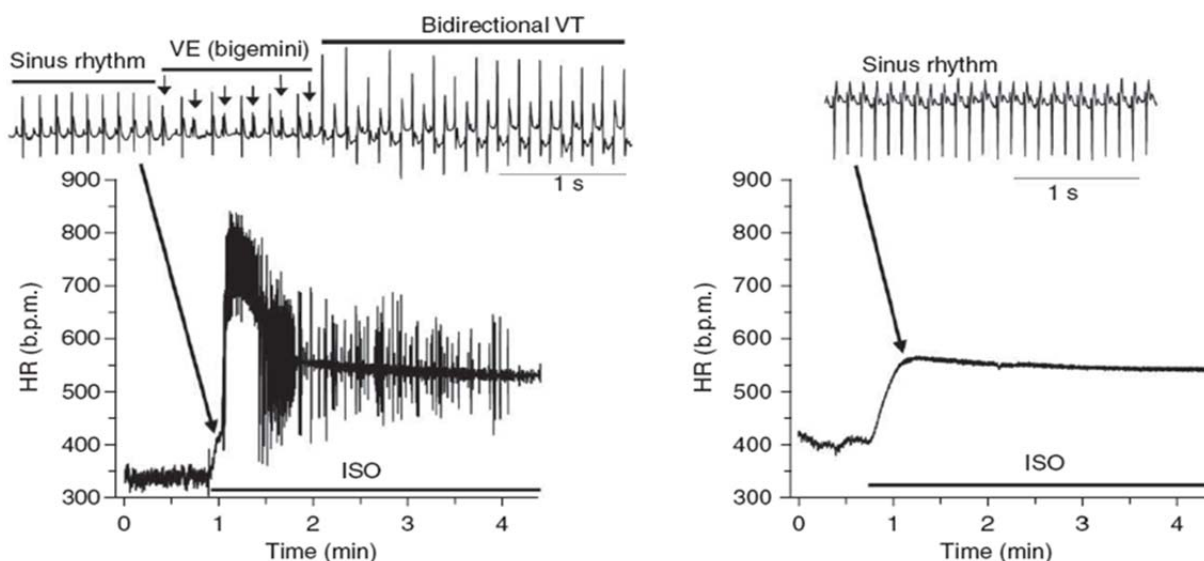


Figure 1. Ventricular arrhythmias during exercise in CASQ null mice (left) are completely abolished with flecainide (right)

97 calsequestrin, an animal model of CPVT.(Watanabe, et al.) Flecainide treatment
 98 completely suppressed ventricular arrhythmias during exercise in this mouse model
 99 (Figure1). Flecainide's mechanism of action can be attributed to an open state block of
 100 *RyR2* channels, thereby directly targeting the molecular defect responsible for the
 101 arrhythmogenic Ca^{2+} waves that trigger CPVT *in vivo*. (Hilliard et al.) Flecainide also
 102 appeared to be effective during short-term treatment in two highly symptomatic CPVT
 103 patients.

104

105 Based on these animal studies, we have collaborated with international centers
 106 to perform an open-label, nonrandomized therapeutic trial of oral flecainide for patients
 107 with CPVT and persistent ventricular ectopy on exercise testing on standard therapy.
 108 In this trial, 86% of patients had improvement of ventricular ectopy on exercise, and
 109 there was no worsening of ectopy with flecainide added to standard therapy.

110

111 **4.0 Inclusion/Exclusion Criteria**

112

113 Inclusion criteria:

- 114 • Clinical diagnosis of CPVT, based on:
 - 115 ○ reproducible polymorphic or bidirectional ventricular tachycardia with
 - 116 exercise
 - 117 OR
 - 118 ○ Ventricular ectopy on exercise test with *RYR2* or *CASQ2* mutation
- 119 • Functioning ICD in place
- 120 • On stable dose of standard therapy defined as the maximal tolerated dose of
- 121 beta-blocker and may include a calcium channel blocker
 - 122 ○ Patients on flecainide are also eligible for enrollment after a 1 week
 - 123 “washout” period during which flecainide is discontinued, and standard
 - 124 therapy alone is used.

125

126 Exclusion criteria:

- 127 • Pregnant females
- 128 • Children < 5 years of age
- 129 • Patients unable to perform treadmill exercise
- 130 • Patients with significant structural heart disease
- 131 • Patients with features consistent with Andersen-Tawil syndrome
 - 132 ○ Periodic paralysis or unexplained weakness
 - 133 ○ Dysmorphic facies
 - 134 ○ Known *KCNJ2* mutation
- 135 • Known hypersensitivity to flecainide
- 136 • Inability to comply with follow-up

137

138 **5.0 Enrollment/Randomization**

139

140 *Patient Enrollment:* The treating physicians at each center will identify potential
 141 subjects and present a brief overview of the study; if the subject (or his/her parent/legal
 142 guardian for patients < 18 years) is interested, an investigator will be contacted and will

143 approach them. Informed consent will be obtained by the investigator after discussing
 144 the study in detail, including the voluntary nature of participation and notification the
 145 subject can withdraw at any time. Ample time for questions and answers will be allowed.
 146 The investigator will give the subject and his/her legal guardian the opportunity to take
 147 the consent home to think about it more, with the option to call or meet with the
 148 investigator to ask additional questions. If the subject and his/her parent/legal guardian
 149 agree to participate, the investigator will ask them to sign a written, informed consent
 150 and assent. A copy of the assent and consent will be given to the subject and his/her
 151 parent/legal guardian. Subjects who are < 18 years of age at the time of enrollment
 152 who turn 18 years of age during the study period will be re-consented after their 18th
 153 birthday. Subjects who wish to participate in the clinical trial without providing a DNA
 154 sample will be allowed to do so.

155
 156 Patients already on flecainide will have a serum level drawn. If the level is > 0.5
 157 mcg/ml, his/her previous dose will be the final study dose (no up titration). If the level is
 158 < 0.5, the previous dose will be the starting dose and up titration will occur as below. All
 159 subjects on flecainide will discontinue it for 1 week prior to the baseline assessment,
 160 continuing on standard therapy alone.

161
 162 *Randomization Procedure:* This will be a single-blind placebo-controlled
 163 randomized crossover study with 2 treatments: oral flecainide or oral placebo. Each
 164 enrolled patient will receive both treatments for 18 months, with a 1 week washout
 165 period between. The order of treatments will be randomized 1:1 across all centers.

