Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial

Kevin Fitzgerald, Maria Frank-Kamenetsky, Svetlana Shulga-Morskaya, Abigail Liebow, Brian R Bettencourt, Jessica E Sutherland, Renta M Hutabarat, Valerie A Clausen, Verena Karsten, Jeffrey Cehelsky, Saraswathy V Nochur, Victor Kotelianski, Jay Horton, Timothy Mant, Joseph Chiesa, James Ritter, Malathy Munisamy, Akshay K Vaishnaw, Jared A Gollob, Amy Simon

Summary

Background Proprotein convertase subtilisin/kexin type 9 (PCSK9) binds to LDL receptors, leading to their degradation. Genetics studies have shown that loss-of-function mutations in *PCSK9* result in reduced plasma LDL cholesterol and decreased risk of coronary heart disease. We aimed to investigate the safety and efficacy of ALN-PCS, a small interfering RNA that inhibits PCSK9 synthesis, in healthy volunteers with raised cholesterol who were not on lipid-lowering treatment.

Methods We did a randomised, single-blind, placebo-controlled, phase 1 dose-escalation study in healthy adult volunteers with serum LDL cholesterol of 3·00 mmol/L or higher. Participants were randomly assigned in a 3:1 ratio by computer algorithm to receive one dose of intravenous ALN-PCS (with doses ranging from 0·015 to 0·400 mg/kg) or placebo. The primary endpoint was safety and tolerability of ALN-PCS. Secondary endpoints were the pharmacokinetic characteristics of ALN-PCS and its pharmacodynamic effects on PCSK9 and LDL cholesterol. Study participants were masked to treatment assignment. Analysis was per protocol and we used ANCOVA to analyse pharmacodynamic endpoint data. This trial is registered with ClinicalTrials.gov, number NCT01437059.

Findings Of 32 participants, 24 were randomly allocated to receive a single dose of ALN-PCS (0·015 mg/kg [n=3], 0·045 mg/kg [n=3], 0·090 mg/kg [n=3], 0·150 mg/kg [n=3], 0·250 mg/kg [n=6], or 0·400 mg/kg [n=6]) and eight to placebo. The proportions of patients affected by treatment-emergent adverse events were similar in the ALN-PCS and placebo groups (19 [79%] *vs* seven [88%]). ALN-PCS was rapidly distributed, with peak concentration and area under the curve (0 to last measurement) increasing in a roughly dose-proportional way across the dose range tested. In the group given 0·400 mg/kg of ALN-PCS, treatment resulted in a mean 70% reduction in circulating PCSK9 plasma protein (p<0·0001) and a mean 40% reduction in LDL cholesterol from baseline relative to placebo (p<0·0001).

Interpretation Our results suggest that inhibition of PCSK9 synthesis by RNA interference (RNAi) provides a potentially safe mechanism to reduce LDL cholesterol concentration in healthy individuals with raised cholesterol. These results support the further assessment of ALN-PCS in patients with hypercholesterolaemia, including those being treated with statins. This study is the first to show an RNAi drug being used to affect a clinically validated endpoint (ie, LDL cholesterol) in human beings.

Funding Alnylam Pharmaceuticals.

Introduction

LDL cholesterol is one of the major risk factors for coronary heart disease, with a continuous and graded association between its plasma concentration and risk—for every 0·78 mmol/L (30 mg/dL) change in LDL cholesterol, the relative risk for coronary heart disease changes by roughly 30%.[1,2] Additionally, in a large meta-analysis[3] of 21 statin studies, the investigators concluded that for every 1·01 mmol/L (39 mg/dL) reduction in LDL cholesterol with statin treatment, cardiovascular events were reduced by about 22%.[3] Despite the extensive use of statins, existing treatments for the management of raised

LDL cholesterol remain inadequate. This is especially true for individuals with pre-existing coronary heart disease or diabetes, who are at the highest risk and require the most aggressive management of hyper-cholesterolaemia.[4] Among high-risk individuals, it is estimated that only 50% achieve the target LDL cholesterol of less than 2·59 mmol/L at 6 months after statin treatment, despite close monitoring and optimi-sation of the drug regimen.[5–9] With the LDL cholesterol target of less than 1·81 mmol/L in high-risk individuals, the number who reach their LDL cholesterol goals is even lower at 30%.[9,10] Thus, a clear unmet medical need

Alnylam Pharmaceuticals, Cambridge, MA, USA
(K Fitzgerald PhD,
M Frank-Kamenetsky PhD,
S Shulga-Morskaya MSc,
A Liebow BS,
B R Bettencourt PhD,
J E Sutherland PhD,
R M Hutabarat PhD,
V A Clausen PhD, V Karsten PhD,
J Cehelsky MBA, S V Nochur PhD,
V Kotelianski MD,
A K Vaishnaw MD, J A Gollob MD,
A Simon MD); **Internal Medicine and Molecular Genetics, University of Texas South Western, Dallas, TX, USA**
(J Horton MD); **Quintiles Drug Research Unit at Guy's Hospital, London, UK**
(T Mant MD, J Ritter MD); **and Covance Clinical Research Unit, Leeds, UK** (J Chiesa MD,
M Munisamy MD)

Correspondence to:
Dr Kevin Fitzgerald, Alnylam
Pharmaceuticals, Cambridge,
MA 02446, USA
kfitzgerald@alnylam.com

exists for hypercholesterolaemia treatments, especially in high-risk patient populations.

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a member of the serine protease family and was first connected to cholesterol metabolism when gain-of-function mutations in *PCSK9* were identified in people with familial hypercholesterolaemia who did not have mutations in the LDL receptor (*LDLR*) or apolipoprotein B (*APOB*) genes.[11] Animal studies subsequently determined that PCSK9 binds hepatocyte LDLRs both intracellularly and extracellularly, leading to their lysosomal degradation (figure 1).[12,13] Loss-of-function mutations in *PCSK9* have also been described in human beings, and are associated with reductions in LDL cholesterol and risk of coronary heart disease.[14,15] Several individuals with no circulating PCSK9 due to compound heterozygous loss-of-function mutations have also been identified. These individuals have very low LDL cholesterol (<0·52 mmol/L), but are otherwise healthy.[16,17] In loss-of-function mouse models for PCSK9, reductions in total cholesterol have been noted,[18] consistent with the human phenotype. Collectively, these genetics studies support the hypothesis that lowering of circulating plasma PCSK9 by inhibiting its synthesis in hepatocytes should lower LDL cholesterol, potentially resulting in reduced risk of coronary heart disease. Additionally, some evidence suggests that statin treatment increases circulating plasma PCSK9, which could limit the effectiveness of statins as the dose is increased.[19–22] Human clinical trials with PCSK9-blocking antibodies have shown significant reductions in LDL cholesterol in healthy volunteers[23–24] and in individuals with hypercholesterolaemia, with and without statins.[25–30]

