Characterization of Autosomal Dominant Hypercholesterolemia Caused by

*PCSK9* Gain of Function Mutations and its Specific Treatment with

Alirocumab, a *PCSK9* Monoclonal Antibody

**Running title:** Hopkins et al.; *PCSK9* Gain of Function Mutations and Treatment

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Abstract:

Background - Patients with PCSK9 gene gain of function (GOF) mutations have a rare form of autosomal dominant hypercholesterolemia. However, data examining their clinical characteristics and geographic distribution are lacking. Furthermore, no randomized treatment study in this population has been reported.

Methods and Results - We compiled clinical characteristics of PCSK9 GOF mutation carriers in a multinational retrospective, cross-sectional, observational study. We then performed a randomized placebo-phase, double-blind study of alirocumab 150 mg administered subcutaneously every 2 weeks to 13 patients representing four different PCSK9 GOF mutations with low-density lipoprotein cholesterol (LDL-C) >70 mg/dL on their current lipid-lowering therapies at baseline. Observational study: Among 164 patients, 16 different PCSK9 GOF mutations distributed throughout the gene were associated with varying severity of untreated LDL-C levels. Coronary artery disease was common (33%; average age of onset 49.4 years) and untreated LDL-C concentrations were higher compared with matched carriers of mutations in the LDLR (n=2126) or apolipoprotein B (n=470) genes. Intervention study: In PCSK9 GOF mutation patients randomly assigned to receive alirocumab, mean percent reduction in LDL-C at 2 weeks was 62.5% (P<0.0001) from baseline, 53.7% compared to placebo-treated PCSK9 GOF mutation patients (P=0.0009; primary endpoint). After all subjects received 8 weeks of alirocumab treatment, LDL-C was reduced by 73% from baseline (P<0.0001).

Conclusions - PCSK9 GOF mutation carriers have elevated LDL-C levels and are at high risk for premature cardiovascular disease. Alirocumab, a PCSK9 antibody, markedly lowers LDL-C levels and appears to be well tolerated in these patients.

Clinical Trial Registration - www.clinicaltrials.gov; Unique Identifier: NCT01604824

Key words: hypercapnia; hypercholesterolemia; cardiovascular disease; genetics; PCSK9, clinical trial, alirocumab
Introduction

Autosomal dominant hypercholesterolemia (ADH), which features high levels of low density lipoprotein cholesterol (LDL-C), is a common monogenic disorder (estimated prevalence 1 in 250–500) that substantially contributes to the worldwide burden of premature cardiovascular disease (CVD). Plasma levels of LDL-C are regulated primarily by apolipoprotein B-mediated binding of LDL particles to hepatic LDL receptors (LDLR) followed by cellular internalization and metabolism. Patients with genetic defects in this pathway have high levels of LDL-C and early-onset CVD, as evident in patients with LDLR (OMIM #606945) or APOB mutations (OMIM #107730) causing familial hypercholesterolemia (FH) and familial defective apolipoprotein B (FDB), respectively.

DNA recombinant mapping in families in France and Utah in which ADH did not cosegregate with markers for LDLR or APOB identified 1p34 as the responsible locus. Shortly thereafter, several gain of function (GOF) mutations in the PCSK9 gene (OMIM #607786) were identified as a third cause of ADH: Ser127Arg and Phe216Leu in 3 French families, and Asp374Tyr in the Utah family and later in Norwegian and English families. Additional PCSK9 GOF mutations were later identified in several small studies from various geographical locations.  

Proprotein convertase subtilisin/kexin type 9 (PCSK9) regulates serum LDL catabolism by binding and targeting LDLR to lysosomal degradation. Thus, increased PCSK9 function leads to reduced hepatic LDLR levels and concomitant high plasma LDL-C levels and vice versa. In several patient populations who cannot achieve target LDL-C levels with currently available lipid-lowering therapies, blockade of PCSK9 with alirocumab, or other human PCSK9 monoclonal antibodies, has demonstrated significant LDL-C reductions.
Despite growing awareness that PCSK9 mutations may cause ADH, no global study has been performed that examines and compares the clinical characteristics of the rare patients with different PCSK9 GOF mutations to each other or to patients with FH and FDB. We report a worldwide comparative compilation of patients known to have varying PCSK9 GOF mutations so as to describe their physical and laboratory manifestations, prevalence of CVD, and lipid response to therapy. We also report results from the first randomized intervention trial in PCSK9 GOF mutation patients treated with alirocumab for which we employed a novel randomized placebo-phase study design to enable a double-blinded comparison of alirocumab with placebo (based upon differential onset of effect between study arms) and the opportunity for all subjects to receive active study medication, and contribute to the analysis of safety and efficacy.24

Methods

Study Designs

The studies were designed by Regeneron Pharmaceuticals Inc. in collaboration with one of the authors (JD for observational study, PNH for treatment study). The study protocols were approved by the investigational review board at each study center and all subjects in the treatment study provided written informed consent. Data were collected at the study sites by several of the co-authors and were analyzed by representatives of Regeneron Pharmaceuticals Inc.

Comparative Observational Study

We conducted a retrospective global comparative compilation study in which individuals known to have PCSK9 GOF mutations were categorized so as to associate mutations with lipid profiles, comorbidity, and response to therapy. All of these patients had also been previously characterized for functional mutations in LDLR, and APOB exons 26 and 29. Data were collected
by supplying the collaborators with a uniform data collection sheet that included untreated and on-treatment lipid profiles; lipid-lowering therapy at the time of treated lipid profiles; presence of xanthoma, xanthelasma, and arcus lipoides corneae; and occurrence and age of onset of CVD.