166 6.0 Study Procedures

167 All patients enrolled in the study will undergo the following baseline assessment and
 168 data collection:

- 169 • Demographics (age, gender, race)
- 170 • Review of data confirming clinical diagnosis of CPVT (recordings of polymorphic
 171 VT, exercise tests, genetic tests). Required components include:
 - 172 ○ Normal imaging study (echocardiogram or cardiac MRI)
 - 173 ○ No evidence of coronary ischemia on ECG or exercise testing, unless
 174 subsequent stress imaging study was not suggestive of ischemia
 - 175 ○ For subjects with no family history of CPVT nor a putative pathogenic
 176 *RYR2* or *CASQ2* mutation who were > 35 years of age *at the time of initial*
 177 *presentation* with polymorphic VT, a coronary angiogram demonstrating
 178 no coronary artery disease must be documented.
- 179 • Previous anti-arrhythmic treatments
- 180 • Prior ECG and echocardiogram reports

181 Randomization

182 There will be a 50:50 randomization across all centers, with half the subjects
 183 randomized to flecainide then placebo, and half randomized to placebo then flecainide.
 184

189
190 Patients previously on flecainide with a serum level > 0.5 mcg/ml will receive a study
191 dose equal to their previous dose. Patients previously on flecainide with a serum level <
192 0.5 mcg/ml will receive a starting dose equal to their previous dose, with up-titration as
193 below. For all subjects not previously on flecainide, the starting dose will be based on
194 age and weight. Children < 12 years of age will be dosed 3 times per day (every 8
195 hours) and patients > 12 years of age will be dosed 2 times per day (every 12 hours),
196 based on plasma elimination half-life (Perry et al.) For children < 12 years of age an
197 oral suspension will be used, the starting dose will be 2 mg/kg/day and the maximum
198 dose will be 8 mg/kg/day. For patients > 12 years of age the starting dose will be 100
199 mg per day (50 mg every 12 hours) and the maximum dose will be 300 mg/day. Study
200 drugs will be prepared and distributed by a central investigational pharmacy; flecainide
201 and placebo will be similar in appearance.

202
203 After enrollment (and after a 1 week washout for subjects previously on flecainide),
204 patients will undergo a baseline exercise test on standard therapy (Exercise test 0).
205 Subjects with baseline ECG or exercise test evidence of ischemia must be excluded,
206 unless stress imaging studies are performed and are not suggestive of ischemia.
207 Patients will then initiate treatment A (flecainide or placebo) in a blinded fashion. After 1
208 week, blood samples for DNA isolation and storage, and determination of a flecainide
209 level will be drawn. Each time a serum sample is obtained for a flecainide level, and
210 ECG will be obtained. For patients on flecainide, the target serum level will be 0.5-0.8
211 mcg/ml. Patients with levels < 0.35 mcg/ml will have the dose doubled, and those with
212 levels between 0.35 and 0.5 will increase the dose by 50%, unless the maximum dose
213 has been achieved. Patients requiring a dose adjustment (and an equal number of
214 randomly chosen subjects on placebo) will have a second serum sample drawn at 1
215 month. Any further dose adjustments and serum samples will be made prior to the 3
216 month visit. Serum levels will be obtained locally and dose adjustments will be done by
217 the central pharmacy, after confirmation with the treating physician that there are no
218 side effects or significant QRS widening.

219
220 The dose escalation will continue until either:

- 221
222 1. The trough flecainide level is > 0.5 mcg/ml
223 2. The QRS width is > 120 ms or prolonged by > 50% of the baseline QRS
224 3. The maximum dose is achieved.

225
226 At 3 months, all subjects undergo a repeat exercise test (exercise test A) and a serum
227 sample drawn for a flecainide level. After 18 months of treatment A, subjects will
228 discontinue the study drug, and after 1 week of standard therapy alone, start treatment
229 B. Determination of flecainide levels will be done as above. After 3 months of
230 treatment B, another exercise test (exercise test B) will be performed. After 18 months
231 of treatment B, the subject will be removed from the study.

232
233 Patients will be followed every 6 months or as clinically indicated for ICD interrogation.
234 Patients who receive therapy (shock or anti-tachycardia pacing) from the ICD will be

235 carefully assessed and the ICD data downloaded. Therapies will be categorized as
236 “appropriate” if delivered for ventricular tachyarrhythmias, or “inappropriate” if delivered
237 for other reasons. In the event of an appropriate ICD therapy, a serum sample will be
238 drawn for a flecainide level. Subjects that have events during treatment A will
239 discontinue treatment A, start the 1 week washout period, and crossover to treatment B.
240 Subjects with events during treatment B will be removed from the study and unblinded.
241 Further treatment will be determined by the treating physician.

242
243 The primary endpoint will be cardiac event defined as appropriate ICD therapy (shock or
244 anti-tachycardia pacing for VT) or death.

245
246 Secondary endpoints include reduction in ventricular ectopy at exercise test compared
247 to baseline during treatment with flecainide but not placebo.

248

249

250 Exercise tests will be scored using the following scale:

251

252 Exercise test scoring system:

253 0= no ventricular ectopy

254 1=PVC's, < 1 in 2 beats, and < 10/min

255 2= PVC's in bigeminal pattern or > 10/minute

256 3= ventricular couplets

257 4= nonsustained VT (3 or more consecutive beats)

258

259

260

261 **7.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to** 262 **Participants or Others**

263

264

265 Any adverse events (AEs) will be recorded on the adverse event form (see attached)
266 and sent to the Data Coordinating Center within 72 hours of the event. AEs will be
267 reported to the IRB according to the IRB policies and procedures. The data coordinating
268 center will notify the DSMB of any major adverse events. Any unanticipated problems
269 involving risk to the participants or others will be discussed with the PI and DSMB.

270

271 Administration of flecainide is associated with the potential for serious side effects. In
272 our preliminary studies, 8% of CPVT patients were unable to take flecainide due to
273 bradycardia or fatigue and dizziness. Patients who discontinue the study drug
274 (flecainide or placebo) due to side-effects will continue to be followed, with an intent-to-
275 treat analysis. Adverse events related to the administration of flecainide will be
276 reported. All unanticipated problems/events such as breach of confidentiality will be
277 reported.

278

279 **Serious Adverse Events (SAEs) will have to be reported according to the**
280 **following special procedure:**

281 The occurrence of serious adverse events will be reported to the Investigator by
282 telephone or fax; they must be reported to him/her within 24 hours after becoming
283 aware of their occurrence. The Investigator will report SAEs to the Vanderbilt
284 Institutional Review Board per policy.

