SUPPLEMENTAL MATERIAL

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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 revascularication 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

			Treatment Period											Follow- up		
Visit Number	SV 1	V2	V 3	PV 4[a]	V 5	PV 5[a]	V 6	V 7	V 8	V 9	V10	V11/ EOT	V 12/E T	V 13	V 14	V15/ EOS
Day (visit window)	-28 to -15	-14 ±1	1	4 ±1	15 ± 3		29 ± 3	43 ±3	57 ±3	71 ±3	85 ±3	99 ±3	113 ±7	127 ±7	141 ±7	155 ±7
Screening/baseline:																
Informed consent & Inclusion/Exclusion	X															
Medical history	X															
Demographics & Height	X															
Randomization			X													
Treatment:																
Administer study drug[b]		X	X		X		X	X	X	X	X	X				
Concomitant Medications & procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Query LLT duration and dosing	X															
Query LLT compliance		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety:																
Weight	X		X													X
Vital signs	X	X	X		X		X	X	X	X	X	X	X	X	X	X
Physical examination	X												X			X
Electrocardiogram	X							X			X		X			X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Laboratory Testing[c]:																
Hematology	X		X		X		X	X	X	X	X	X	X			X
Chemistry	X	X	X		X		X	X	X	X	X	X	X			X
Troponin	X		X		X		X	X	X	X	X	X	X	X	X	X
Lipid panel	X	X	X		X		X	X	X	X	X	X	X	X	X	X
hs-CRP		X	X		X			X		X		X		X		
Urinalysis	X				X											X
HbA1c, Hepatitis B & C serology	X															
Sensitive TSH	X															
Pregnancy Test[d]	S		U				U		U		U		U			U
Research samples (biomarker)	X		X		X					X		X				
PCSK9 levels	X	X	X		X		X	X	X	X	X	X	X	X	X	X
Required DNA sample for PCSK9 genotyping[e]	X															

Visit Number	SV 1	V2	Treatment Period											Follow- up		
			V 3	PV 4[a]	V 5	PV 5[a]	V 6	V 7	V 8	V 9	V10	V11/ EOT	V 12/E T	V 13	V 14	V15/ EOS
Day (visit window)	-28 to -15	-14 ±1	1	4 ±1	15 ± 3		29 ± 3	43 ±3	57 ±3	71 ±3	85 ±3	99 ±3	113 ±7	127 ±7	141 ±7	155 ±7
Optional DNA consent			X													
Optional DNA sample[f]			X													
Alirocumab PK sample			X		X		X	X	X	X	X	X	X	X	X	X
Anti-alirocumab antibody sample	X		X				X		X				X			X

LLT = lipid-lowering therapy; SV1 = screening visit 1; PV4 = phone visit 4; PV5 = phone visit 5; EOT = end-of-treatment; ET = early termination; TSH = thyroid stimulating hormone

[a] The study site will contact patients by telephone on visit 4 (PV4)/day 4 and phone visit 5 (PV5) to assess AEs. Phone visit 5 (PV5) will occur 3 (±1) days after the previous visit (visit 5).

[b] Patients will be monitored at the clinical site for at least 30 minutes following each study drug injection.

[c] All laboratory samples will be collected before the injection of study drug at dosing visits.

[d] S = serum and U = urine

[e] Patients will be required to provide written informed consent for the collection of their DNA sample for PCSK9 genotyping. The blood sample for DNA extraction should be collected at the screening visit.

[f] For patients who provide written informed consent for the optional DNA sample collection, the DNA sample should be collected on day 1 (baseline), but can be collected at any visit during the study.

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*.