We compared lipid profiles and other clinical characteristics of patients with PCSK9 GOF mutations to patients with FH and FDB. For this comparison we selected molecularly proven carriers of pathological LDLR or APOB mutations from the Dutch Familial Hypercholesterolemia Registry who had untreated lipid levels available.25,26 Each patient with a PCSK9 GOF mutation was matched by gender and age (± 2 years) to all available FH and FDB patients Dutch Familial Hypercholesterolemia Registry. This approach yielded a cohort with an average of 3 FDB and 16 FH patients for each PCSK9 carrier. LDLR mutations were characterized as ‘defective’ (missense, small in-frame indel, synonymous with added splice site) or ‘deficient’ (large or frame-shifting indel, nonsense, splice site, promoter variant). In comparisons of the effect of different PCSK9 GOF mutations on LDL cholesterol, we only performed statistical tests for a particular variant when five or more individuals were observed to carry that variant, and we compared that variant to all non-carriers of that particular variant.

Treatment Study

The treatment study was conducted at 3 sites in France and one in Utah. We included men and women age 18–70 with PCSK9 GOF mutations verified by DNA sequencing and serum LDL-C levels ≥70 mg/dL at screening on a stable lipid-lowering regimen, and considered not at goal by the investigator. Subjects had body mass index 18.0–40.0 kg/m² and no cardiovascular event, heart failure, or uncontrolled diabetes within 6 months of enrolment. Patients continued to take their pre-study lipid-lowering therapies throughout the study. Additional enrolment criteria are provided in the Data Supplement.
We utilized a novel double-blind, randomized, placebo-phase design instead of an open-label non-randomized study design in order to enable a double-blinded comparison of alirocumab with placebo (Figure I in the Data Supplement). This study design also provided on-drug treatment data for all subjects in this small group of unique patients. All participants received a single-blind dose of placebo at week 2. After subsequent randomization, group A received alirocumab (150 mg subcutaneously) at weeks 0, 2, 4, 6, and 10 and placebo at weeks 8, 12, and 14; group B received alirocumab at weeks 2, 4, 6, 8, and 12 and placebo at weeks 0, 10, and 14 (Figure I in the Data Supplement). Follow-up visits were conducted at weeks 16, 18, 20, and 22. Accordingly, the number of alirocumab doses was equal in the 2 groups but the dosing schedule for group B was shifted by 2 weeks compared to group A.

The primary endpoint was a comparison in percent change of measured serum LDL-C from pre-treatment to 2 weeks between group A (single alirocumab dose) and group B (placebo). Secondary efficacy endpoints included changes in other lipids at week 2 and changes in lipid measures from baseline to each study visit. Safety assessments included a physical exam, the evaluation of vital signs, electrocardiography, and blood tests. Further details and the schedule of assessments are provided in the Data Supplement.

**Statistical Analysis**

**Comparative Observational Study**

For the comparative observational study, we used analysis of variance to assess differences in mean lipoprotein levels between each of the individual PCSK9 mutations and all other PCSK9 GOF mutations combined. This methodology was also used to compare lipoprotein levels in all patients with PCSK9 GOF mutations (without LDLR mutations) combined and patients with FH and FDB. To determine the effect of medication, a paired t-test was performed on lipoprotein
levels before and after treatment.

**Treatment Study**

Power analysis for the treatment study were based on prior efficacy data and suggested that approximately 6 patients per dose group in this rare patient population would provide at least 80% power to detect a treatment difference of 30% (standard deviation [SD]15%) versus placebo for the primary endpoint at a 5% significance level. Continuous primary and secondary efficacy variables were analyzed using an analysis of covariance (ANCOVA) model with treatment arm as the fixed effect and using the relevant baseline value as a covariate. The rank-based ANCOVA was used for triglycerides and lipoprotein (a) [Lp(a)]. The results for the remaining lipid parameters were also confirmed using a non-parametric method (Kruskal-Wallis). There were no missing data points.

**Results**

**Comparative Observational Study**

During 2012, 200 lipid specialty centers around the world were contacted and 164 patients (83 men and 81 women, aged <1–79 years) heterozygous with previously identified PCSK9 GOF mutations were compiled from 12 centers in 8 countries (Table 1). The patients carried 16 different missense mutations, 6 of which were previously undescribed (Table 1). Individual PCSK9 GOF mutations generally had restricted geographic distributions and were found in a small number of pedigrees (Figure II in the Data Supplement). Examples include 22 patients with Arg215His found only in 2 pedigrees in Norway, and 12 patients with Val4Ile and 30 patients with Glu32Lys found only in Japan. Obligate carrier founders in the Utah pedigree were migrants from the United Kingdom. For pooled PCSK9 GOF mutation patients, mean untreated total and LDL-C were 359 and 272 mg/dL, respectively. Eleven patients were double
heterozygotes for mutations in PCSK9 and LDLR; these patients tended to have higher untreated lipids compared to patients with the same GOF mutation alone, as previously reported for the three Glu32Lys double heterozygote patients.12

GOF mutations were found in all structural protein domains and 5 of 9 coding exons (Figure 1), and were associated with varying degrees of lipid abnormalities (Table 1). Untreated lipid levels associated with each mutation were compared to the entire PCSK9 GOF mutation population: Asp374Tyr and Ser127Arg carriers had severe dyslipidemia while Glu32Lys, Arg215His, and Ser465Leu carriers were comparatively mild, although substantial variation was present in patients carrying the same mutation (Figure 1).

The physical stigmata of elevated cholesterol were frequent (Table 1), with prevalence similar to previous reports for FH and FDB (Table 1 in the Data Supplement). Also similar to FH and FDB,3,27 44% of patients had a history of CVD. Coronary artery disease was the most prevalent manifestation (33%) with an average age of onset of 49.4±13.8 years (Table 1 and Table 1 in the Data Supplement).