285

286 **8.0 Study Withdrawal/Discontinuation**

287

288 Subjects may withdraw from the study at any time. Subjects will be unblinded at the
289 time of withdrawal.

290

291 **9.0 Statistical Considerations**

292

293 **Sample Size Estimation and Power Analysis**

294 The primary endpoint of this randomized controlled 2x2 cross-over trial will be cardiac
295 event defined as appropriate ICD therapy (shock or anti-tachycardia pacing for VT) or
296 death. The objective is to demonstrate decreased event rate in patients treated with
297 flecainide in addition to standard therapy compared to patients treated with standard
298 therapy plus placebo. Previous studies of CPVT patients with ICD's reveal an event rate
299 ranging from 50% over 20 months (Piori et al) to 25% over 3.9 years (Hayashi et al).
300 This wide range results in an estimated event rate during 18 months of treatment with
301 placebo plus standard therapy between 10 and 45%. In our small series of patients
302 receiving open-label flecainide, we observed 1 event in 23 patients (4%), which was
303 likely due to noncompliance. The event rate of the control group is expected to be 15%
304 based on the above data. The sample size estimation was carried out using the
305 Pearson chi-square test for paired proportions. With a sample size of 55, the study will
306 have 80% power to detect a 10% difference in the primary endpoint with a two-sided
307 test at 5% significance level. To compensate for reduced power caused by the
308 noncompliance (the anticipated dropout rate over 36 months is 8% based on our pilot
309 data), 60 patients will be enrolled.

310

311

312 **Statistical Analysis Plan**

313 Descriptive statistics, including means, standard deviations, and ranges for continuous
314 variables, as well as percentages and frequencies for categorical variables, will be
315 provided to describe the study sample. Pearson chi-square test or Fisher's exact test
316 will be used to assess the categorical variables. Differences between group means for
317 continuous variables will be examined using ANOVA or Kruskal-Wallis Test. The
318 Mainland-Gart's test will be used for univariate analysis of the primary outcome, when
319 the assumption of no carry-over effect holds. For multivariate analysis, the Generalized
320 Linear Mixed Model will be used to assess the treatment effect, period effect, and
321 treatment-by-period interaction effect, and to adjust for other risk factors such as gender
322 or age. The analysis of survival data will be carried out if such data are available, using
323 the Kaplan-Meier method with log-rank test to compare time-to-event between the two
324 arms and the proportional hazard Cox model to investigate potential prognostic factors.
325 The alpha-spending function of O'Brien-Fleming will be applied for the interim analysis
326 to maintain an overall significance level at 0.05. Point estimates along with the

327 corresponding p-values and 95% confidence intervals will be reported. The adjusted p-
328 values and the corresponding 95% confidence interval will be reported for multivariate
329 analyses. Statistical analysis will be done with R for Windows, version 2.9.2 and SAS
330 9.2.

331
332 Secondary analysis will include comparison of the exercise treadmill tests after 3
333 months of placebo vs. 3 months of flecainide, and quantified as follows for comparison:
334

335 Ventricular arrhythmia score:

336 0 = no ventricular ectopic beats

337 1 = single PVC's

338 2 = PVC's in bigeminal pattern

339 3 = PVC pairs (couplets)

340 4 = nonsustained VT (≥ 3 beats, but < 30 seconds)

341 5 = sustained VT (> 30 seconds)

342

343 Quantification of arrhythmias:

344 1. Total number of ventricular ectopic beats during entire exercise test (rest,
345 exercise, and recovery)

346 2. Number of ectopic beats during worst 10 second period of exercise test (rest,
347 exercise, and recovery)

348 3. Ratio of ectopic to sinus beats during worst 10 second period of exercise test
349 (rest, exercise, and recovery)

350

351 Based on pilot human studies, we anticipate a reduction in the number of ectopic beats
352 during worst 10 second period of exercise from 13 ± 5 beats to 5 ± 5 beats. Assuming
353 an α of 0.05 and 80% power, we would need only 5 subjects to show a statistically
354 significant reduction, as this is a paired analysis.

355

356

357 **10.0 Privacy/Confidentiality Issues**

358

359 Only individuals directly involved with the study will have access to data. Information is
360 for research purposes only and will be used for publication purposes. All participants
361 will have their names concealed. Access to identified patient information will be limited
362 to the investigators listed within this IRB application. De-identified information with
363 HIPPA identifiers removed will be available to other investigators following appropriate
364 IRB approval. Confidentiality and security will be maintained for the database. The
365 database is stored behind a firewall (in addition to the institutional firewall) with the
366 highest level of protection, i.e. the same level of protection as the on-line hospital
367 information system at Vanderbilt. This means that users must logon to a web server
368 that sits between the institutional firewall and the firewall to the database, and only this
369 application server is allowed to query the database. Only users approved through our
370 institutional review board will be allowed access to patient identifiers. Other levels of
371 authorization may exist for future approved users following IRB approval, e.g. access to
372 de-identified data.

373
374 Data is initially collected in the medical record for each individual study participant. The
375 information will be extracted from the patient's medical record and then transferred into
376 the Case Report Form (CRF).

377
378 The CRFs will include personal identifiers for participant. However, this data will not be
379 accessible as numbers and initials are assigned for each participant and these will
380 become the identifying information for each study participant. A master list with patient
381 demographics will only be accessible to the principle investigator and his senior co-
382 investigator. This data will not be available to others.

383

384

385

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423 **A Prospective Randomized Crossover trial of Oral**
424 **Flecainide for Catecholaminergic Polymorphic**
425 **Ventricular Tachycardia**
426

427

428 **Version 5/29/2015**

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447 **1.0 Background**

448 Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a genetic arrhythmia
449 syndrome characterized by frequent ventricular ectopy and polymorphic, classically bidirectional
450 ventricular tachycardia with physical or emotional stress, which also carries a risk of ventricular
451 fibrillation and sudden death, despite no structural heart abnormality.(Liu et al.) The disease is
452 caused by mutations in the cardiac ryanodine receptor gene (RYR2) or cardiac calsequestrin
453 gene (CASQ2).(Priori et al.;Postma et al.) Exercise can elicit ectopy or VT in the majority of
454 CPVT patients. Treatment consists of beta-blockers and/or calcium channel blockers, but up to
455 30% of patients require implantable cardioverter-defibrillators (ICDs) due to recurrent symptoms
456 on medical therapy(Priori et al.).