Small interfering RNA (siRNA) can direct sequence-specific degradation of messenger RNA, leading to suppression of synthesis of the corresponding proteins, as part of the natural biological process known as RNA interference (RNAi).[31,32] Our group has previously reported the acute, hepatocyte-specific lowering of synthesis and plasma concentrations of PCSK9 by treatment with a PCSK9-specific siRNA formulated in a lipid nanoparticle in several preclinical models.[33] This treatment resulted in substantial and durable lowering of LDL cholesterol, without an effect on HDL cholesterol. Additionally, we confirmed in these models that the reduction in PCSK9 was based on an RNAi mechanism and resulted in increased numbers of LDLRs on hepatocyte membranes.[33] We aimed to investigate the safety and efficacy in human beings of ALN-PCS, an siRNA that inhibits PCSK9 synthesis formulated in a novel lipid nanoparticle for delivery.[34]

Methods

Study design and participants

We undertook a randomised, single-blind, placebo-controlled, phase 1 clinical trial at two phase 1 units in the UK (Covance Clinical Research Unit, Leeds, and Quintiles Drug Research Unit at Guy's Hospital, London). Eligible participants were healthy adults aged 18–65 years with an LDL cholesterol higher than 3·00 mmol/L. Inclusion criteria required that they had received no lipid-lowering treatments in the 30 days before screening and that they had fasting triglyceride concentrations of 2·8 mmol/L or less. Exclusion criteria included a body-mass index of 35 kg/m² or higher, use of dietary supplements known to affect lipids in the 4 weeks before screening, and a change in exercise regimen in the 4 weeks before screening.

We obtained written informed consent from all participants. The study protocol is available from the Alnylam website and includes the full list of inclusion and exclusion criteria. The protocol was approved by an ethics committee, and the study was done in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki. The trial was continued to completion.

Randomisation and masking

Treatment assignment was masked only from study participants, and not from those investigators who gave the interventions, those who assessed the outcomes, or those who analysed the data. At each site, the investigator enrolled volunteers, who were randomly assigned by the site's research pharmacist. Within each dose cohort of four volunteers, the first two eligible participants were assigned by a computer-generated, predetermined, randomisation algorithm, with one assigned to ALN-PCS and the other to placebo (normal saline). The remaining two participants of each cohort received ALN-PCS. Because ALN-PCS is not identical in appearance to saline, the infusion sets (intravenous bag with drug and tubing) were covered so that the contents of the infusion could not be seen by the participants.

Procedures

The appendix (pp 9–12) summarises the study measurements used, and detailed methods are provided in the study protocol. All participants were admitted to the phase 1 unit 3 days before dosing and remained at the unit site for 4 days after dosing. During this time at the phase 1 unit, all participants received a standard diet designed to meet the nutritional requirements necessary to promote health and prevent diet-related diseases.

On the evening before and the morning of dosing, all participants received oral corticosteroids, histamine receptor (H1 and H2) blockers, and paracetamol to reduce the potential for infusion-related reactions. Specifically, participants received dexamethasone (8 mg the evening before dosing and 20 mg 60 min before the start of infusion of ALN-PCS or placebo) and paracetamol (500 mg orally the evening before dosing and again 30–60 min before the start of infusion). They also received an oral H2 blocker (ranitidine 150 mg or famotidine 20 mg) and an H1 blocker (10 mg cetirizine) the evening before dosing and again 30–60 min before the start of infusion.

A single 1 h intravenous infusion of ALN-PCS or placebo (normal saline) was given to all participants. Six different

doses (0·015, 0·045, 0·090, 0·150, 0·250, and 0·400 mg/kg) of ALN-PCS were assessed. Each dose cohort consisted of four participants randomly assigned in a 3:1 ratio of ALN-PCS treatment to placebo. Two additional cohorts of four participants (randomly assigned in the same ratio) were given the 0·250 and 0·400 mg/kg doses of ALN-PCS (or placebo). Dose was escalated after satisfactory review of all safety information by the safety review committee.

Participants were seen for prespecified follow-up visits at various times during the 180 days after study drug dosing. Data for adverse events were collected for 28 days after dosing. Clinical laboratory safety tests, physical examinations, and medically important events were assessed up to day 180. Safety reviews were done by a safety review committee between each ascending dose cohort before dose escalation. These reviews consisted of assessment of treatment-emergent adverse events, 12-lead electrocardiographs (ECGs), lead II ECG monitoring, arterial oxygen saturation (SaO_2) via pulse oximetry, vital signs (blood pressure, pulse, oral body temperature, and respiration rate), clinical laboratory safety tests (haematology, serum chemistry, coagulation parameters, urinalysis, cytokines, complement activation fragment [Bb], and troponin), and physical examinations.

Concentrations of interferon α, interferon γ, interleukin 6, interleukin 12, tumour necrosis factor α, interleukin 1β, interleukin 1 receptor antagonist, granulocyte colony-stimulating factor, and chemokine (C-X-C motif) ligand 10 were also measured, with analysis done by Charles River Laboratories Preclinical Services (Montreal, QC, Canada).

PCSK9 and LDL cholesterol samples for screening and on-study assessments (obtained after an overnight fast) were analysed at accredited centralised laboratories: plasma PCSK9 was measured by ELISA (Charles River Laboratories, Montreal, QC, Canada) and LDL cholesterol, total cholesterol, and HDL cholesterol were measured directly by β-quantification (Medpace Reference Laboratories, Cincinnati, OH, USA and Leuven, Belgium).

For exploratory purposes, changes in very low-density lipoprotein (VLDL) cholesterol, ApoB, and lipoprotein A were also examined in the higher-dose groups (≥0·150 mg/kg; ApoB and lipoprotein A analyses were not prespecified).

The primary endpoint was safety and tolerability. Safety was assessed by the frequency and severity of adverse events, including the protocol-defined stopping rules. The numbers and proportions of participants with any treatment-emergent adverse event, any treatment-emergent adverse event assessed by the treating investigator as related to study drug, and any serious treatment-emergent adverse event were summarised by dose. Adverse events were coded with the Medical Dictionary for Regulatory Activities (version 14.1).