In a comparison with FH and FDB patients drawn from the Dutch Hypercholesterolemia Registry, PCSK9 GOF mutation patients had the highest, and FDB patients the lowest, mean untreated LDL-C levels (Table 2). Among patients with FH, those with deficient mutations had higher untreated LDL-C levels than those with defective mutations (Table 2). Although lipid-lowering therapy (primarily statins, Figure III in the Data Supplement) improved lipid profiles, a substantial proportion failed to achieve guideline LDL cholesterol levels (Figure III in the Data Supplement).

Treatment Study

Six Asp374Tyr mutation carriers were enrolled in Utah, and 4 Ser127Arg, 2 Leu108Arg, and 1
Arg218Ser carriers in France (Figure IV in the Data Supplement). Baseline characteristics of the subjects in groups A and B were mostly similar (Table 3) although some differences are apparent.

**Lipid and Lipoprotein Response**

In PCSK9 GOF mutation patients randomly assigned to receive alirocumab, mean percent reduction in LDL-C at 2 weeks was 62.5% ($P<0.0001$) from baseline and 53.7% compared to control PCSK9 GOF mutation patients treated with placebo for 2 weeks ($P=0.0009$; primary endpoint). Changes in LDL-C levels in response to alirocumab were similar but temporally delayed by 2 weeks in group B compared to group A due to the placebo-phase study design (Figure 2A). After 8 weeks of alirocumab treatment, mean percent change in LDL-C was 73.3% ($P<0.0001$) and 12 of 13 subjects achieved an LDL-C level <70 mg/dL (Table 4). Reductions of LDL-C in the 2 groups were temporally related to reductions of free PCSK9 (Figure 2B). Pooled analysis of 8-week lipid changes in apolipoprotein B, triglycerides, very low-density lipoprotein (VLDL) cholesterol, and Lp(a) were significantly reduced (Table 4). In an exploratory analysis, we examined changes in levels of LDL-C and free PCSK9 from baseline as a function of PCSK9 GOF genotype. Alirocumab treatment resulted in marked reductions in LDL-C levels from baseline in all patients with all PCSK9 genotypes (Figure 2C). Potential differences in the rate of LDL-C reduction between the genotypes appeared to correlate with kinetics of free PCSK9 reduction (Figure 2D).

**Safety**

No patient discontinued early from the study for any reason. The most common treatment-emergent adverse events were infections and included non-serious upper and lower respiratory tract infections and gastroenteritis (Table II in the Data Supplement). No patient experienced an
elevation of hepatic enzymes or creatinine kinase 3-fold above the upper limit of normal; no trends were observed in hepatic enzymes, creatinine kinase, or fasting blood glucose over the course of the study. Five patients experienced one or more fasting blood glucose levels above 126 mg/dL during the course of the trial. All of these patients had a history of abnormal fasting blood glucose or an elevated level at screening. One subject experienced a serious adverse event of chest pain. Evidence for a myocardial infarction was not found and a follow-up stress test did not reveal cardiac ischemia.

**Discussion**

Gain of function PCSK9 mutations are a third, rare cause of ADH, but knowledge of the clinical attributes of mutation carriers and their response to therapy have heretofore been limited. In an observational study we characterized the PCSK9 GOF mutation phenotype. Compared to FH or FDB, these patients had similarly frequent physical stigmata and premature CVD, but higher LDL-C levels. Although we report evidence that these patients respond to available lipid-lowering treatments, most did not attain optimal lipid profiles on their current regimen of statins plus other lipid-lowering therapies, thus establishing the need for additional therapies. We then demonstrated in a clinical intervention trial that patients with four different PCSK9 GOF mutations achieved a marked additional reduction in LDL-C (up to 73%) after the addition of alirocumab to their current regimen, and nearly all attained the goal of 70 mg/dL. The results of this small, randomized, placebo-phase trial suggest that PCSK9 antibodies may become a specific and effective treatment for PCSK9 GOF mutation patients.

Our observational study demonstrated that PCSK9 GOF variants had mostly restricted geographical distributions, were found in a limited number of pedigrees, and exhibited significant phenotypic variability in associated disease severity. While GOF mutations were
found throughout the PCSK9 coding sequence, our study confirms that carriers of either Asp374Tyr or Ser127Arg mutations had significantly higher untreated LDL-C levels than the other PCSK9 GOF mutation carriers. This result is not unexpected given that these 2 mutations were among the first to be described, and extends the results from a smaller study suggesting that the Asp374Tyr variant may be associated with a severe form of ADH. However, because most mutations were reported in a limited number of pedigrees, in our comparison of the different variants we are unable to define the portion of the phenotype contributed by background genetics. The geographic isolation of GOF variants suggests they are likely due to private mutations in different populations, and is consistent with a relatively recent origin of many or all of them. As cascade screening was used to enrich for the presence of PCSK9 mutations, we are unable to obtain a true prevalence of GOF mutations in the general population. However, extensive efforts were undertaken to define the genetic architecture of ADH in Holland and Japan but no overlap in the variants was found, supporting the geographical isolation of these mutations.