457

458 **2.0 Rationale and Specific Aim**

459

460 Additional forms of therapy for CPVT are needed, as current medications are not completely
461 effective in all patients. By studying mouse models of CPVT (CASQ null mice), we have
462 observed beneficial effects of flecainide, a class IC sodium channel blocker. In a retrospective
463 clinical study in patients with CPVT we have also shown improvement of ventricular ectopy on
464 exercise tests when flecainide is added to standard therapy.

465 The Specific Aim of this protocol is to test the hypothesis that ventricular ectopy and/or VT on
466 treadmill exercise test in patients with CPVT on standard therapy is reduced by flecainide, but
467 not by placebo.

468 This will be a single-blind (blinded subjects) randomized cross-over study, in which each
469 patient will receive treatment A (flecainide or placebo), undergo an exercise test,
470 crossover to treatment B (placebo or flecainide) and undergo another exercise treadmill
471 test.

472

473 **3.0 Animal Studies and Previous Human Studies**

474

475 Since the causal association between stress and arrhythmic symptoms of CPVT was
476 recognized, beta--blockers have been the mainstay of therapy. Moreover, since
477 polymorphic VT is reproducibly induced with exercise in the majority of patients with
478 CPVT, it is common practice to use repeated exercise testing to evaluate the efficacy of
479 β -blocker therapy. While beta-blockers are very effective for preventing exercise-
480 induced sustained polymorphic VT, the majority of patients with CPVT continue to have
481 different degrees of ventricular ectopy during exercise despite maximally tolerated

482

483 dosages. In addition, studies report high mortality rates and a high incidence of
484 recurrent polymorphic VT despite beta-blocker therapy.

485 The addition of calcium channel blockers to beta-blockers was reported in 6 children
486 with CPVT, with an improvement in exercise-induced ectopy in all, and a marked clinical
487 improvement in 1 (reduction in ICD shocks).(Rosso et al.) Left cardiac sympathetic
488 denervation has also been reported as an effective alternative in 5 patients when
489 symptoms persist despite the maximum tolerable dose of β -blocker. (Wilde et al.,
490 Collura et al.)

491 Oral flecainide has also been reported to reduce ventricular tachycardia in another
492 genetic arrhythmia syndrome, Andersen-Tawil syndrome (ATS). (Bokenkamp et al.)
493 Two siblings with ATS who failed therapy with beta-blockers and calcium channel
494 blockers had marked improvement with oral flecainide. ATS shares some features with
495 CPVT, in that the characteristic bidirectional ventricular tachycardia (only seen in CPVT,
496 ATS, and digoxin toxicity) is frequently observed, again with minimal symptoms, yet
497 patients are at risk for ventricular fibrillation.

498 Recently, we discovered that the class 1C antiarrhythmic agent flecainide directly
499 blocked *RyR2* channels, prevented *RyR2*-mediated premature Ca^{2+} release, and suppressed
500 triggered beats in myocytes isolated from mouse hearts lacking calsequestrin, an animal model
501 of CPVT.(Watanabe, et al.) Flecainide treatment completely suppressed ventricular arrhythmias
502 during exercise in this mouse model (Figure1). Flecainide's mechanism of action can be
503 attributed to an open state block of *RyR2* channels, thereby directly targeting the molecular
504 defect responsible for the arrhythmogenic Ca^{2+} waves that trigger CPVT *in vivo*. (Hilliard et al.)
505 Flecainide also appeared to be effective during short-term treatment in two highly symptomatic

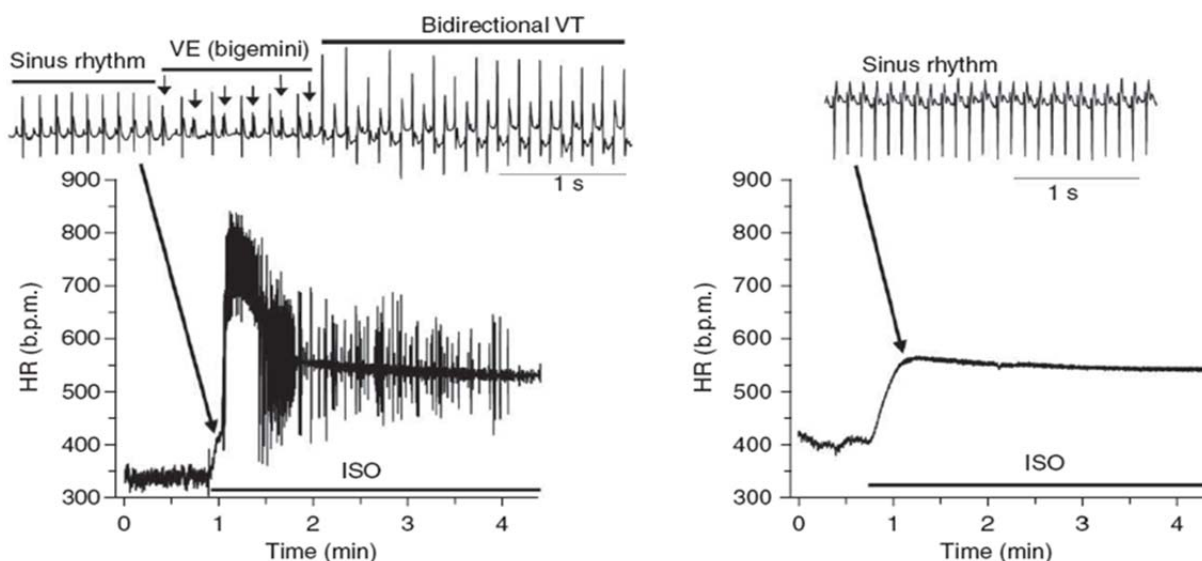


Figure 1. Ventricular arrhythmias during exercise in CASQ null mice (left) are completely abolished with flecainide (right)

506 CPVT patients.

507 Based on these animal studies, we have collaborated with international centers to
 508 perform an open-label, nonrandomized therapeutic trial of oral flecainide for patients with CPVT
 509 and persistent ventricular ectopy on exercise testing on standard therapy. In this trial, 86% of
 510 patients had improvement of ventricular ectopy on exercise, and there was no worsening of
 511 ectopy with flecainide added to standard therapy.