Secondary endpoints were the pharmacokinetics of ALN-PCS and its pharmacodynamic effects on fasting plasma PCSK9 and serum LDL cholesterol. Changes in
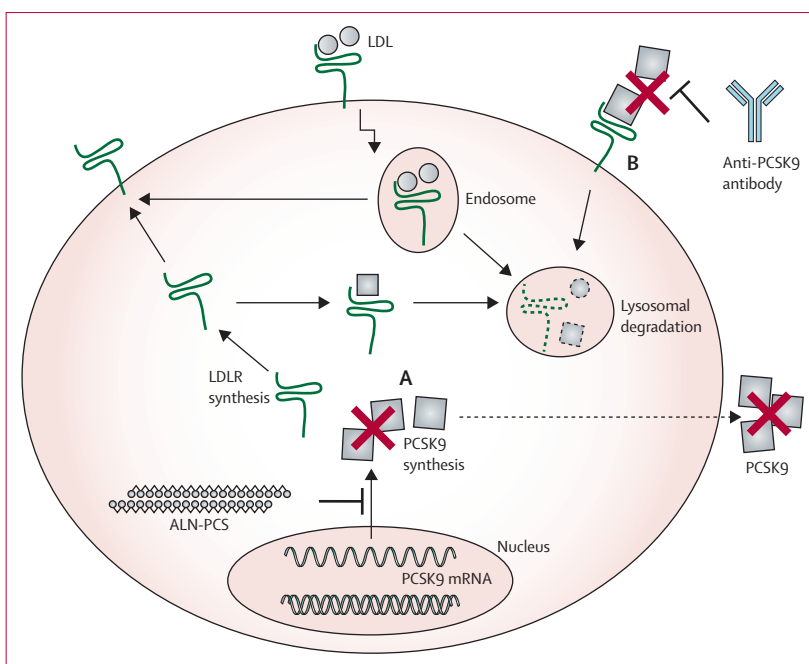
Figure 1: PCSK9 pathway and RNA interference synthesis-inhibitor approach
PCSK9 has a role in both intracellular and extracellular degradation of the LDL receptor (LDLR). PCSK9 synthesis inhibitors such as ALN-PCS inhibit PCSK9 synthesis (A) and therefore both intracellular and extracellular functions, whereas PCSK9 blockers (such as anti-PCSK9 antibodies) inhibit only extracellular function (B). mRNA=messenger RNA.
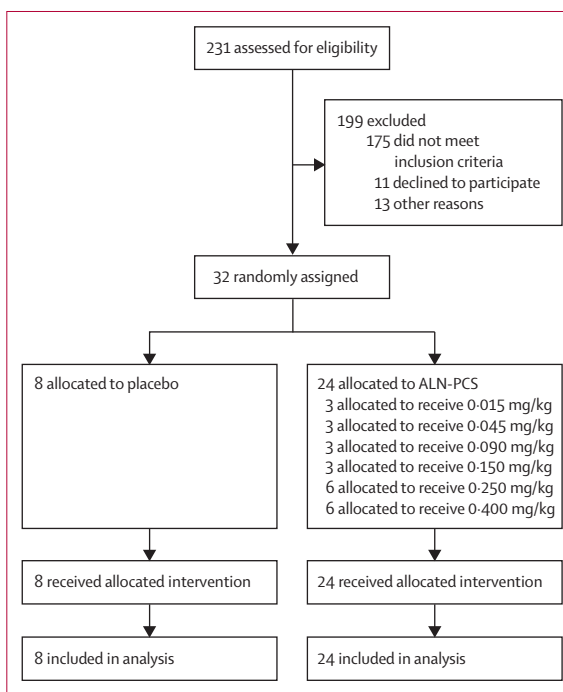
Figure 2: Trial profile

fasting HDL cholesterol, total cholesterol, and VLDL cholesterol were also measured (post-hoc analyses). In the higher-dose groups, lipoprotein A and ApoB were also examined post hoc.

Statistical analysis

All statistical analyses were done with SAS (version 9.2 or later) or R (version 2.13.2 or later). Ratios of PCSK9 protein and LDL cholesterol, both relative to baseline and relative to baseline and placebo (individual reductions scaled to placebo-group mean per day), were log-transformed before analysis. For all traits for which pairwise comparisons of individual dose groups at particular timepoints were to be done, repeated-measures ANCOVA first established significant time, dose, and time-by-dose interaction effects. The ANCOVA models included dose group, time, and individual participant as factors; trait baseline as a covariate; and dose by time as the sole interaction term.

Significance of pairwise comparisons was then assessed by t tests (with Bonferroni correction for multiple comparisons), and ratios were back-transformed to percentages for presentation. Percentage change versus placebo (the difference between dose group and placebo group percentage reduction relative to their respective baselines) was estimated from a similar ANCOVA model that used percentage without transformation, and p values were determined in the same way as for pairwise comparisons of dose groups.

Single-factor ANOVAs with Tukey's post-hoc tests were used to determine the significance of changes in total cholesterol and HDL cholesterol. ANCOVA models examined PCSK9 and LDL cholesterol reduction at per-individual nadir (days 1–28). The models included baseline PCSK9 or LDL cholesterol as covariates, and dose group as a factor. Least-square means and 95% CIs were estimated from the ANCOVA models. Tukey's tests (with Bonferroni correction) assessed the significance of pairwise comparisons of individual dose groups' least-square means versus placebo.

The trial is registered with ClinicalTrials.gov, number NCT01437059.

Role of the funding source

The sponsor contributed to the study at all stages, including trial design, sample testing, statistical analysis, data interpretation, and writing of this report. Prometrika (Cambridge, MA, USA) was used as a contract organisation for some of the statistical analysis. All authors had access to the primary data. KF and AS had primary responsibility for the decision to submit for publication.

Results

32 eligible participants were recruited between Sept 21, 2011, and Sept 11, 2012. 24 were randomly assigned to receive ALN-PCS and eight to receive placebo (figure 2). One participant was excluded after random assignment because their lipid concentrations proximal to dosing were outside of those defined by the inclusion criteria. Table 1 lists the baseline characteristics of all participants who received study treatment and therefore were included in the analyses.

Overall, ALN-PCS was safe and well tolerated. No drug-related serious adverse events occurred in any of the participants who received ALN-PCS. One serious adverse event occurred during the study in a participant given the 0·045 mg/kg dose, who was diagnosed with bilateral pulmonary emboli and a deep vein thrombosis on day 3 of the study. This event was at one of the lower doses in

	ALN-PCS group (n=24)	Placebo group (n=8)
Median age (years)	51·0 (25·0–60·0)	41·5 (33·0–61·0)
Sex		
Male	22	8
Female	2	0
Mean body-mass index	28·7 (23·4–33·9)	28·9 (24·7–33·4)
Mean baseline PCSK9 (ng/mL)	1014·7 (453·2–1528·7)	1067·0 (788·9–1355·1)
Mean baseline LDL cholesterol (mmol/L)	3·7 (2·9–4·8)	3·9 (3·0–6·0)
Mean baseline HDL cholesterol (mmol/L)	1·3 (0·8–2·3)	1·2 (0·8–1·6)
Mean baseline total cholesterol (mmol/L)	5·7 (4·3–6·6)	5·7 (4·5–8·1)

Data are n, median (range), or mean (range). PCSK9 plasma values are based on ELISA. Cholesterol values are based on β-quantification measurements.

