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Lipids

The impact of proprotein convertase subtilisin-kexin type 9 serine protease inhibitors on lipid levels and outcomes in patients with primary hypercholesterolaemia: a network meta-analysis

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Aims We performed a network meta-analysis of randomized controlled trials (RCTs) in patients with primary hypercholesterolaemia to compare the impact of proprotein convertase subtilisin-kexin type 9 serine protease (PCSK9) inhibitors with placebo and ezetimibe on lipid levels and outcomes.

Methods and results MEDLINE/PubMed, Cochrane CENTRAL, and ClinicalTrials.gov were searched for RCTs assessing PCSK9 inhibitors vs. other therapies in patients with primary hypercholesterolaemia. Network meta-analysis with both a frequentist approach and a Bayesian framework was performed to directly and indirectly compare PCSK9 inhibition on lipid levels with ezetimibe and placebo. Odds ratios with 95% confidence intervals (OR [95% CIs]) were generated with random-effects models to compare outcomes. Our meta-analysis included 17 RCTs with 13 083 patients that were randomized to PCSK9 inhibitors ($n = 8250$), placebo ($n = 3957$), ezetimibe ($n = 846$), or PCSK9 inhibitors and ezetimibe ($n = 30$). The mean age was 59 $\pm$ 10, 52% were male, 34% had coronary artery disease, 51% had hypertension, 19% had diabetes mellitus, baseline LDL of 122 $\pm$ 36 mg/dL, total cholesterol of 199 $\pm$ 39 mg/dL, and HDL of 51 $\pm$ 14 mg/dL. inhibitors significantly reduced LDL cholesterol by 57% relative to placebo ($P < 0.001$) and 36.1% relative to ezetimibe ($P < 0.001$). Proprotein convertase subtilisin-kexin type 9 serine protease inhibitors reduced the incidence of all-cause mortality [OR 0.43 (95% CI 0.22–0.82), $P = 0.01$] but was associated with an increased incidence of neurocognitive adverse events [OR 2.34 (95% CI 1.11–4.93), $I^2 = 4\%$, $P = 0.02$] when compared with placebo.

Conclusion Proprotein convertase subtilisin-kexin type 9 serine protease inhibition significantly improved lipid profiles and reduced the incidence of all-cause mortality compared with placebo but had a higher rate of neurocognitive adverse events. Thus, PCSK9 inhibitor therapy may serve as an alternative for patients with statin intolerance and for those who do not respond to other lipid reduction therapy.

Keywords PCSK9 inhibitors • Lipid levels • Outcomes • Hypercholesterolemia

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Introduction

The development of HMG-CoA reductase inhibitors, or statins, resulted in a major shift in medical therapy over the last 20 years for patients with atherosclerosis and its clinical manifestations of myocardial infarction and stroke. Data not only support secondary prevention but also the implementation of statin therapy for primary prevention to reduce the development of major adverse cardiovascular and cerebrovascular events (MACCE), especially in patients at higher genetic risk.[1] Interestingly, a large meta-analysis of the major statin trials demonstrated that further reductions in LDL cholesterol resulted in incremental reduction in MACCE during follow-up.[2] However, while pre-clinical and phase 2 data may support the beneficial effects of lipid-lowering therapies, it is critical that these findings are confirmed in large phase 3 randomized controlled trials (RCTs) with adequate follow-up to assess outcomes. Notable examples include torcetrapib[3] and niacin,[4] where there were significant reductions in LDL cholesterol and increases in HDL cholesterol but no improvement in outcomes and evidence of harm. The LDL strategy of 'lower is better' was recently supported by the results of the IMProved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT),[5] in which further LDL reduction with ezetimibe beyond statin therapy was associated with improved cardiovascular (CV) outcomes.