512

513 **4.0 Inclusion/Exclusion Criteria**

514

515 Inclusion criteria:

- 516 • Clinical diagnosis of CPVT, based on:
 - 517 ○ reproducible polymorphic or bidirectional ventricular tachycardia with exercise
 - 518 OR
 - 519 ○ Ventricular ectopy on exercise test with *RYR2* or *CASQ2* mutation
- 520 • Functioning ICD in place
- 521 • On stable dose of standard therapy defined as the maximal tolerated dose of beta-
 522 blocker and may include a calcium channel blocker. On stable doses of CYP2D6
 523 inhibitors (quinidine, fluoxetine, paroxetine, bupropion, cimetidine) or inducers (rifampin,
 524 carbamazepine, phenytoin, Phenobarbital). If CYP2D6 inhibitor/inducer doses require
 525 changes during the course of the subject's treatment with active flecainide, serum
 526 flecainide levels will be monitored
 - 527 ○ Patients on flecainide are also eligible for enrollment after a 1 week "washout"
 528 period during which flecainide is discontinued, and standard therapy alone is
 529 used.

530

531 Exclusion criteria:

- 532 • Pregnant females
- 533 • Children < 5 years of age
- 534 • Patients unable to perform treadmill exercise
- 535 • Patients with significant structural heart disease
- 536 • Patients with features consistent with Andersen-Tawil syndrome
 - 537 ○ Periodic paralysis or unexplained weakness
 - 538 ○ Dysmorphic facies
 - 539 ○ Known *KCNJ2* mutation
- 540 • Known hypersensitivity to flecainide
- 541 • Inability to comply with follow-up

542

543 **5.0 Enrollment/Randomization**

544

545 *Patient Enrollment:* The treating physicians at each center will identify potential subjects
546 and present a brief overview of the study; if the subject (or his/her parent/legal guardian for
547 patients < 18 years) is interested, an investigator will be contacted and will approach them.
548 Informed consent will be obtained by the investigator after discussing the study in detail,
549 including the voluntary nature of participation and notification the subject can withdraw at any
550 time. Ample time for questions and answers will be allowed. The investigator will give the
551 subject and his/her legal guardian the opportunity to take the consent home to think about it
552 more, with the option to call or meet with the investigator to ask additional questions. If the
553 subject and his/her parent/legal guardian agree to participate, the investigator will ask them to
554 sign a written, informed consent and assent. A copy of the assent and consent will be given to
555 the subject and his/her parent/legal guardian. Subjects who are < 18 years of age at the time of
556 enrollment who turn 18 years of age during the study period will be re-consented after their 18th
557 birthday. Subjects who wish to participate in the clinical trial without providing a DNA sample
558 will be allowed to do so.

559

560 Patients already on flecainide will have a serum level drawn. If the level is > 0.5
561 mcg/ml, his/her previous dose will be the final study dose (no up titration). If the level is
562 < 0.5, the previous dose will be the starting dose and up titration will occur as below. All
563 subjects on flecainide will discontinue it for 1 week prior to the baseline assessment,
564 continuing on standard therapy alone.

565

566 *Randomization Procedure:* This will be a single-blind placebo-controlled randomized
567 crossover study with 2 treatments: oral flecainide or oral placebo. Each enrolled patient will
568 receive both treatments for at least 3 months, with a 1 week washout period between. The
569 order of treatments will be randomized 1:1 across all centers.

570

571 **6.0 Study Procedures**

572

573 All patients enrolled in the study will undergo the following baseline assessment and data
574 collection:

575

- 576 • Demographics (age, gender, race)
- 577 • Review of data confirming clinical diagnosis of CPVT (recordings of polymorphic
578 VT, exercise tests, genetic tests). Required components include:
 - 579 ○ Normal imaging study (echocardiogram or cardiac MRI)
 - 580 ○ No evidence of coronary ischemia on ECG or exercise testing, unless
581 subsequent stress imaging study was not suggestive of ischemia
 - 582 ○ For subjects with no family history of CPVT nor a putative pathogenic
583 *RYR2* or *CASQ2* mutation who were > 35 years of age *at the time of initial*

584 *presentation* with polymorphic VT, a coronary angiogram demonstrating
585 no coronary artery disease must be documented.

- 586 • Previous anti-arrhythmic treatments
- 587 • Prior ECG and echocardiogram reports

588

589 Randomization

590

591 There will be a 50:50 randomization across all centers, with half the subjects randomized to
592 flecainide then placebo, and half randomized to placebo then flecainide.

593

594 Patients previously on flecainide with a serum level > 0.5 mcg/ml will receive a study
595 dose equal to their previous dose. Patients previously on flecainide with a serum level <
596 0.5 mcg/ml will receive a starting dose equal to their previous dose, with up-titration as
597 below. For all subjects not previously on flecainide, the starting dose will be based on
598 age and weight. Children < 12 years of age will be dosed 3 times per day (every 8
599 hours) and patients > 12 years of age will be dosed 2 times per day (every 12 hours),
600 based on plasma elimination half-life (Perry et al.) For children < 12 years of age an
601 oral suspension will be used, the starting dose will be 2 mg/kg/day and the maximum
602 dose will be 8 mg/kg/day. For patients > 12 years of age the starting dose will be 100
603 mg per day (50 mg every 12 hours) and the maximum dose will be 400 mg/day. Study
604 drugs will be prepared and distributed by a central investigational pharmacy; flecainide
605 and placebo will be similar in appearance.

606

607 After enrollment (and after a 1 week washout for subjects previously on flecainide),
608 patients will undergo a baseline exercise test on standard therapy (Exercise test 0).
609 Subjects with baseline ECG or exercise test evidence of ischemia must be excluded,
610 unless stress imaging studies are performed and are not suggestive of ischemia.
611 Patients will then initiate treatment A (flecainide or placebo) in a blinded fashion. After 1
612 week, blood samples for DNA isolation and storage, and determination of a flecainide
613 level will be drawn. Each time a serum sample is obtained for a flecainide level, and
614 ECG will be obtained. For patients on flecainide, the target serum level will be 0.5-0.8
615 mcg/ml. Patients with levels < 0.35 mcg/ml will have the dose doubled, and those with
616 levels between 0.35 and 0.5 will increase the dose by 50%, unless the maximum dose
617 has been achieved. Patients requiring a dose adjustment (and an equal number of
618 randomly chosen subjects on placebo) will have a second serum sample drawn at 1
619 month. Any further dose adjustments and serum samples will be made prior to the 3
620 month visit. Serum levels will be obtained locally and dose adjustments will be done by
621 the central pharmacy, after confirmation with the treating physician that there are no
622 side effects or significant QRS widening.

623

624 The dose escalation will continue until either:

625

- 626 1. The trough flecainide level is > 0.5 mcg/ml
- 627 2. The QRS width is > 120 ms or prolonged by > 50% of the baseline QRS
- 628 3. The maximum dose is achieved.