Despite variability in disease severity of individual mutations, pooled analyses revealed significantly greater LDL-C levels in PCSK9 GOF mutation patients compared to patients with FH or FDB. FH patients are found worldwide, and LDLR variants causing FH are distributed throughout the gene (over 1700 reported) with greater disease severity associated with individual mutations. In contrast, FDB is also found worldwide, though a single APOB variant (Arg3527Gln), found primarily in northern Europeans, is responsible for the vast majority of FDB cases (>95%). For our comparison, we matched the PCSK9 GOF mutation patients with FH and FDB patients from the Dutch Familial Hypercholesterolemia Registry, the largest such resource in the world. It is possible that these patients have more or less severe disease than
patients from other parts of the world due to genetics or shared environment, and additional comparisons to other large collections of patients will be of interest. However, because this registry includes a large number of patients identified by cascade screening, it may better reflect the phenotype of patients with FH and FDB in a population-based sample than many other registries that consist mostly of index patients and their first-degree relatives. Although relative severity of these patients bear future investigation, it is clear that the severity of the PCSK9 GOF phenotype warrants maximizing lipid-lowering therapies in these patients.

In our intervention study, alirocumab administration significantly reduced LDL-C levels in all patients enrolled, and this was temporally correlated with free PCSK9 reductions (Figure 2B and 2D). The magnitude of LDL-C reduction was similar to that observed in previous studies of PCSK9 monoclonal antibodies administered to different patient populations. By utilizing a randomized placebo-phase design, each patient contributed to the safety and efficacy data while still enabling comparison of alirocumab administration to placebo. During the 2-week placebo-controlled portion of the trial, alirocumab administration also significantly reduced apolipoprotein B and triglycerides. While some difference in the baseline LDL-C levels and other characteristics was present between groups A and B (not unexpected given the small size and international design of the study), a post-hoc pooled analysis of all subjects after 8 weeks of alirocumab treatment revealed statistically significant reductions of LDL-C, apolipoprotein B, triglycerides, Lp(a), and VLDL cholesterol levels. We conclude that inhibition of PCSK9 in patients with PCSK9 GOF mutations greatly reduces LDL-C levels. While all mutation carriers responded to treatment, our results suggest that the rate of reduction in LDL-C may differ in patients carrying different GOF mutations, and this may correlate with the rate of free PCSK9 reduction after alirocumab administration, providing interesting future avenues of research into
the biochemical mechanisms of PCSK9.

Our study has limitations. While we endeavored to obtain all available PCSK9 GOF mutation carriers from a wide selection of lipid research and specialty clinics around the world, we believe that additional PCSK9 GOF mutations will be found. Furthermore, the collection of clinical information on the PCSK9 GOF mutation carriers was necessarily limited by the retrospective study design. While additional information regarding other CAD risk factors and course of lipid management would be desirable, such data may best be collected in the setting of prospective follow-up. While we found that carriers of either Asp374Tyr or Ser127Arg mutations had higher LDL cholesterol levels than carriers of other mutations as a whole, our analysis was constrained by available sample size, which may limit the generalizability of our findings. Finally, we note some clinical differences in the two randomized intervention groups, a result not surprising given the relatively small group of mutation carriers included in the intervention trial. While imbalance in baseline factors may have had some unexpected effect on lipid response at 2 weeks (the time for the placebo-controlled primary endpoint determination), the large and essentially universal change from baseline at 8 weeks make an important contribution to responses at 2 weeks less likely.

In conclusion, PCSK9 GOF mutation is characterized by a high prevalence of premature CVD and higher untreated LDL-C levels than FH and FDB. Intervention in these patients with alirocumab, a monoclonal antibody against PCSK9, was well-tolerated and resulted in marked reductions in LDL-C levels, suggesting PCSK9 antibodies may become an important targeted treatment option for these patients.

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References:


### Table 1: Summary of Clinical Data of Patients with a Familial GOF Mutation in PCSK9

<table>
<thead>
<tr>
<th>Protein (DNA)</th>
<th>Exon</th>
<th>N</th>
<th>Countries (n)</th>
<th>Total Cholesterol (mg/dL) Mean±SD (n)</th>
<th>LDL-C (mg/dL) Mean±SD (n)</th>
<th>CAD</th>
<th>Stroke</th>
<th>PVD</th>
<th>Arcus</th>
<th>Xanthoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp374Tyr (1120G&gt;T)†</td>
<td>7</td>
<td>44</td>
<td>United Kingdom (13)</td>
<td>419.6±105.2*** (42)</td>
<td>329.1±102.5*** (35)</td>
<td>13/39</td>
<td>0/10</td>
<td>1/10</td>
<td>3/22</td>
<td>14/22</td>
</tr>
<tr>
<td>Ser465Leu (1685C&gt;T)‡</td>
<td>9</td>
<td>10</td>
<td>Netherlands</td>
<td>269.9±58.8* (7)</td>
<td>186.4±56.8* (7)</td>
<td>4/10</td>
<td>0/10</td>
<td>0/10</td>
<td>-/0</td>
<td>-/0</td>
</tr>
<tr>
<td>Arg496Trp (1777C&gt;T)‡</td>
<td>9</td>
<td>9</td>
<td>Netherlands</td>
<td>300.5±48.3 (3)</td>
<td>337.6±184.5 (3)</td>
<td>0/9</td>
<td>0/4</td>
<td>0/4</td>
<td>-/0</td>
<td>-/0</td>
</tr>
<tr>
<td>All mutations</td>
<td>164</td>
<td>All countries</td>
<td>358.9±107.9 (144)</td>
<td>272.2±109.8 (116)</td>
<td>(13%)(61%)(3%)(2%)(22%)(53%)</td>
<td></td>
<td></td>
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</tbody>
</table>

CAD indicates coronary artery disease; LDL-C, low-density lipoprotein cholesterol; PVD, peripheral vascular disease; and SD, standard deviation.

*P<0.05; **P<0.01; ***P<0.001 compared the all other subjects combined.

†Val441le, Glu48Lys, Pro71Leu, Arg96Cys, Asp129Asn, Ser465Leu mutations were previously unreported.