	Current Study	Published Literature	
	***PCSK9* GOF Mutation (n=89)**	**FH[1]**	**FDB[2]**
Cardiovascular events			
CAD	33% (n=126)	33% (n=1940)	37% (n=516)
Age of onset (mean ±SD)	49·4±13·8	48·2	46·6±9·8
Peripheral	2% (n=96)	-	21% (n=516)
Age of onset	62	-	-
Cerebrovascular	6% (n=98)	-	-
Age of onset (mean ±SD)	60·0±8·4	-	-
Physical stigmata of elevated cholesterol		FH[3]	FDB[4]
Xanthoma	53%	44%	36%
Xanthelasmata	15%	-	-
Arcus lipoides corneae	22%	31%	38%

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

Event	n out of 13
Any TEAE	11
Discontinuations due to TEAEs	0
Infections (URI, LRI, gastroenteritis)	7
Nervous system (headache, peripheral neuropathy (1), sciatica)	5
Respiratory (sore throat, cough)	2
Gastrointestinal (abdominal pain, cankers, constipation, haemorrhoids)	3
Musculoskeletal (back pain, arthralgia (1), muscle spasm or pain)	3
General (chest pain)	1
Patients with at least 1 double-blind TEAE related to study drug	3
Laboratory values	
AST or ALT ≥3 times upper limit at any time	0
Creatine kinase ≥3 times upper limit at any time	0
Glucose ≥200 mg/dl (11·1 mmol/L) non-fasting, or ≥126 mg/dL (7·0 mmol/L) fasting at any time	5*

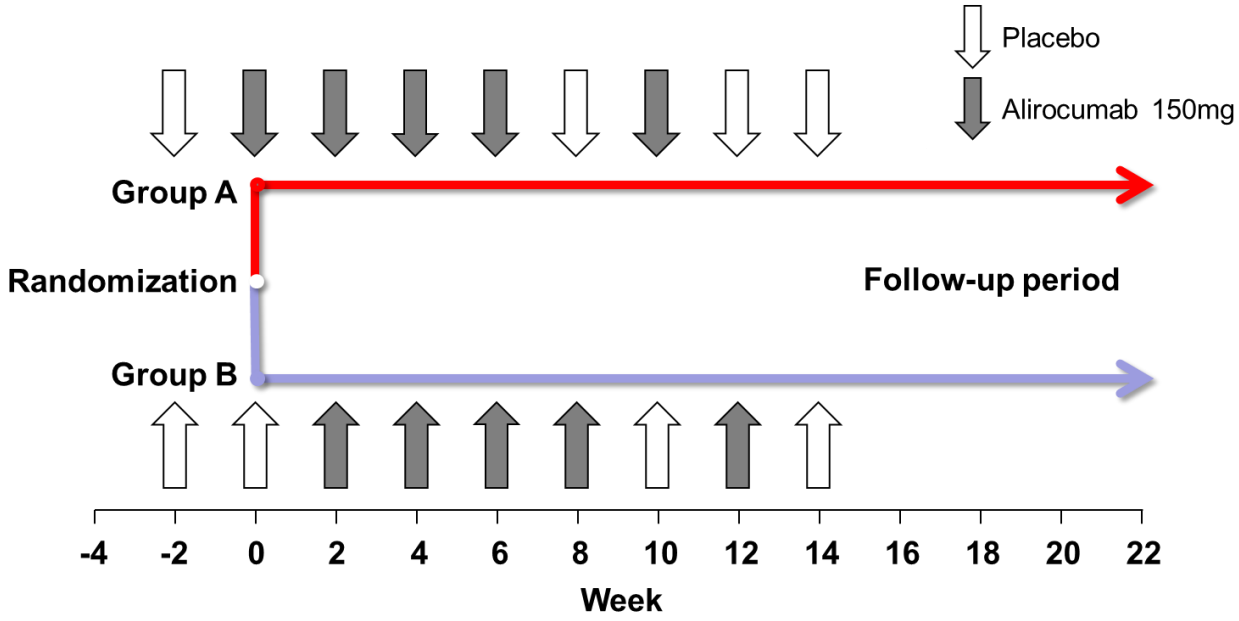
*Three patients had a prior history of diabetes mellitus, 1 had a history of glucose intolerance, and 1 had elevated serum glucose (above laboratory upper limit of normal) at baseline.

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.