Proprotein convertase subtilisin-kexin type 9 serine protease (PCSK9) plays an important role in regulating LDL cholesterol and sequence variants in the PCSK9 gene are associated with significant reductions in not only LDL cholesterol but also lower risk of coronary artery disease (CAD).[6,7] Given the improvement in LDL cholesterol and outcomes in patients with defective PCSK9, multiple companies are pursuing therapies to inhibit PCSK9. Over the past 4 years, data from multiple RCTs suggest PCSK9 inhibitors improve lipid levels and may improve outcomes. Furthermore, many of these RCTs included patients not only randomized to placebo or PCSK9 inhibitors but also to ezetemibe or both PCSK9 inhibitors and ezetimibe. Thus, we performed a network meta-analysis to compare the impact of PCSK9 inhibitors on lipid levels and outcomes with placebo or ezetimibe.

Methods

Objectives, study selection, and data abstraction

The primary objective of this network meta-analysis was to assess the lipid-lowering effects of PCSK9 inhibitors compared with placebo or ezetimibe and determine if PCSK9 inhibitors significantly reduce all-cause mortality or CV events. Two independent reviewers (M.J.L. and R.O.E.) systematically searched (August 2004 to March 2015) MEDLINE/PubMed, Cochrane CENTRAL, and ClinicalTrials.gov, applying the search terms 'PCSK9' or 'Evolocumab' or 'AMG 145' or 'Alirocumab' or 'REGN727' or 'SAR236553'. We apply the pre-specified inclusion criteria to include only: (i) patients from RCTs comparing PCSK9 therapy, (ii) from phase 2 studies or higher, (iii) utilizing high-dose PCSK9 regimens as evaluated by Amgen (Evolocumab 140 mg every 2 weeks or 420 mg monthly) and Regeneron/Sanofi (Alirocumab150 mg every 2 weeks or 300 mg monthly), and (iv) provided lipid and outcomes data which could be abstracted. We excluded any study or group that (i) included patients with homozygosity for genes implicated in familial hypercholesterolaemia or (ii) studies only available in abstract form. Data were abstracted by the same two investigators (M.J.L. and R.O.E.). The percent change in lipid and PCSK9 levels from baseline to follow-up for each treatment group was abstracted when available. We also obtained the absolute change in LDL and the post-therapy LDL levels. Data demonstrating percent change compared with placebo could not be employed as the change from baseline for each treatment group was necessary for analysis. We collected outcomes data for all-cause mortality, CV death, CV events, any adverse events, serious adverse events, events leading to drug discontinuation, a three-fold elevation of liver function tests (LFT) including aspartate aminotransferase or alanine aminotransferase, and an elevation of creatine kinase (a five-fold elevation was preferentially employed). We accepted the study definitions for CV events and death and all studies had event adjudication by physicians.

Quality assessment

The Cochrane Collaboration tool for assessing risk of bias[8] was utilized to assess for different forms of bias within the included studies in our meta-analysis and study quality was assessed with the GRADE system.[9]

Data synthesis and analysis

Dichotomous variables are reported as percentages while continuous variables were reported as mean $\pm$ SD or median (interquartile range). Network meta-analysis with both a frequentist approach and a Bayesian framework with non-informative priors were used to compare lipid and PCSK9 levels between different therapies. Network meta-analysis with a frequentist approach[10] was performed using Netmeta R package version 8.0 (http://CRAN.R-project.org/package=netmeta) to calculate point estimates with 95% confidence intervals and generate forest plots using fixed-effects and random-effects models comparing the effect estimates of different therapies relative to placebo. *P*-rank scores were generated to determine probability scores to rank which therapies result in the greatest change in lipid levels. Heterogeneity and inconsistency were assessed and heat plots were also generated which is a matrix visualization proposed by Krahn and colleagues[11] that highlights hot spots of inconsistency between specific direct evidence in the whole network and renders transparent possible drivers. As a means of confirming our findings with a sensitivity analysis, mixed treatment comparison model generation was performed to directly and indirectly compare the percent change in lipid and PCSK9 levels for therapies from baseline using GeMTC 0.14.3 software (GeMTC, http://drugis.org/mtc). Bayesian hierarchical random-effects model with directed acyclic graph model for general-purpose Markov chain Monte Carlo analysis was performed with 50 000 tuning iterations and 100 000 simulation iterations. Data are presented as mean difference (credible intervals). Convergence was appraised graphically according to Gelman and Rubin.[12] Data from a consistency model are presented and direction of findings was confirmed with an inconsistency model to serve as a sensitivity analysis. Binary outcomes from individual studies to assess pre-specified treatment groups were combined with random-effects models, leading to computations of odds ratios with 95% confidence intervals [OR (95% CIs)]. The variable I^2 was calculated as a measure of statistical heterogeneity; I^2 values of 25, 50, and 75% represented mild, moderate, and severe inconsistency, respectively. Small study or publication bias was explored with funnel plots and Peters' test.[13] Meta-regression analysis using random-effects models was performed to assess the correlation of certain variables with outcomes. Statistical analysis was performed using Review Manager (RevMan) 5 version 5.2.11 freeware package (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008) and Comprehensive