‡LDL-C levels for 2 mutations (Glu48Lys and Phe216Leu) are provided on lipid-lowering therapy because either the patient’s medication history was unknown or the only data available were on medication. Cholesterol levels refer to untreated values. To convert values for cholesterol to mmol/L, multiply by 0.02586.
Table 2: Comparison of untreated lipid profiles (means±SD) of heterozygous patients with familial GOF mutation in PCSK9, FDB and defective and deficient LDLR mutations in FH

<table>
<thead>
<tr>
<th></th>
<th>PCSK9 GOF Mutation (n)</th>
<th>FDB (n=470)</th>
<th>All FH (n=2126)</th>
<th>Defective LDLR (n=1398)</th>
<th>Deficient LDLR (n=728)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.7±18.6 (135)</td>
<td>32.1±16.9</td>
<td>28.1±16.5</td>
<td>29.2±16.4</td>
<td>26.1±16.5</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>351.9±104.4 (134)</td>
<td>254.8±50.7***</td>
<td>290.0±82.8***</td>
<td>277.3±74.2***</td>
<td>314.8±92.8</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>266.8±108.3 (108)</td>
<td>184.8±43.3***</td>
<td>219.6±76.6***</td>
<td>206.5±67.3***</td>
<td>245.2±86.2</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>54.2±27.1 (108)</td>
<td>48.7±16.2</td>
<td>46.4±14.3**</td>
<td>46.8±15.1**</td>
<td>45.2±13.1***</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>150.6±115.1 (108)</td>
<td>111.6±65.5**</td>
<td>121.3±76.2</td>
<td>122.2±77.1*</td>
<td>120.5±75.3</td>
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</tbody>
</table>

To convert cholesterol to mmol/L, multiply by 0.02586. To convert triglycerides to mmol/L, multiply by 0.01129.
HDL-C indicates high-density lipoprotein cholesterol; FDB, familial defective apolipoprotein B; FH, familial hypercholesterolemia; GOF, gain-of-function; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; and SD, standard deviation.

*P<0.05; **P<0.01; ***P<0.001 when compared to PCSK9 GOF mutation carriers. The 11 patients who were double heterozygotes for mutations in PCSK9 and LDLR were excluded from the analysis.
Table 3: Baseline Characteristics of Patients with Familial GOF Mutation in PCSK9 in the Randomized Alirocumab 150 mg Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group A (n=6)</th>
<th>Group B (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42.3±14.7</td>
<td>46.6±13.3</td>
</tr>
<tr>
<td>Race (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Indian Ocean Islander</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sex (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.5±6.6</td>
<td>30.4±6.7</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>97.7±10.8</td>
<td>107.6±17.8</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>5.45±0.40</td>
<td>6.14±0.55</td>
</tr>
<tr>
<td>Prior history of diabetes mellitus</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Prior history of glucose intolerance</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PCSK9 GOF mutation (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp374Tyr</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Ser127Arg</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Leu108Arg</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Arg218Ser</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Lipid-lowering therapy (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statin</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Niacin</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Fibrate</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Bile acid sequestrant</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>History of cardiovascular disease (n)</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

Continuous variables are shown as mean ±SD.
BMI indicates body mass index; GOF, gain-of-function; and SD, standard deviation.
Table 4: Lipid Parameters in Patients in the Randomized Study at Baseline, at Week 2, and After 8 weeks of Alirocumab 150 mg Treatment

There were 6 Participants Randomized to Group A and 7 Randomized to Group B for 13 Total Combined Participants

<table>
<thead>
<tr>
<th>Lipid parameter</th>
<th>Baseline Group A (n=6)</th>
<th>Baseline Group B (n=7)</th>
<th>Study Week 2 Group A</th>
<th>Study Week 2 Group B</th>
<th>P-value Combined (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C (measured, mg/dL)</td>
<td>108.8±33.8</td>
<td>144.3±68.4</td>
<td>45.2±42.0</td>
<td>126.3±43.2</td>
<td>32.3±21.4</td>
</tr>
<tr>
<td>% change from baseline</td>
<td>-62.5±8.2</td>
<td>-8.8±7.6</td>
<td>-73.3±16.1 (&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% difference group A vs B*</td>
<td>-53.7±11.5</td>
<td>0.0009</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>57.2±19.4</td>
<td>50.4±14.7</td>
<td>58.7±22.6</td>
<td>47.0±15.1</td>
<td>55.8±18.0</td>
</tr>
<tr>
<td>% change from baseline</td>
<td>1.0±4.6</td>
<td>-6.1±4.3</td>
<td>7.9±13.7 (0.0603)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% difference group A vs B</td>
<td>7.2±6.4</td>
<td>0.2864</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dL, median [IQR])</td>
<td>84.5 (61.0:112.0)</td>
<td>144.0 (66.0:170.0)</td>
<td>55.0 (41.0:76.0)</td>
<td>167.0 (72.0:199.0)</td>
<td>64.0 (42:86)</td>
</tr>
<tr>
<td>% change from baseline</td>
<td>-27.9 (-33.3:-6.1)</td>
<td>12.9 (-27:29:7)</td>
<td>-37.8 (-46:27) (0.0002)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% difference group A vs B</td>
<td>-40.8</td>
<td>0.0461</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-C (measured, mg/dL)</td>
<td>22.8±18.8</td>
<td>28.9±15.0</td>
<td>19.2±20.7</td>
<td>29.3±12.9</td>
<td>14.4±8.1</td>
</tr>
<tr>
<td>% change from baseline</td>
<td>-23.8±10.1</td>
<td>6.7±9.3</td>
<td>-39.5±17.5 (&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% difference group A vs B</td>
<td>-30.5±13.9</td>
<td>0.0526</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo B-100 (mg/dL)</td>
<td>89.2±27.3</td>
<td>101.0±15.8</td>
<td>42.8±43.4</td>
<td>99.4±16.5</td>
<td>32.4±15.3</td>
</tr>
<tr>
<td>% change from baseline</td>
<td>-55.3±8.7</td>
<td>-3.8±8.0</td>
<td>-65.0±16.6 (&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% difference group A vs B</td>
<td>-49.6±12.1</td>
<td>0.0021</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lp(a) (mg/dL, median [IQR])</td>
<td>56.6 (34.4:69.1)</td>
<td>19.4 (10.0:56.1)</td>
<td>37.2 (16.6:77.4)</td>
<td>13.0 (9.0:57.6)</td>
<td>11.9 (4:51)</td>
</tr>
<tr>
<td>% change from baseline</td>
<td>-21.0</td>
<td>0.0 (-10:3.0)</td>
<td>-43.3 (-65:4) (0.0020)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% difference group A vs B</td>
<td>-21.0</td>
<td>0.1317</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For week 2 results, baseline refers to values obtain just prior to the first dosing of alirocumab (group A) or placebo (group B) and week 2 is the patients’ nominal week 2 visit. For week 8 results, the 2w groups are combined and baseline refers to blood drawn just prior to the first dosing of alirocumab; for group A, the baseline remains as before, but for group B, a 2-week shift is adjusted so the nominal week 2 value becomes baseline and the nominal week 10 value becomes the week 8 value.