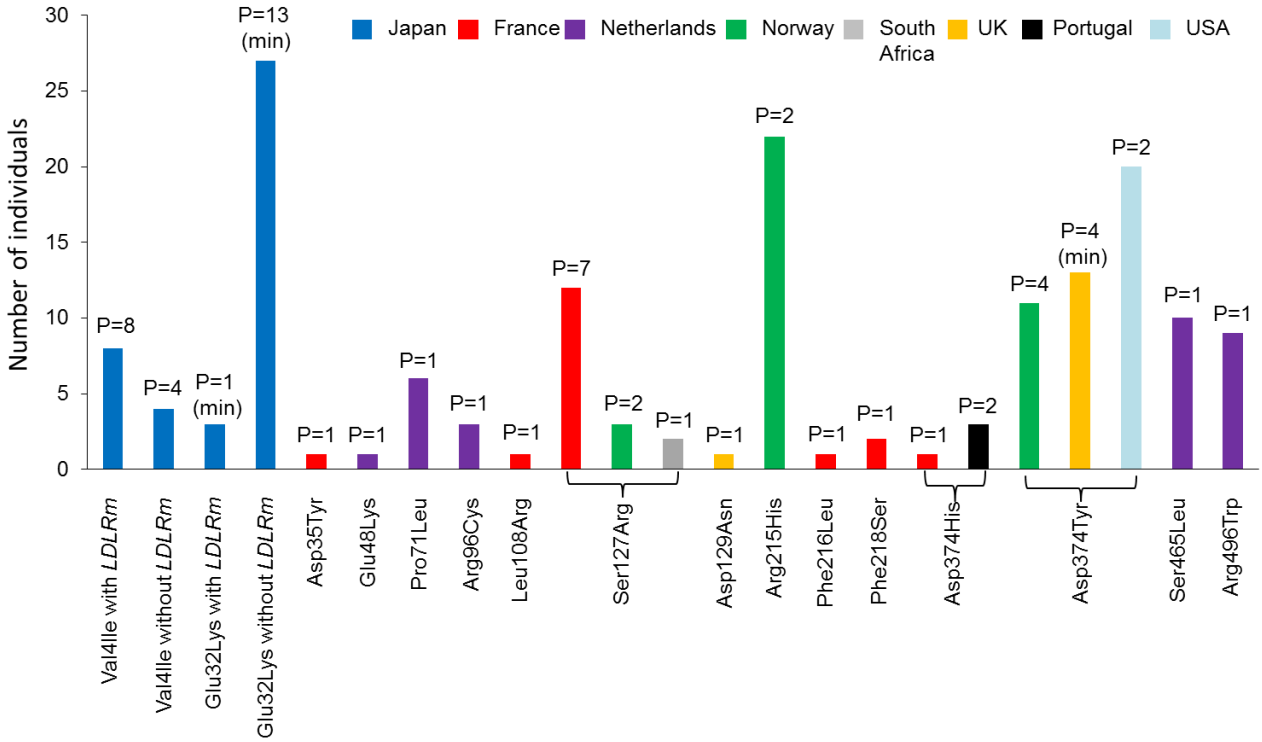
Low titer treatment-emergent ADAs were found in 3 of 13 patients (23%). There was no apparent impact of ADA on systemic concentrations of alirocumab.