629

630 At 3 months, all subjects undergo a repeat exercise test (exercise test A) and a serum
631 sample drawn for a flecainide level. Subjects will then immediately discontinue the
632 Treatment A study drug, and after 1 week of standard therapy alone, start treatment B.
633 Determination of flecainide levels will be done as above. After at least 3 months of
634 treatment B, another exercise test (exercise test B) will be performed. Treatment B
635 study drug will be discontinued at the time of exercise test B, and the subject's
636 participation in the study will be complete.
637 Patients who receive therapy (shock or anti-tachycardia pacing) from their ICD during the
638 course of their participation in the study will be carefully assessed and the ICD data
639 downloaded. Therapies will be categorized as "appropriate" if delivered for ventricular
640 tachyarrhythmias, or "inappropriate" if delivered for other reasons. In the event of an
641 appropriate ICD therapy, a serum sample will be drawn for a flecainide level. Subjects that
642 have events during treatment A will discontinue treatment A, start the 1 week washout period,
643 and crossover to treatment B. Subjects with events during treatment B will be removed from the
644 study and unblinded. Further treatment will be determined by the treating physician.

645 The primary endpoint will be reduction in ventricular ectopy at exercise test compared to
646 baseline during treatment with flecainide but not placebo.

647
648 Exercise tests will be scored using the following scale:
649

650 Exercise test scoring system:
651 0= no ventricular ectopy
652 1=PVC's, < 1 in 2 beats, and < 10/min
653 2= PVC's in bigeminal pattern or > 10/minute
654 3= ventricular couplets
655 4= nonsustained VT (3 or more consecutive beats)

656 657 658 **7.0 Reporting of Adverse Events or Unanticipated Problems Involving Risk to** 659 **Participants or Others** 660

661 Any adverse events (AEs) will be recorded on the adverse event form (see attached) and sent
662 to the Data Coordinating Center within 72 hours of the event. AEs will be reported to the IRB
663 according to the IRB policies and procedures. The data coordinating center will notify the DSMB
664 of any major adverse events. Any unanticipated problems involving risk to the participants or
665 others will be discussed with the PI and DSMB.

666 Administration of flecainide is associated with the potential for serious side effects. In our
667 preliminary studies, 8% of CPVT patients were unable to take flecainide due to bradycardia or
668 fatigue and dizziness. Patients who discontinue the study drug (flecainide or placebo) due to
669 side-effects will continue to be followed, with an intent-to-treat analysis. Adverse events related
670 to the administration of flecainide will be reported. All unanticipated problems/events such as
671 breach of confidentiality will be reported.

672

673 **Serious Adverse Events (SAEs) will have to be reported according to the**
 674 **following special procedure:**

675 The occurrence of serious adverse events will be reported to the Investigator by
 676 telephone or fax; they must be reported to him/her within 24 hours after becoming
 677 aware of their occurrence. The Investigator will report SAEs to the Vanderbilt
 678 Institutional Review Board per policy.

679

680 **8.0 Study Withdrawal/Discontinuation**

681

682 Subjects may withdraw from the study at any time. Subjects will be unblinded at the
 683 time of withdrawal.

684

685 **9.0 Statistical Considerations**

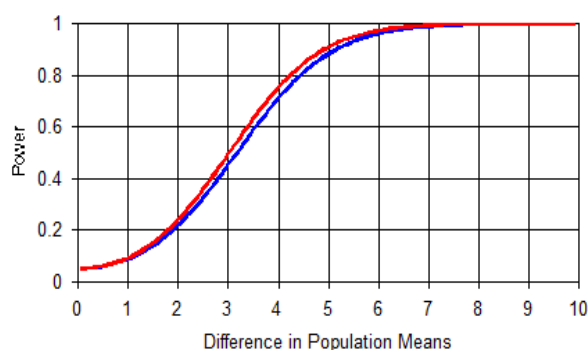
686

687 **Statistical Analysis Plan**

688 Our previous open-label comparison of flecainide to standard therapy showed a 7 beat
 689 reduction in number of ectopic beats in worst 10 seconds of exercise test (from 12 ± 5 beats to
 690 5 ± 5 beats).¹ A second study in 12 genotype-negative CPVT patients demonstrated a 6 beat
 691 reduction in number of ectopic beats in worst 10 seconds of exercise test (from 12 ± 7 beats to
 692 6 ± 7 beats).² **A target of 14** enrolled subjects provide adequate statistical power (80% power
 693 with an alpha of 0.05) to detect the previously observed differences (6-7) in number of PVC's in
 694 worst 10 second period during exercise testing.³ Graphs of Power (Y-axis) vs observed
 695 difference in means (X-axis) are provided below. The 2 panels represent power calculations
 696 using different values for the within-group standard deviation (SD). These values are informed
 697 from the 2 previous studies in CPVT patients using the same methodology (left, within group SD
 698 = 5, observed in reference 1; right within group SD = 7, observed in reference 2). Red lines
 699 indicate N=13, and blue lines indicate N=12, allowing for potential subject dropout.

700 References for statistical analysis plan

- 701 1. van der Werf C, Kannankeril PJ, Sacher F, Krahn AD, Viskin S, Leenhardt A, Shimizu W,
 702 Sumitomo N, Fish FA, Bhuiyan ZA, Willems AR, van der Veen MJ, Watanabe H, Laborderie J,
 703 Haïssaguerre M, Knollmann BC, Wilde AA. Flecainide therapy reduces exercise-induced
 704 ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. *J*
 705 *Am Coll Cardiol*. 2011 May 31;57(22):2244-54.
- 706 2. Watanabe H, van der Werf C, Roses-Noguer F, Adler A, Sumitomo N, Veltmann C, Rosso R,
 707 Bhuiyan ZA, Bikker H, Kannankeril PJ, Horie M, Minamino T, Viskin S, Knollmann BC, Till J,
 708 Wilde AA. Effects of flecainide on exercise-induced ventricular arrhythmias and recurrences in
 709 genotype-negative patients with catecholaminergic polymorphic ventricular tachycardia. *Heart*
 710 *Rhythm*. 2013 Apr;10(4):542-7.
- 711 3. Dupont WD, Plummer WD. Power and Sample Size Calculations: A Review and Computer
 712 Program. *Controlled Clinical Trials*. 1990;11:116-28.