To convert cholesterol to mmol/L, multiply by 0.02586. To convert triglycerides to mmol/L, multiply by 0.11129. To convert Lp(a) to μmol/L, multiply by 0.0357.

Apo indicates apolipoprotein; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); SD, standard deviation; SE, standard error; and VLDL-C, very low-density lipoprotein cholesterol.

*Primary endpoint. All lipid values are shown as mg/dL. Mean ±SD are given for lipid parameters at baseline, 2 weeks, and 8 weeks as well as percent changes from baseline. Least-square means ±SE are given for differences between group A and B at 2 weeks. Significance of 8-week changes from baseline were tested with 2-sided paired t-tests. Median % change from baseline and IQR (Q1:Q3) are shown for triglycerides and Lp(a).
Figure Legends:

Figure 1: Distribution of untreated LDL-C for patients with familial gain-of-function mutations in PCSK9 without LDLR mutations (A), and position of the mutations and the 12 exons of the PCSK9 gene relative to the protein domains (B)

\(^\dagger\)P-value indicates reduction for mutation versus overall mean.

\(^\ddagger\)P-value indicates increase for mutation versus overall mean. Dotted line represents mean LDL-C level of all PCSK9 mutation carriers from whom untreated LDL-C levels were available.

1.1 mmol/L = 70 mg/dL; 2.59 mmol/L = 100 mg/dL

LDL-C indicates low-density lipoprotein cholesterol; SD, standard deviation.

Figure 2: Change in LDL-C and free PCSK9 for patients with familial GOF mutation in PCSK9 in the randomized alirocumab study. (A) Mean (±SE) LDL-C values and (B) mean (±SE) percent change from baseline in free plasma PCSK9 are shown by study group together with an indication of the dosing schedules. (C) Mean (+SE) percent change from baseline in LDL-C and (D) free plasma PCSK9 are shown by PCSK9 gain of function mutation. In panels (C) and (D), results from groups A and B were combined by shifting group A visits forward 2 weeks, thereby aligning the dosing schedule in the 2 groups.

LDL-C indicates low-density lipoprotein cholesterol; and SE, standard error.
Characterization of Autosomal Dominant Hypercholesterolemia Caused by PCSK9 Gain of Function Mutations and its Specific Treatment with Alirocumab, a PCSK9 Monoclonal Antibody

Paul N. Hopkins, Joep Defesche, Sigrid W. Fouchier, Eric Bruckert, Gérald Luc, Bertrand Cariou, Barbara Sjouke, Trond P. Leren, Mariko Harada-Shiba, Hiroshi Mabuchi, Jean-Pierre Rabès, Alain Carrié, Charles van Heyningen, Valérie Carreau, Michel Farnier, Yee P. Teoh, Mafalda Bourbon, Masa-aki Kawashiri, Atsushi Nohara, Handrean Soran, A. David Marais, Hayato Tada, Marianne Abifadel, Catherine Boileau, Bernard Chanu, Shoji Katsuda, Ichiro Kishimoto, Gilles Lambert, Hisashi Makino, Yoshihiro Miyamoto, Matthieu Pichelin, Kunimasa Yagi, Masakazu Yamagishi, Yassine Zair, Scott Mellis, George D. Yancopoulos, Neil Stahl, Johanna Mendoza, Yunling Du, Sara Hamon, Michel Krempf and Gary D. Swergold

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Supplemental Methods

Enrollment Criteria in the Treatment Study

Inclusion Criteria
A patient must meet the following criteria to be eligible for inclusion in the study:

1. Man or woman between the ages of 18 and 70 years, inclusive
2. A history of molecularly confirmed PCSK9 GOFm
3. Plasma LDL-C levels ≥70 mg/dL (x 0.0259 mmol/L) at the screening visit (visit 1 [day -28 to -15]) on an LLT regimen stable for at least 28 days; LDL-C must be considered to be not at goal by the investigator
   LLT regimen may include, but is not limited to:
   - Statins
   - Ezetimibe
   - Fibrates
   - Niacin
   - Omega-3 fatty acids
   - Bile acid resins
   - Red yeast rice
4. Body mass index ≥18.0 and ≤40.0 kg/m\(^2\) at the screening visit (visit 1 [day -28 to -15])
5. Systolic blood pressure (BP) ≤150 mm Hg and diastolic BP ≤95 mm Hg at the screening visit (visit 1 [day -28 to -15])
6. Willing to refrain from the consumption of no more than 2 standard alcoholic drinks in any 24-hour period for the duration of the study. A standard alcoholic drink is the equivalent of 12 ounces beer, 5 ounces of wine, or 1.5 ounces of hard liquor
7. Willing to refrain from the consumption of alcohol for 24 hours before each study visit
8. Willing to maintain their usual stable diet and exercise regimen throughout the study
9. Willing and able to comply with clinic visits and study-related procedures
10. Provide signed informed consent
Note: A patient who is out of the specified time window criterion for 1 or more inclusion or exclusion criteria may be re-screened once they fall within the required time window.