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



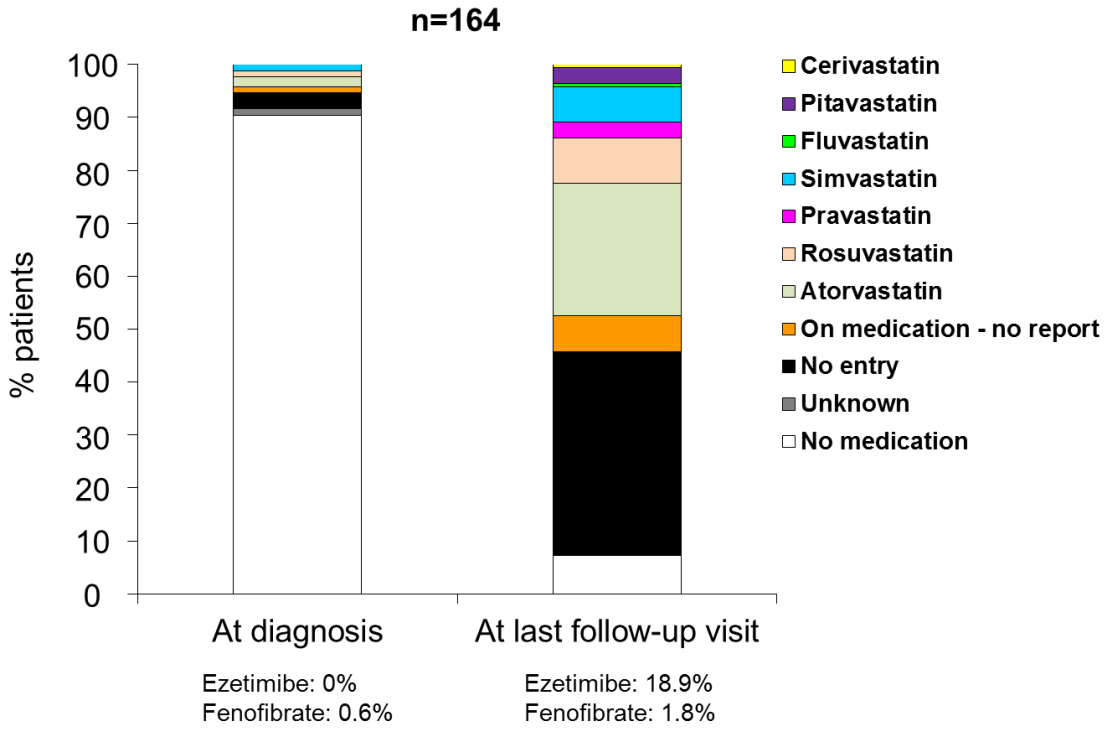
Min indicates the minimum number of pedigrees (some individuals did not have pedigree indicated).

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

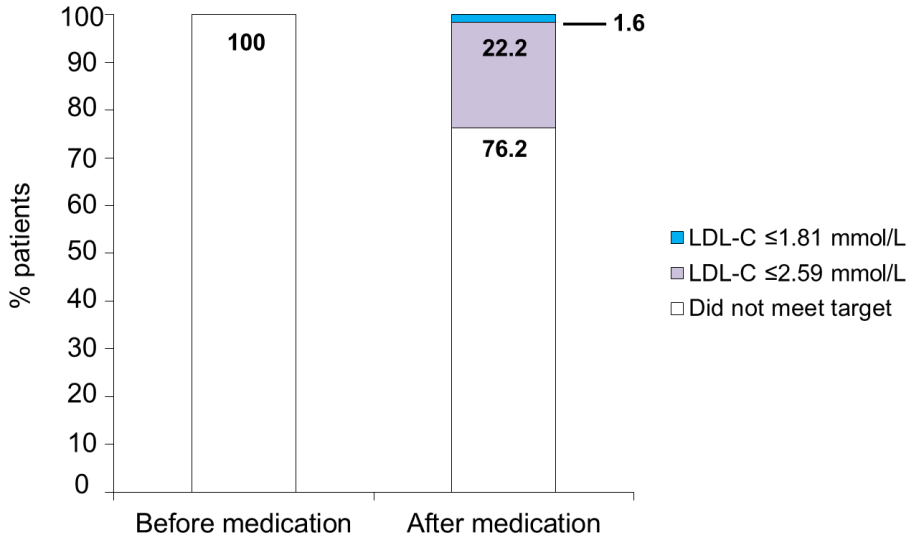
LDLRm indicates low-density lipoprotein receptor mutation; P, pedigree.

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.