713

714 Descriptive statistics, including means, standard deviations, and ranges for continuous
 715 variables, as well as percentages and frequencies for categorical variables, will be provided to
 716 describe the study sample. Pearson chi-square test or Fisher's exact test will be used to assess
 717 the categorical variables. Differences between group means for continuous variables will be
 718 examined using ANOVA or Kruskal-Wallis Test. Point estimates along with the corresponding p-
 719 values and 95% confidence intervals will be reported. The adjusted p-values and the
 720 corresponding 95% confidence interval will be reported for multivariate analyses. Statistical
 721 analysis will be done with R for Windows, version 2.9.2 and SAS 9.2.

722

723 Analysis will consist of a comparison of the exercise treadmill tests after 3 months of
 724 placebo vs. 3 months of flecainide, and quantified as follows for comparison:

725

726 Ventricular arrhythmia score:

727 0 = no ventricular ectopic beats

728 1 = single PVC's

729 2 = PVC's in bigeminal pattern

730 3 = PVC pairs (couplets)

731 4 = nonsustained VT (≥ 3 beats, but < 30 seconds)

732 5 = sustained VT (> 30 seconds)

733

734 Quantification of arrhythmias:

735 1. Total number of ventricular ectopic beats during entire exercise test (rest,
 736 exercise, and recovery)

737 2. Number of ectopic beats during worst 10 second period of exercise test (rest,
 738 exercise, and recovery)

739 3. Ratio of ectopic to sinus beats during worst 10 second period of exercise test
 740 (rest, exercise, and recovery)

741

742

743 10.0 Privacy/Confidentiality Issues

744

745 Only individuals directly involved with the study will have access to data. Information is
 746 for research purposes only and will be used for publication purposes. All participants
 747 will have their names concealed. Access to identified patient information will be limited
 748 to the investigators listed within this IRB application. De-identified information with

749 HIPPA identifiers removed will be available to other investigators following appropriate
750 IRB approval. Confidentiality and security will be maintained for the database. The
751 database is stored behind a firewall (in addition to the institutional firewall) with the
752 highest level of protection, i.e. the same level of protection as the on-line hospital
753 information system at Vanderbilt. This means that users must logon to a web server
754 that sits between the institutional firewall and the firewall to the database, and only this
755 application server is allowed to query the database. Only users approved through our
756 institutional review board will be allowed access to patient identifiers. Other levels of
757 authorization may exist for future approved users following IRB approval, e.g. access to
758 de-identified data.

759

760 Data is initially collected in the medical record for each individual study participant. The
761 information will be extracted from the patient's medical record and then transferred into the
762 Case Report Form (CRF).

763

764 The CRFs will include personal identifiers for participant. However, this data will not be
765 accessible as numbers and initials are assigned for each participant and these will
766 become the identifying information for each study participant. A master list with patient
767 demographics will only be accessible to the principle investigator and his senior co-
768 investigator. This data will not be available to others.

769

770

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773 with Andersen-Tawil syndrome." Heart Rhythm. 4.4 (2007): 508-11.

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775 long QT syndrome and catecholaminergic polymorphic ventricular tachycardia using video-
776 assisted thoracic surgery. Heart Rhythm 2009;6:752-9.

777 Hilliard FA, Steele DS, Laver D, et al. Flecainide inhibits arrhythmogenic Ca(2+) waves by open state block
778 of ryanodine receptor Ca(2+) release channels and reduction of Ca(2+) spark mass. J Mol Cell
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781 premature Ca²⁺ release, and catecholaminergic polymorphic ventricular tachycardia."
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786 world literature on efficacy, safety, and dosing. *Am Heart J* 1992 Dec; 124(6): 1614-21.
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- 788 Postma, A. V., et al. "Absence of calsequestrin 2 causes severe forms of catecholaminergic
789 polymorphic ventricular tachycardia." Circ.Res. 91.8 (2002): e21-e26.
- 790 Priori, S. G., et al. "Clinical and molecular characterization of patients with catecholaminergic
791 polymorphic ventricular tachycardia." Circulation 106.1 (2002): 69-74.
- 792 Priori, S. G., et al. "Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie
793 catecholaminergic polymorphic ventricular tachycardia." Circulation 103.2 (2001): 196-
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- 795 Rosso, R., et al. "Calcium channel blockers and beta-blockers versus beta-blockers alone for
796 preventing exercise-induced arrhythmias in catecholaminergic polymorphic ventricular
797 tachycardia." Heart Rhythm. 4.9 (2007): 1149-54.
- 798 Watanabe H, Chopra N, Laver D, et al. Flecainide prevents catecholaminergic polymorphic
799 ventricular tachycardia in mice and humans. *Nat Med* 2009;15:380-3.
- 800 Wilde AA, Bhuiyan ZA, Crotti L, et al. Left cardiac sympathetic denervation for
801 catecholaminergic polymorphic ventricular tachycardia. *N Engl J Med* 2008;358:2024-9.

802

803 **Summary of changes to protocol:**

804 The reasons for changes to the protocol are reflected in the manuscript Methods:

805 “The primary endpoint was appropriate ICD therapy, and the secondary endpoint was degree of
806 ventricular arrhythmias induced on exercise testing. Adequate power for the primary endpoint
807 required enrolling 60 subjects. In June 2015, after only 14 of the desired 60 subjects were able to
808 be enrolled, the investigators, with approval from the funding source and the Data and Safety
809 Monitoring Board (DSMB), modified the study protocol to evaluate only the secondary endpoint
810 (ventricular arrhythmias on exercise test). At that point, subjects who had completed the
811 Treatment A exercise test crossed over, without the 18 month treatment duration. Similarly,
812 subjects discontinued Treatment B once the three month exercise test was complete.”

813 **The protocols differ in the following sections:**814 **Section 2:** Aims 1 and 3 removed. Aim 2 becomes primary aim.

815 **Section 6:** Study procedures: Duration of therapy shortened from 18 months to 3 months.
816 Primary endpoint changed from cardiac event to ventricular ectopy at exercise test.

817 **Section 9:** Statistical considerations are significantly revised, with original and final statistical
818 plans copied below.