Table 1: Baseline characteristics

|	ALN-PCS dose group						ALN-PCS (all doses) (n=24)	Placebo (n=8)
	0·015 mg/kg (n=3)	0·045 mg/kg (n=3)	0·090 mg/kg (n=3)	0·150 mg/kg (n=3)	0·250 mg/kg (n=6)	0·400 mg/kg (n=6)		
Rash	1	1	0	0	4	6	12	4
Headache	1	1	2	0	0	1	5	2
Hiccups	2	0	0	0	0	2	4	2
Cold symptoms	0	1	0	2	1	0	4	1
Paraesthesia	1	0	0	0	0	2	3	0
Polyuria or dysuria	1	0	0	0	0	1	2	1
Infusion-site haematoma	0	0	0	1	0	0	1	1

Data are numbers of treatment-emergent adverse events that were believed to be related to ALN-PCS and occurred with an overall frequency of 10% or higher. All treatment-emergent adverse events were mild or moderate in severity.

Table 2: Treatment-emergent adverse events

the study and was determined by the investigator and safety review committee to be unrelated to study drug, in view of the individual's medical history of a similar episode of chest pain 3 months previously, and the presence of symptoms of thigh pain before dosing (both of which were undisclosed by the participant during the study screening assessments), as well as ultrasound evidence of collateral circulation pathways circumventing the deep vein thrombosis of the leg, which suggested a chronic disease process. Similar neutral lipid nanoparticles have been given at higher doses and chronically in patients with no evidence of thrombosis.[35,36] All treatment-emergent adverse events were mild to moderate in severity, with similar proportions affected in the ALN-PCS and placebo groups (19 [79%] vs seven [88%]; table 2). A mild, macular, erythematous rash occurred with equal frequency in participants given ALN-PCS and

those given placebo. The rash was transient, occurring on the first day after treatment, and was asymptomatic without pain or pruritus. We noted no clinically significant dose-dependent changes in any laboratory indices, including liver function tests, creatine phosphokinase, C-reactive protein, and haematological measures. No clinically significant dose-dependent changes occurred in concentrations of the nine cytokines measured. Finally, we noted no clinically significant safety findings during the 6 month follow-up period.

The plasma pharmacokinetic profiles of the ALN-PCS siRNA showed rapid distribution in the plasma, with peak concentration occurring at the end of infusion or shortly thereafter. Peak concentration and area under the curve (0 to last measurement) increased in a roughly dose-proportional manner across the dose range tested (appendix p 7).
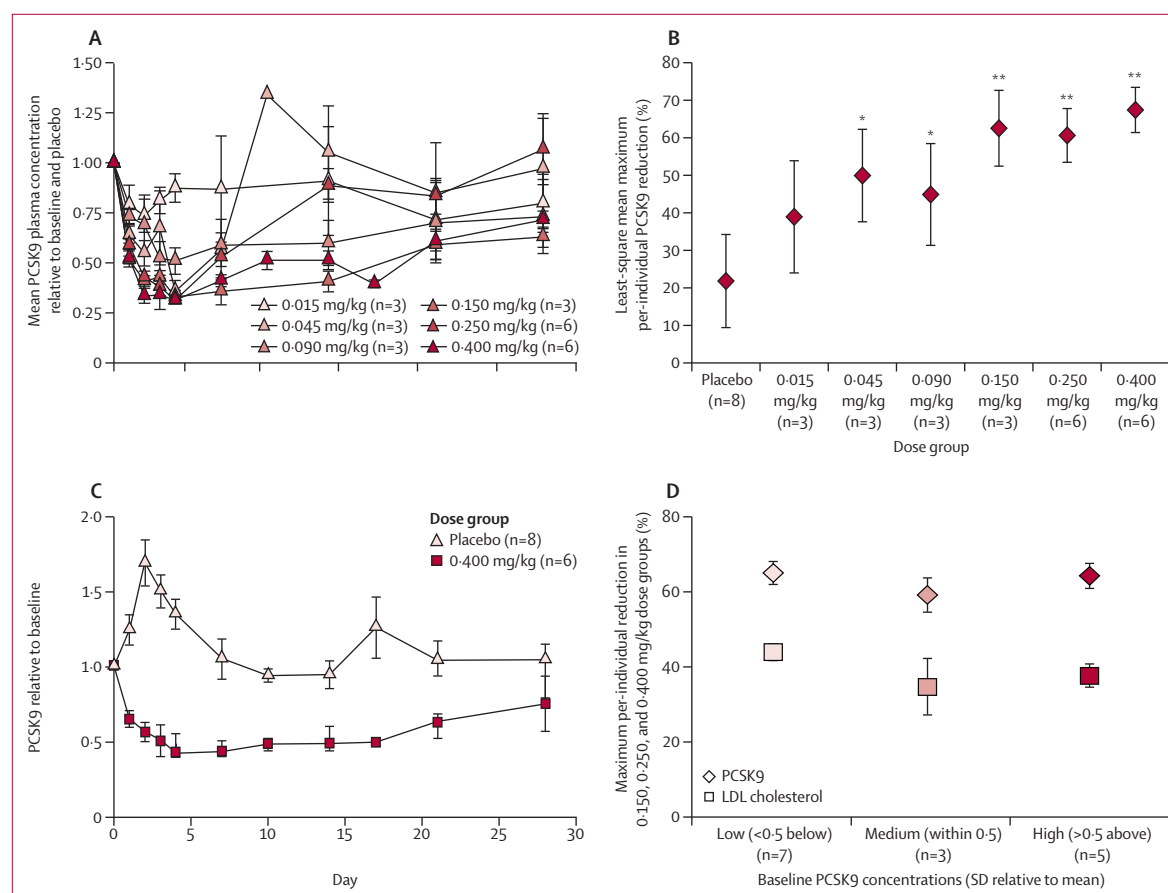
Figure 3: **Effect of ALN-PCS treatment on plasma PCSK9 concentration**
(A) Mean plasma PCSK9 concentrations relative to baseline and placebo. Error bars are SEs. PCSK9 concentrations were normalised per-individual to baseline and then per-day to placebo group means. For 0·045 mg/kg group at day 10, data from only one of three participants were available; for days 10 and 17, blood samples were only available for the 0·250 mg/kg and 0·400 mg/kg dose groups and their affiliated placebo participants, so values at these days are derived from or normalised to data from two of eight placebo participants. (B) Maximum plasma PCSK9 percentage reduction after treatment with ALN-PCS. Maximum reductions were determined per individual, at lowest PCSK9 value from days 1–28. Data are least-square means and error bars are 95% CIs, determined from analysis of covariance with baseline PCSK9 as covariate. *p<0·05; **p<0·001 (Tukey's post-hoc tests vs placebo). Least-square mean PCSK9 reductions per dose group were estimated via a linear model with dose group as a factor and baseline PCSK9 as a covariate. The overall model was significant (F=8·821; p=2·307x10⁻⁵). (C) Mean plasma PCSK9 concentrations relative to baseline. Error bars are SEs. (D) Mean maximum percentage reductions in PCSK9 and LDL cholesterol in participants given 0·150, 0·250, and 0·400 mg/kg ALN-PCS, grouped by low, intermediate, and high baseline PCSK9 concentrations (<0·5, within 0·5, and >0·5 SDs from the mean, respectively). Error bars are SEs.