Exclusion Criteria
A patient who meets any of the following criteria will be excluded from the study:
1. Serum TG >350 mg/dL (x 0.01129 mmol/L) at the screening visit (visit 1 [day -28 to day -15]) measured after an 8 to 12 hour fast

2. History of heart failure (New York Heart Association Class II-IV) within the 12 months before the screening visit (visit 1 [day -28 to -15])

3. History of myocardial infarction, acute coronary syndrome (ACS), unstable angina pectoris, stroke, peripheral vascular disease, transient ischemic attack, or cardiac revascularization within the 6 months before the screening visit (visit 1 [day -28 to -15])

4. History of uncontrolled, clinically significant cardiac dysrhythmias or clinically significant recent changes in ECG 6 months before the screening visit (visit 1 [day -28 to -15])

5. Known history of active optic nerve disease

6. History of undergoing LDL apheresis within 3 months before the screening visit (visit 1 [day -28 to -15])

7. Uncontrolled diabetes mellitus with hemoglobin A1C (HbA1c) >8.5% at the screening visit (visit 1 [day -28 to -15])

8. Thyroid stimulating hormone (TSH) >1.5 x upper limit of normal (ULN) at the screening visit (visit 1 [day -28 to -15])

9. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2 x ULN at the full screening visit (visit 1 [day -28 to -15]) (1 repeat lab is allowed)

10. Creatine phosphokinase (CPK) >3 x ULN at the screening visit (visit 1 [day -28 to -15]) (1 repeat lab is allowed)

11. Known sensitivity to monoclonal antibody therapeutics

12. Participation in a clinical research study evaluating an investigational drug within 30 days, or at least 5 half-lives of the investigational drug, before the screening visit (visit 1 [day -28 to -15]), whichever is longer

13. Known to be positive for human immunodeficiency virus (HIV), hepatitis B virus, or hepatitis C virus

14. History of malignancy of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years, regardless of whether there is evidence of local recurrence or metastases

15. Pregnant or breast-feeding women

16. Sexually active man* or woman of childbearing potential** who is unwilling to practice adequate contraception during the study (adequate contraceptive measures include stable use of oral contraceptives or other prescription pharmaceutical contraceptives for 2 or more menstrual cycles prior to screening; intrauterine device; bilateral tubal ligation; vasectomy; condom plus contraceptive sponge, foam, or jelly, or diaphragm plus contraceptive sponge, foam, or jelly)

17. Any medical or psychiatric condition which, in the opinion of the investigator, would place the patient at risk, interfere with patient’s participation in the study or interfere with the interpretation of the study results.
Contraception is not required for men with documented vasectomy.

**Postmenopausal women must be amenorrheic for at least 12 months in order not to be considered of childbearing potential. Pregnancy testing and contraception are not required for women with documented hysterectomy or tubal ligation.

### Schedule of Events in the Treatment Study

<table>
<thead>
<tr>
<th>Visit Number</th>
<th>SV 1</th>
<th>V2</th>
<th>V 3</th>
<th>PV 4</th>
<th>V 5</th>
<th>PV 5</th>
<th>V 6</th>
<th>V 7</th>
<th>V 8</th>
<th>V 9</th>
<th>V10</th>
<th>V11/EOT</th>
<th>V12/EOT</th>
<th>V13</th>
<th>V 14</th>
<th>V15/EOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day (visit window)</td>
<td>-28 ±1</td>
<td>-14 ±1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>9</td>
<td>43</td>
<td>57</td>
<td>71</td>
<td>85</td>
<td>99</td>
<td>113 ±7</td>
<td>12 ±7</td>
<td>14 ±7</td>
</tr>
</tbody>
</table>

**Screening/baseline:**
- Informed consent & Inclusion/Exclusion
- Medical history
- Demographics & Height
- Randomization

**Treatment:**
- Administer study drug
- Concomitant Medications & procedures
- Query LLT duration and dosing
- Query LLT compliance

**Safety:**
- Weight
- Vital signs
- Physical examination
- Electrocardiogram
- Adverse events

**Laboratory Testing:**
- Hematology
- Chemisty
- Troponin
- Lipid panel
- hs-CRP
- Urinalysis
- HbA1c, Hepatitis B & C serology
- Sensitive TSH
- Pregnancy Test
- Research samples (biomarker)
- PCSK9 levels
- Required DNA sample for PCSK9 genotyping
### Procedures in the Treatment Study

Fasting blood samples (after an 8 to 12-hour fast) were collected at each clinic visit starting with the screening visit (visit 1 [day -28 to -15]). Planned lipid assessments included LDL-C measured by ultracentrifugation and estimated by Friedewald equation, total cholesterol, non-HDL-C, ApoB100, HDL-C, ApoA1, Lp(a), and triglycerides.

The treatment study protocol called for measurement of low-density lipoprotein cholesterol (LDL-C) by ultracentrifugation. Instead, due to a laboratory error, LDL-C was measured from serum samples using LDL-C plus second-generation reagents manufactured by Roche Diagnostics, Indianapolis, IN, USA.