Meta-Analysis version 3 (trial version) with statistical significance for hypothesis testing set at the 0.05 two-tailed level and for heterogeneity testing at the 0.10 two-tailed level.

Results

Using Medline/PubMed, Cochrane CENTRAL, and ClinicalTrials.gov, we identified 920 citations using the previously defined search terms. Implementing our inclusion/exclusion criteria, we evaluated 89 abstracts, of which we assessed 34 as full-text publications. We excluded studies due to duplication of data,[14–19] patients with homozygosity for genes implicated in familial hypercholesterolaemia,[20,21] phase 1 RCT design,[22–24] or publication of a study design.[25,26] One study by Roth and colleagues[27] could not be included in our network meta-analysis as it did not share a comparable group with the other included studies to provide an appropriate comparison (initiation of statin therapy compared with initiation of PCSK9 inhibitor and statin therapy). Our search flow diagram can be found in *Figure 1* and our network profile in Supplementary material online, *Figure S1*. For our meta-analysis, we included 17 RCTs[28–44] with 13 083 patients that were randomized to PCSK9 inhibitors ($n = 8250$), placebo ($n = 3957$), ezetimibe ($n = 846$), or PCSK9 inhibitors and ezetimibe ($n = 30$). All included studies had a low risk of bias as assessed by the Cochrane Collaboration tool for assessing risk of bias[8] (see Supplementary material online, *Table S1*) and all studies were deemed High Quality by the GRADE system.[9] Baseline study characteristics can be found in *Table 1*. All studies were conducted at multiple centres. Baseline patient characteristics can be found in *Table 2*. The included patients in our study had a mean age of 59 ± 10, 52% were male, 34% had coronary artery disease, 51% had hypertension, 19% had diabetes mellitus, and 17% were current smokers. As seen in *Table 3*, the included patients had a mean baseline LDL of 122 ± 36 mg/dL, total cholesterol of 199 ± 39 mg/dL, ApoB level of 98 ± 24 mg/dL, free PCSK9 level of 362 ± 114 ng/mL, HDL of 51 ± 14 mg/dL, and ApoA1 level of 151 ± 27 mg/dL. When compared with baseline LDL levels, patients treated with PCSK9 inhibitors had an absolute reduction in LDL of 71 mg/dL and the post-therapy LDL level was 51 ± 30 mg/dL.

Proprotein convertase subtilisin-kexin type 9 serine protease inhibition and lipid levels