Single-dose administration of ALN-PCS resulted in a rapid and dose-dependent reduction in plasma PCSK9 protein (figures 3A, 3B). The duration of PCSK9 lowering was also dose-dependent, with higher doses resulting in more prolonged reduction (figures 3A, 3B). At the highest dose of 0·400 mg/kg, PCSK9 fell by 70% from baseline relative to placebo on day 3 post-dose (p<0·0001; figure 3A, table 3), with an individual maximum reduction of 84%. Similar to the arithmetic mean data, the least-square mean individual maximum reduction in plasma PCSK9 protein at the 0·400 mg/kg dose was 68% (figure 3B).

Plasma PCSK9 increased significantly in the placebo group, beginning on day 1, peaking on day 2, and returning to baseline values on day 7 (figure 3C, table 3). This increase in the placebo group was attributed to the premedication given to all participants, and was clearly

overcome in a dose-dependent fashion by ALN-PCS treatment (figure 3A, 3C, table 3, appendix p 8). The effect of ALN-PCS was sustained well beyond day 7, when the effects of the premedication were absent (figure 3C, table 3, figure 4A). To confirm that the effect of ALN-PCS was specific for PCSK9, we also measured changes in plasma transthyretin, a liver-secreted protein that was not expected to change with liver silencing of PCSK9. We noted no significant change in plasma transthyretin concentration after treatment with ALN-PCS at 0·400 mg/kg (appendix p 8), suggesting that the effect of ALN-PCS on plasma PCSK9 concentration was target specific. Baseline values of PCSK9 can vary by a factor of more than 100 across the population;[37] however, since RNAi drugs function by a catalytic mechanism and ALN-PCS blocks PCSK9 synthesis, we predicted that the reductions in plasma PCSK9 would be independent of the baseline PCSK9

	0·015 mg/kg (n=3)	0·045 mg/kg (n=3)	0·090 mg/kg (n=3)	0·150 mg/kg (n=3)	0·250 mg/kg (n=6)	0·400 mg/kg (n=6)	Placebo (n=8)
Fasting plasma PCSK9							
Mean percentage change from baseline*	–30·8%	–52·9%	–45·9%	–64·2%	–58·5%	–58·6%	–8·7%
Time to group nadir (days)†	21	4	14	7	4	4	4
p value vs baseline‡	0·2901	0·1335	0·0974	0·0391	<0·0001	<0·0001	0·3546
Mean per-individual maximum percentage change from baseline§	–36·4%	–47·8%	–44·5%	–65·1%	–59·6%	–65·6%	–21·4%
p value vs placebo¶	0·1360	0·0109	0·0387	0·0002‖	<0·0001‖	<0·0001‖	··
Mean percentage change from baseline relative to placebo**	–30·9%	–63·9%	–48·7%	–64·9%	–69·2%	–69·7%	··
Time to group nadir relative to placebo (days)††	21	4	4	4	4	3	··
p value relative to placebo**	0·0563	<0·0001‖	0·0007‖	<0·0001‖	<0·0001‖	<0·0001‖	··
Mean percentage change vs placebo‡‡	–45·3%	–86·0%	–71·5%	–96·2%	–98·3%	–114·5%	··
Time to group nadir vs placebo (days)§§	2	4	3	2	2	2	··
p value vs placebo‡‡	0·0093	<0·0001‖	<0·0001‖	<0·0001‖	<0·0001‖	<0·0001‖	··
Fasting serum LDL cholesterol							
Mean percentage change from baseline*	–14·4%	–19·3%	–30·4%	–35·0%	–35·5%	–36·1%	–24·0%
Time to group nadir (days)†	3	3	3	4	4	4	3
p value vs baseline‡	0·0700	0·0579	0·1115	0·0218	0·0033	0·0036	0·0004
Mean per-individual maximum percentage change from baseline§	–17·6%	–20·6%	–35·9%	–37·6%	–39·7%	–41·8%	–26·8%
p value vs placebo¶	0·1602	0·2948	0·1221	0·0759	0·0084	0·0021‖	··
Mean percentage change from baseline relative to placebo**	–6·2%	–11·4%	–24·7%	–22·2%	–27·8%	–40·1%	··
Time to group nadir relative to placebo (days)††	1	14	14	14	14	10	··
p value relative to placebo**	0·5037	0·2138	0·0036‖	0·0099	<0·0001‖	<0·0001‖	··
Mean percentage change vs placebo‡‡	–6·6%	–13·4%	–27·2%	–24·0%	–30·1%	–47·2%	··
Time to group nadir vs placebo (days)§§	1	14	14	14	14	10	··
p value vs placebo‡‡	0·4078	0·0966	0·0008‖	0·0031	<0·0001‖	<0·0001‖	··

*Nadir for percentage change from baseline was derived from the log of the fold change from baseline for each dose group at each timepoint; fold change from baseline was defined as the ratio of each participant's post-baseline value to their baseline value; for this table, the minimum log of the fold change value was converted back to percentage change by exp(log(fold change)–1)×100. †Study day at which the minimum mean (log) fold change occurred for each group. ‡At nadir, p value was generated from a paired t test, testing log(fold change)=0 or log(value at nadir)=log(baseline value). §Per-individual maximum percentage change from baseline is calculated at each participant's nadir (log) fold change from baseline, at any day from 1 to 28. ¶p values for pairwise comparisons were derived from analysis of covariance, modelling the log of the fold change from baseline as a function of treatment with baseline as a covariate and individual participant as a random effect. ‖p values that passed Bonferroni correction for multiple comparison. **Nadir for fold change relative to placebo is derived from the estimated values for the log of the active group mean fold change minus the log of placebo group mean fold change, generated from a repeated-measure analysis of covariance (modelling log of fold change from baseline as a function of treatment, visit, and treatment-by-visit interaction, with baseline as a covariate, and individual participant as a random effect; p values for pairwise comparison with the placebo group were generated from the same model. ††Study day at which the minimum of the difference between the log of the active group mean fold change and the log of the placebo group mean fold change occurred. ‡‡Nadir for percentage change versus placebo is derived from the estimated values of the active group mean percentage change minus the placebo group mean percentage change, generated from a repeated-measure analysis of covariance (modelling percentage change from baseline as a function of treatment, visit, and treatment-by-visit interaction, with baseline as a covariate, and individual participant as a random effect; p values for pairwise comparison with the placebo group were generated from the same model. §§Study day at which the greatest negative difference between mean active group percentage change and mean placebo group percentage change occurred.