### Further Statistical Details

For the observational study, no adjustments were made for multiple testing.
In the treatment study, the alpha level of the primary comparisons (group A compared to group B) of the primary and key secondary efficacy endpoints was controlled by the hierarchical testing procedure.
## Supplemental Table I. Clinical Characteristics of Patients with Familial Gain-of-Function Mutation in PCSK9.

<table>
<thead>
<tr>
<th></th>
<th>Current Study</th>
<th>Published Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCSK9 GOF Mutation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=89)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>33% (n=126)</td>
<td>33% (n=1940)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37% (n=516)</td>
</tr>
<tr>
<td>Age of onset (mean ±SD)</td>
<td>49.4±13.8</td>
<td>48.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>46.6±9.8</td>
</tr>
<tr>
<td>Peripheral</td>
<td>2% (n=96)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21% (n=516)</td>
</tr>
<tr>
<td>Age of onset</td>
<td>62</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Cerebrovascular</td>
<td>6% (n=98)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Age of onset (mean ±SD)</td>
<td>60.0±8.4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Physical stigmata of elevated cholesterol</td>
<td>FH³</td>
<td>FDB⁴</td>
</tr>
<tr>
<td>Xanthoma</td>
<td>53%</td>
<td>44%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36%</td>
</tr>
<tr>
<td>Xanthelasmata</td>
<td>15%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Arcus lipoides corneae</td>
<td>22%</td>
<td>31%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38%</td>
</tr>
</tbody>
</table>

A dash (·) indicates that information is unavailable.

CAD indicates coronary artery disease; FDB, familial defective apolipoprotein B; FH, familial hypercholesterolemia; GOF, gain-of-function; SD, standard deviation.
Supplemental Table II. Adverse Events in Patients with Familial Gain-of-Function Mutation in PCSK9 in the Randomized Alirocumab 150 mg Study

<table>
<thead>
<tr>
<th>Event</th>
<th>n out of 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any TEAE</td>
<td>11</td>
</tr>
<tr>
<td>Discontinuations due to TEAEs</td>
<td>0</td>
</tr>
<tr>
<td>Infections (URI, LRI, gastroenteritis)</td>
<td>7</td>
</tr>
<tr>
<td>Nervous system (headache, peripheral neuropathy (1), sciatica)</td>
<td>5</td>
</tr>
<tr>
<td>Respiratory (sore throat, cough)</td>
<td>2</td>
</tr>
<tr>
<td>Gastrointestinal (abdominal pain, cankers, constipation, haemorrhoids)</td>
<td>3</td>
</tr>
<tr>
<td>Musculoskeletal (back pain, arthralgia (1), muscle spasm or pain)</td>
<td>3</td>
</tr>
<tr>
<td>General (chest pain)</td>
<td>1</td>
</tr>
<tr>
<td>Patients with at least 1 double-blind TEAE related to study drug</td>
<td>3</td>
</tr>
</tbody>
</table>

Laboratory values

<table>
<thead>
<tr>
<th>Test</th>
<th>n out of 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST or ALT ≥3 times upper limit at any time</td>
<td>0</td>
</tr>
<tr>
<td>Creatine kinase ≥3 times upper limit at any time</td>
<td>0</td>
</tr>
<tr>
<td>Glucose ≥200 mg/dl (11.1 mmol/L) non-fasting, or ≥126 mg/dL (7.0 mmol/L) fasting at any time</td>
<td>5*</td>
</tr>
</tbody>
</table>

*Three patients had a prior history of diabetes mellitus, 1 had a history of glucose intolerance, and 1 had elevated serum glucose (above laboratory upper limit of normal) at baseline.

No apparent trends in other laboratory values or vital signs such as blood pressure or heart rate were reported.

One serious adverse event of chest pain with left bundle branch block was reported. Cardiac workup was negative and event was considered non-related by the investigator.

Low titer treatment-emergent ADAs were found in 3 of 13 patients (23%). There was no apparent impact of ADA on systemic concentrations of alirocumab.
ADA indicates anti-drug antibodies; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LRI, lower respiratory tract infection; TEAE, treatment-emergent adverse event; URI, upper respiratory tract infection.

**Supplemental Figure I.** Design of the randomized alirocumab 150 mg Q2W study
Supplemental Figure II. Number of individuals with each *PSCK9* mutation specifically from each country and number of pedigrees

Min indicates the minimum number of pedigrees (some individuals did not have pedigree indicated).

Mutations previously unreported: Val4Ile, Glu48Lys, Pro71Leu, Arg96Cys, Asp129Asn, Ser465Leu.

*LDLRm* indicates low-density lipoprotein receptor mutation; *P*, pedigree.
Supplemental Figure III. Reported medication use (A), and proportion of patients not reaching LDL-C targets (baseline and on-treatment LDL-C, n=63) (B) in patients with familial gain-of-function mutation in PCSK9 alone (without LDLR mutations)

A.
B.

1.81 mmol/l = 70 mg/dl; 2.59 mmol/l = 100 mg/dl

LDL-C indicates low-density lipoprotein cholesterol
Supplemental Figure IV. Patient flow (CONSORT) in the randomized alirocumab 150 mg study

**Enrollment**
- Assessed for eligibility (n=14)
- Excluded (n=1)
  - 1 patient did not meet inclusion criteria
- Randomized (n=13)

**Allocation**
- Allocated to Group A (n=6)
  - Received allocated intervention (n=6)
- Allocated to Group B (n=7)
  - Received allocated intervention (n=7)

**Follow-up**
- Completed follow-up (n=6)
- Completed follow-up (n=7)

**Analysis**
- Analyzed (n=6)
- Analyzed (n=7)
Supplemental References