819

820 **Original Statistical Considerations:**

821

822 **Sample Size Estimation and Power Analysis**

823 The primary endpoint of this randomized controlled 2x2 cross-over trial will be cardiac
824 event defined as appropriate ICD therapy (shock or anti-tachycardia pacing for VT) or
825 death. The objective is to demonstrate decreased event rate in patients treated with
826 flecainide in addition to standard therapy compared to patients treated with standard
827 therapy plus placebo. Previous studies of CPVT patients with ICD's reveal an event rate
828 ranging from 50% over 20 months (Piori et al) to 25% over 3.9 years (Hayashi et al).
829 This wide range results in an estimated event rate during 18 months of treatment with
830 placebo plus standard therapy between 10 and 45%. In our small series of patients
831 receiving open-label flecainide, we observed 1 event in 23 patients (4%), which was
832 likely due to noncompliance. The event rate of the control group is expected to be 15%
833 based on the above data. The sample size estimation was carried out using the
834 Pearson chi-square test for paired proportions. With a sample size of 55, the study will
835 have 80% power to detect a 10% difference in the primary endpoint with a two-sided
836 test at 5% significance level. To compensate for reduced power caused by the
837 noncompliance (the anticipated dropout rate over 36 months is 8% based on our pilot
838 data), 60 patients will be enrolled.

839

840 **Statistical Analysis Plan**

841 Descriptive statistics, including means, standard deviations, and ranges for continuous
842 variables, as well as percentages and frequencies for categorical variables, will be provided to
843 describe the study sample. Pearson chi-square test or Fisher's exact test will be used to assess
844 the categorical variables. Differences between group means for continuous variables will be
845 examined using ANOVA or Kruskal-Wallis Test. The Mainland-Gart's test will be used for
846 univariate analysis of the primary outcome, when the assumption of no carry-over effect holds.
847 For multivariate analysis, the Generalized Linear Mixed Model will be used to assess the
848 treatment effect, period effect, and treatment-by-period interaction effect, and to adjust for other
849 risk factors such as gender or age. The analysis of survival data will be carried out if such data
850 are available, using the Kaplan-Meier method with log-rank test to compare time-to-event
851 between the two arms and the proportional hazard Cox model to investigate potential prognostic
852 factors. The alpha-spending function of O'Brien-Fleming will be applied for the interim analysis
853 to maintain an overall significance level at 0.05. Point estimates along with the corresponding p-
854 values and 95% confidence intervals will be reported. The adjusted p-values and the
855 corresponding 95% confidence interval will be reported for multivariate analyses. Statistical
856 analysis will be done with R for Windows, version 2.9.2 and SAS 9.2.

857

858 Secondary analysis will include comparison of the exercise treadmill tests after 3 months of
859 placebo vs. 3 months of flecainide, and quantified as follows for comparison:

860

861 Ventricular arrhythmia score:

862 0 = no ventricular ectopic beats

863 1 = single PVC's

- 864 2 = PVC's in bigeminal pattern
865 3 = PVC pairs (couplets)
866 4 = nonsustained VT (≥ 3 beats, but < 30 seconds)
867 5 = sustained VT (> 30 seconds)

868

869 Quantification of arrhythmias:

- 870 1. Total number of ventricular ectopic beats during entire exercise test (rest, exercise, and
871 recovery)
872 2. Number of ectopic beats during worst 10 second period of exercise test (rest, exercise,
873 and recovery)
874 3. Ratio of ectopic to sinus beats during worst 10 second period of exercise test (rest,
875 exercise, and recovery)

876

877 Based on pilot human studies, we anticipate a reduction in the number of ectopic beats during
878 worst 10 second period of exercise from 13 ± 5 beats to 5 ± 5 beats. Assuming an α of 0.05
879 and 80% power, we would need only 5 subjects to show a statistically significant reduction, as
880 this is a paired analysis.

881

882 Final Statistical Analysis Plan

883 Our previous open-label comparison of flecainide to standard therapy showed a 7 beat
 884 reduction in number of ectopic beats in worst 10 seconds of exercise test (from 12 ± 5 beats to
 885 5 ± 5 beats).¹ A second study in 12 genotype-negative CPVT patients demonstrated a 6 beat
 886 reduction in number of ectopic beats in worst 10 seconds of exercise test (from 12 ± 7 beats to
 887 6 ± 7 beats).² **A target of 14** enrolled subjects provide adequate statistical power (80% power
 888 with an alpha of 0.05) to detect the previously observed differences (6-7) in number of PVC's in
 889 worst 10 second period during exercise testing.³ Graphs of Power (Y-axis) vs observed
 890 difference in means (X-axis) are provided below. The 2 panels represent power calculations
 891 using different values for the within-group standard deviation (SD). These values are informed
 892 from the 2 previous studies in CPVT patients using the same methodology (left, within group SD
 893 = 5, observed in reference 1; right within group SD = 7, observed in reference 2). Red lines
 894 indicate N=13, and blue lines indicate N=12, allowing for potential subject dropout.

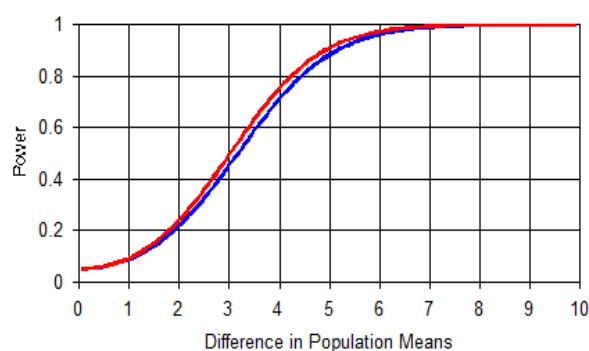
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 898 Sumitomo N, Fish FA, Bhuiyan ZA, Willems AR, van der Veen MJ, Watanabe H, Laborderie J,
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 903 Bhuiyan ZA, Bikker H, Kannankeril PJ, Horie M, Minamino T, Viskin S, Knollmann BC, Till J,
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909

910



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917 values and 95% confidence intervals will be reported. The adjusted p-values and the
918 corresponding 95% confidence interval will be reported for multivariate analyses. Statistical
919 analysis will be done with R for Windows, version 2.9.2 and SAS 9.2.

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921 Analysis will consist of a comparison of the exercise treadmill tests after 3 months of placebo vs.
922 3 months of flecainide, and quantified as follows for comparison:

923

924 Ventricular arrhythmia score:

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932 Quantification of arrhythmias:

933 1. Total number of ventricular ectopic beats during entire exercise test (rest, exercise, and
934 recovery)

935 2. Number of ectopic beats during worst 10 second period of exercise test (rest, exercise,
936 and recovery)

937 3. Ratio of ectopic to sinus beats during worst 10 second period of exercise test (rest,
938 exercise, and recovery)

939

940 **Summary of changes to statistical plan:**

941 The originally proposed secondary analysis becomes the primary analysis, and power
942 calculations are presented for the enrolled subjects, with new references added.

943