Table 3: **Percentage change from baseline for PCSK9 and LDL cholesterol, by dose group**

concentrations for each individual participant, and this prediction was confirmed by our results (figure 3D).

As expected from the significant lowering of plasma PCSK9, single-dose administration of ALN-PCS also resulted in a rapid and dose-dependent reduction in serum LDL cholesterol (figures 4A, 4B). The duration of LDL cholesterol lowering was also dose-dependent, with higher doses resulting in more prolonged reduction (figures 4A, 4B). Administration of the highest dose (0·400 mg/kg) resulted in a mean 40% reduction (p<0·0001) from baseline relative to placebo (figure 4A, table 3), with an individual maximum reduction of 57%. Similar to the arithmetic mean data, the least-square mean individual maximum redwuction in LDL cholesterol was 42% (p=0·0021; figure 4B). In addition to the effects of premedication on PCSK9, ALN-PCS treatment also had an apparent effect on LDL cholesterol at early timepoints, with a reduction noted on day 2 that reached a nadir at day 4 and recovered to baseline by day 7

(figure 4C, table 3, appendix p 8). The effects of ALN-PCS on LDL cholesterol were clearly sustained well past the time at which LDL cholesterol concentrations in participants given placebo had recovered to baseline (figure 4C, table 3). The number of participants who reached an LDL cholesterol concentration less than 2·6mmol/L increased with increasing dose (figure 4D). The appendix (p 5) lists the actual values of LDL cholesterol for individual participants. We also noted changes in total cholesterol, but not in HDL cholesterol (appendix pp 1–4).

Discussion

ALN-PCS was well tolerated, with similar proportions of mild to moderate treatment-emergent adverse events occurring in the treatment and placebo groups. Although some participants developed a rash, it was mild, resolved spontaneously, and occurred with equal frequency in the in placebo and ALN-PCS-treated participants. The rash was identical in appearance and nature in both groups,
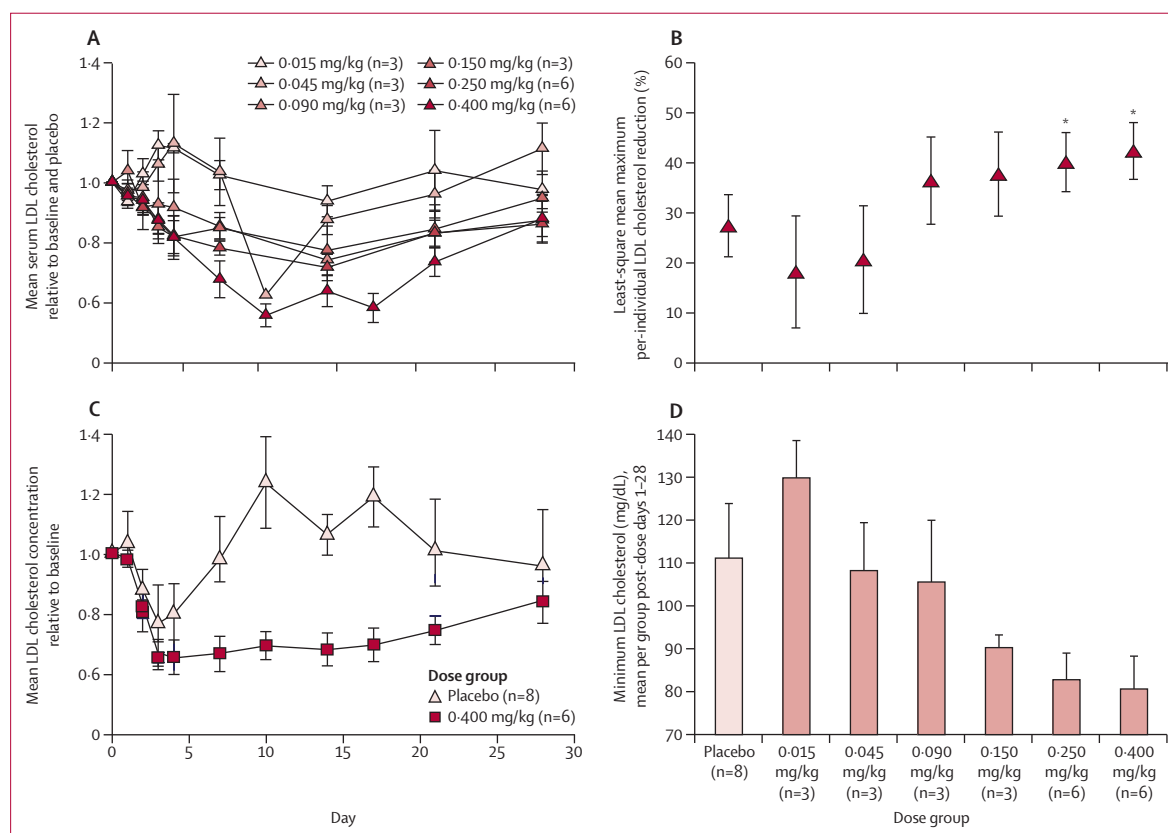
Figure 4: Effect of ALN-PCS treatment on serum LDL cholesterol

(A) Mean serum LDL cholesterol concentrations relative to baseline and placebo. Error bars are SEs. LDL cholesterol concentrations were normalised per-individual to baseline and then per-day to placebo group means. For 0·045 mg/kg group at day 10, data from only one of three participants were available; for days 10 and 17, blood samples were only available for the 0·250 mg/kg and 0·400 mg/kg dose groups and their affiliated placebo participants, so values at these days are derived from or normalised to data from two of eight placebo participants. (B) Maximum serum LDL cholesterol percentage reductions after treatment with ALN-PCS. Maximum reductions were determined per individual, at nadir LDL cholesterol value from days 1–28. Data are least-square means and error bars are 95% CIs, determined from analysis of covariance with baseline LDL cholesterol as covariate. *p<0·01 (Tukey's post hoc test vs placebo). Least-square mean LDL cholesterol reductions per dose group were estimated via a linear model with dose group as a factor and baseline LDL cholesterol as a covariate. The overall model was significant (F=4·888; p=0·0015). (C) Mean serum LDL cholesterol concentrations relative to baseline and placebo. Error bars are SEs. For days 10 and 17, blood samples were only available for the 0·250 mg/kg and 0·400 mg/kg dose groups and their affiliated placebo participants, so values at these days are derived from or normalised to data from two of eight placebo participants. (D) Mean serum LDL cholesterol concentrations at per-individual nadir from days 1–28. Error bars are SEs.