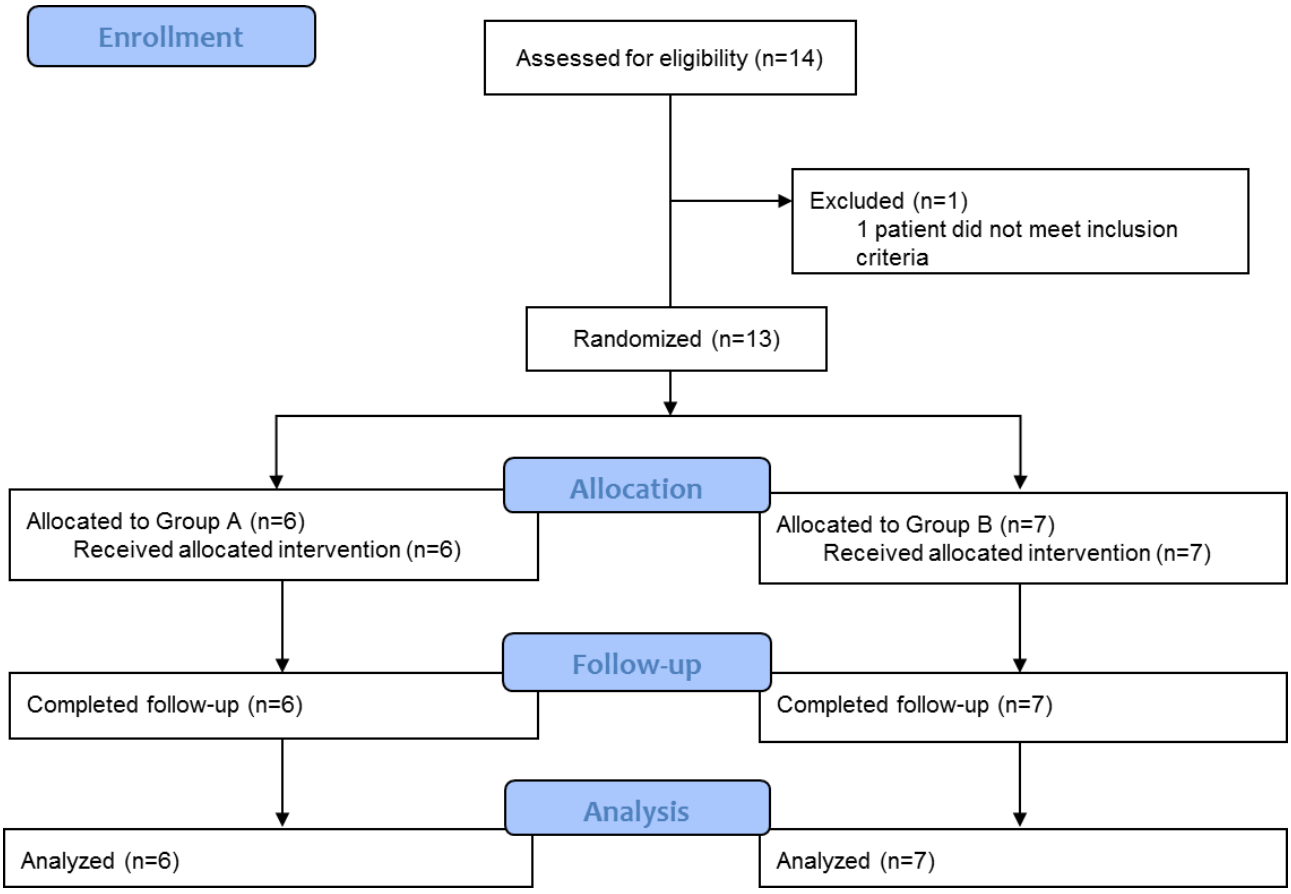
B.



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



Supplemental References

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2. Fouchier SW, Defesche JC, Kastelein JJ, Sijbrands EJ. Familial defective apolipoprotein B versus familial hypercholesterolemia: an assessment of risk. *Semin Vasc Med.* 2004;4:259–264. doi:10.1055/s-2004-861493.

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