Systematic review

In 2006, Cohen and colleagues,[14] first reported that heterozygous loss-of-function mutations in *PCSK9* resulted in individuals having lower than average LDL cholesterol and a greatly reduced risk of cardiovascular disease. Since that report investigators have focused on the development of drugs to lower the activity of PCSK9 and thereby increase LDL receptor concentrations and decrease plasma cholesterol. We searched PubMed for all articles published in English up to June 12, 2013, that contained the MeSH terms "PCSK9" or "proprotein convertase subtilisin kexin" in the abstract or title. We identified several publications that described PCSK9 inhibitors that had been assessed in clinical trials.[23–29] These reports showed that inhibiting PCSK9 by blocking its activity with an antibody lowers LDL cholesterol in healthy volunteers and in patients with hyperlipidaemia with or without concomitant treatment with statin treatment. We found no reports of RNA interference (RNAi)-based synthesis inhibitors of PCSK9.

Interpretation

ALN-PCS, an RNAi drug designed to inhibit the synthesis of the *PCSK9* transcript and thus reduce plasma PCSK9, was well tolerated and significantly lowered PCSK9 and LDL cholesterol after a single intravenous dose in healthy volunteers with raised cholesterol. Our study is the first systemic trial of an RNAi drug to report an effect on a clinically validated endpoint (LDL cholesterol). Future trials are needed to fully assess the benefit and long-term safety of ALN-PCS in various patient populations, including those on statins, those who are statin intolerant, and those with high baseline PCSK9 due to defects in LDL receptor function.

and was probably a result of the premedications given to all participants, which can cause skin flushing (due to vasodilatation) or a skin rash. Ascertainment bias might have led to an increase in the reported frequency of rash in both groups as the dose of ALN-PCS increased, giving the impression of a dose-responsive effect; the higher-dose cohorts (≥0·150 mg/kg) were treated exclusively at one site (Covance Clinical Research Unit), and this site reported a higher frequency of rash than the second site in both the treatment and placebo groups. We noted no clinically significant changes in liver function tests, troponin, or inflammatory markers (ie, cytokines and C-reactive protein). Despite these encouraging safety findings, additional, larger, multidose studies are needed to confirm the safety and tolerability of ALN-PCS.

Although this was a small phase 1 study that was not powered to detect changes in PCSK9 or LDL cholesterol, we did note a significant lowering of plasma PCSK9 and serum LDL cholesterol in the higher-dose groups. These effects were rapid and durable after a single dose, potentially supporting a dosing schedule of once or twice per month in future multidose studies (panel).

The data in human beings were well predicted from preclinical studies in non-human primates (appendix p 6), and our results suggest that dosing translated from primates to human beings on a roughly 1:1 mg/kg basis. Because this study was the first time ALN-PCS was given to human beings, participants were given premedication to prevent any potential infusion-related reactions that can occur with lipid-formulated drugs. The premedication regimen resulted in a substantial increase in serum

PCSK9 concentrations and a small reduction in serum LDL cholesterol in the placebo group at early timepoints, with these measures returning to baseline by day 7. Although this effect was fully accounted for in our statistical analysis, we suggest a cautious approach to interpreting the absolute size of the changes in PCSK9 and LDL cholesterol concentrations seen at the early timepoints of the study.

Although working by very different mechanisms (figure 1), antibodies and ALN-PCS both seem to be effective at blocking PCSK9 function and thereby inducing a reduction in LDL and total cholesterol. ALN-PCS was given intravenously in this study and its effect on LDL cholesterol was less than that reported for the highest dose groups of anti-PCSK9 antibodies, with AMG 145 lowering LDL cholesterol by up to 67% at the highest dose when given to healthy volunteers.[23,24] Statins such as atorvastatin range in efficacy with respect to the lowering of LDL cholesterol from 36% to 53%, dependent on the patient population and the dose given.[38] A maximum tolerated dose of ALN-PCS was not reached in this study and premedication resulted in artificially high values of PCSK9 and reduced LDL cholesterol at early timepoints. Therefore, future studies that use higher doses of ALN-PCS without premedication and in patients on statin treatment are warranted.

Studies with anti-PCSK9 antibodies in healthy individuals have shown increased HDL cholesterol and decreased lipoprotein A.[23,24] In this study we noted no significant changes in either in response to ALN-PCS (appendix pp 1–4), although the number of participants assessed at efficacious doses was small.

The seminal discovery of RNAi as a conserved biological mechanism[39] took place in 1998 and was awarded the Nobel Prize in Physiology or Medicine in 2006. This study is the first definitive clinical proof of concept in human beings of an RNAi drug being used to lower a liver-derived protein (PCSK9), thereby resulting in favourable changes in a clinically validated endpoint (LDL cholesterol). These results pave the way for additional siRNA treatments targeted at liver-expressed genes implicated in human disease.

References

1 Grundy SM, Cleeman JI, Merz CN, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *J Am Coll Cardiol* 2004; **44:** 720–32.

2 Delahoy PJ, Magliano DJ, Webb K, Grobler M, Liew D. The relationship between reduction in low-density lipoprotein cholesterol and reduction in risk of cardiovascular outcomes: an updated meta-analysis. *Clin Ther* 2009; **31:** 236–44.

3 Sniderman A, Thanassoulis G, Couture P, Williams K, Alam A, Furberg CD. Is lower and lower better and better? A re-evaluation of the evidence from the Cholesterol Treatment Trialists' Collaboration meta-analysis for low-density lipoprotein lowering. *J Clin Lipidol* 2012; **6:** 303–09.

4 Davidson MH, Maki KC, Pearson TA, et al. Results of the National Cholesterol Education (NCEP) Program Evaluation ProjecT Utilizing Novel E-Technology (NEPTUNE) II survey and implications for treatment under the recent NCEP Writing Group recommendations. *Am J Cardiol* 2005; **96:** 556–63.

5 Foley KA, Simpson RJ Jr, Crouse JR 3rd, Weiss TW, Markson LE, Alexander CM. Effectiveness of statin titration on low-density lipoprotein cholesterol goal attainment in patients at high risk of atherogenic events. *Am J Cardiol* 2003; **92:** 79–81.

6 Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins. *Lancet* 2005; **366:** 1267–78.

7 Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy of cholesterol-lowering therapy in 18 686 people with diabetes in 14 randomised trials of statins: a meta-analysis. *Lancet* 2008; **371:** 117–25.

8 Genest J, McPherson R, Frohlich J, et al. 2009 Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult—2009 recommendations. *Can J Cardiol* 2009; **25:** 567–79.

9 Foody JM, Sajjan SG, Hu XH, et al. Loss of early gains in low-density lipoprotein cholesterol goal attainment among high-risk patients. *J Clin Lipidol* 2010; **4:** 126–32.

10 Thomas JE, Tershakovec AM, Jones-Burton C, Sayeed RA, Foody JM. Lipid lowering for secondary prevention of cardiovascular disease in older adults. *Drugs Aging* 2010; **27:** 959–72.

11 Abifadel M, Varret M, Rabes JP, et al. Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003; **34:** 154–56.

12 Horton JD, Cohen JC, Hobbs HH. Molecular biology of *PCSK9:* its role in LDL metabolism. *Trends Biochem Sci* 2007; **32:** 71–77.

13 Poirier S, Mayer G, Poupon V, et al. Dissection of the endogenous cellular pathways of PCSK9-induced low density lipoprotein receptor degradation: evidence for an intracellular route. *J Biol Chem* 2009; **284:** 28856–64.

14 Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in *PCSK9,* low LDL, and protection against coronary heart disease. *N Engl J Med* 2006; **354:** 1264–72.

15 Horton JD, Cohen JC, Hobbs HH. PCSK9: a convertase that coordinates LDL catabolism. *J Lipid Res* 2009; **50** (suppl)**:** S172–77.

16 Zhao Z, Tuakli-Wosornu Y, Lagace TA, et al. Molecular characterization of loss-of-function mutations in *PCSK9* and identification of a compound heterozygote. *Am J Hum Genet* 2006; **79:** 514–23.

17 Hooper AJ, Marais AD, Tanyanyiwa DM, Burnett JR. The C679X mutation in *PCSK9* is present and lowers blood cholesterol in a Southern African population. *Atherosclerosis* 2007; **193:** 445–48.

18 Rashid S, Curtis DE, Garuti R, et al. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking *Pcsk9. Proc Natl Acad Sci USA* 2005; **102:** 5374–79.

19 Careskey HE, Davis RA, Alborn WE, Troutt JS, Cao G, Konrad RJ. Atorvastatin increases human serum levels of proprotein convertase subtilisin/kexin type 9. *J Lipid Res* 2008; **49:** 394–98.

20 Davignon J, Dubuc G. Statins and ezetimibe modulate plasma proprotein convertase subtilisin kexin-9 (PCSK9) levels. *Trans Am Clin Climatol Assoc* 2009; **120:** 163–73.

21 Costet P, Hoffmann MM, Cariou B, et al. Plasma PCSK9 is increased by fenofibrate and atorvastatin in a non-additive fashion in diabetic patients. *Atherosclerosis* 2010; **212:** 246–51.

22 Welder G, Zineh I, Pacanowski MA, Troutt JS, Cao G, Konrad RJ. High-dose atorvastatin causes a rapid sustained increase in human serum PCSK9 and disrupts its correlation with LDL cholesterol. *J Lipid Res* 2010; **51:** 2714–21.

23 Stein EA, Mellis S, Yancopoulos GD, et al. Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. *N Engl J Med* 2012; **366:** 1108–18.

24 Dias CS, Shaywitz AJ, Wasserman SM, et al. Effects of AMG 145 on low-density lipoprotein cholesterol levels: results from 2 randomized, double-blind, placebo-controlled, ascending-dose phase 1 studies in healthy volunteers and hypercholesterolemic subjects on statins. *J Am Coll Cardiol* 2012; **60:** 1888–98.

25 Raal F, Scott R, Somaratne R, et al. Low-density lipoprotein cholesterol-lowering effects of AMG 145, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease in patients with heterozygous familial hypercholesterolemia: the Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) randomized trial. *Circulation* 2012; **126:** 2408–17.

26 Sullivan D, Olsson AG, Scott R, et al. Effect of a monoclonal antibody to PCSK9 on low-density lipoprotein cholesterol levels in statin-intolerant patients: the GAUSS randomized trial. *JAMA* 2012; **308:** 2497–506.

27 Roth EM, McKenney JM, Hanotin C, Asset G, Stein EA. Atorvastatin with or without an antibody to PCSK9 in primary hypercholesterolemia. *N Engl J Med* 2012; **367:** 1891–900.

28 Banerjee Y, Shah K, Al-Rasadi K. Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. *N Engl J Med* 2012; **366:** 2425–26.

29 Milazzo L, Antinori S. Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. *N Engl J Med* 2012; **366:** 2425.

30 Hooper AJ, Burnett JR. Anti-PCSK9 therapies for the treatment of hypercholesterolemia. *Expert Opin Biol Ther* 2013; **13:** 429–35.

31 Meister G, Tuschl T. Mechanisms of gene silencing by double-stranded RNA. *Nature* 2004; **431:** 343–49.

32 Vaishnaw AK, Gollob J, Gamba-Vitalo C, et al. A status report on RNAi therapeutics. *Silence* 2010; **1:** 14.

33 Frank-Kamenetsky M, Grefhorst A, Anderson NN, et al. Therapeutic RNAi targeting PCSK9 acutely lowers plasma cholesterol in rodents and LDL cholesterol in nonhuman primates. *Proc Natl Acad Sci USA* 2008; **105:** 11915–20.

34 Jayaraman M, Ansell SM, Mui BL, et al. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angew Chem Int Ed Engl* 2012; **51:** 8529–33.

35 Tabernero J, Shapiro GI, LoRusso PM, et al. First-in-humans trial of an RNA interference therapeutic targeting VEGF and KSP in cancer patients with liver involvement. *Cancer Discov* 2013; **3:** 406–17.

36 Barros SA, Gollob JA. Safety profile of RNAi nanomedicines. *Adv Drug Deliv Rev* 2012; **64:** 1730–37.

37 Lakoski SG, Lagace TA, Cohen JC, Horton JD, Hobbs HH. Genetic and metabolic determinants of plasma PCSK9 levels. *J Clin Endocrinol Metab* 2009; **94:** 2537–43.

38 Adams SP, Tsang M, Wright JM. Lipid lowering efficacy of atorvastatin. *Cochrane Database Syst Rev* 2012; **12:** CD008226.

39 Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans. Nature* 1998; **391:** 806–11.