Network meta-analysis with a frequentist approach was performed to compare the change in different lipid and PCSK9 levels from baseline to follow-up with different therapies (PCSK9 inhibitors alone, ezetimibe alone, and combination of PCSK9 inhibitors and ezetimibe). Forest plots were generated for analysis using both fixed-effects and random-effects models. The fixed-effect data serve as a sensitivity analysis and confirms the findings of the random-effects data. As seen in *Figure 2*, the combination of PCSK9 inhibitors and ezetimibe resulted in the greatest reduction of LDL cholesterol (69%), total cholesterol (46%), apolipoprotein B (53%), and PCSK9 levels (59%) compared with placebo. Proprotein convertase subtilisin-kexin type 9 serine protease inhibitors alone led to a 57% reduction in LDL cholesterol, a 36% reduction in total cholesterol, a 46% reduction in apolipoprotein B, and a 47% reduction in PCSK9 levels compared with placebo. Ezetimibe led to a 21% reduction in LDL cholesterol, a 16% reduction in total cholesterol, a 16% reduction in apolipoprotein B, and no impact on PCSK9 levels compared with placebo. Proprotein convertase subtilisin-kexin type 9 serine protease inhibitors also led to a significant increase in HDL and apolipoprotein A1. *P*-rank scores confirm the ranking of these therapies (*Table 4*). Measures of heterogeneity and inconsistency can be found in Supplementary material online, *Table S2*. Heat plots, which are matrix visualizations that highlight hot spots of inconsistency between specific direct evidence in the whole network, can be found in Supplementary material online, *Table S3*. These data suggest that there was less inconsistency and heterogeneity in the change of HDL cholesterol and PCSK9 levels between studies than for apolipoprotein B and total cholesterol levels. Sensitivity analysis with removal of individual studies did not change the direction or magnitude of these findings.

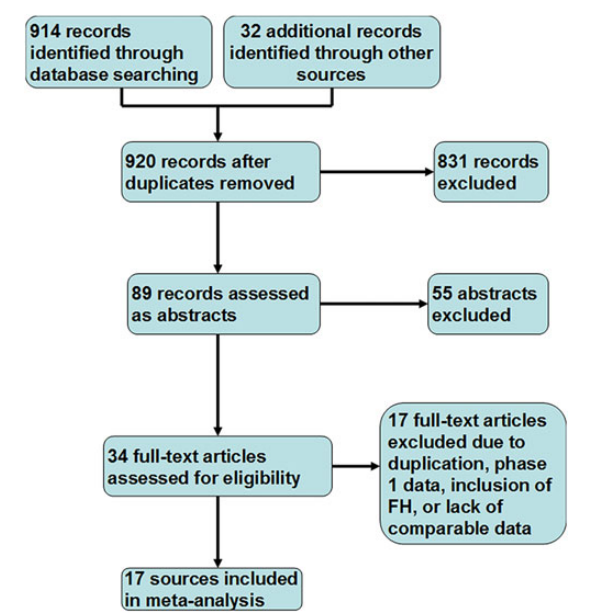

Figure 1 Study flow diagram.

As a sensitivity analysis, we performed Bayesian network meta-analysis using mixed treatment comparison models to confirm the impact of different treatment groups on lipid levels and present the data relative to placebo in Supplementary material online, *Figure S2*. The degree of reduction in lipid levels were similar as that seen with the frequentist approach. Compared with placebo, lipoprotein(a) was reduced by 24.3% with PCSK9 inhibitors and 25.7% with PCSK9 inhibitors and ezetimibe. Ezetimibe, however, did not significantly lower Lp(a). As a further sensitivity analysis, use of an inconsistency model as well as use of different priors for mean and standard deviation confirmed the direction of our findings.

We next performed a subgroup analysis to compare the impact of PCSK9 inhibitors on LDL levels in studies with high baseline statin use[28,31–33,36–38,40–44] and low baseline statin use.[29,30,34,35,39] In

Table 1 Study characteristics of included trials

	Publication year	Study type	Included patients	Patient groups	Groups for analysis	Follow-up length	Statin intolerance
DESCARTES	2014	Phase 3	901	302 Placebo, 599 Evolocumab (420 mg Q4W or could be divided into Q2W)	Placebo PCSK9	52 weeks	No
GAUSS	2012	Phase 2	94	32 Ezetimibe, 30 Evolocumab and Ezetimibe (420 Q4W), 32 Evolocumab (420 mg Q4W)	Ezetimibe PCSK9 Ezetimibe + PCSK9	12 weeks	Yes
GAUSS 2	2014	Phase 3	307	102 Ezetimibe, 205 Evolocumab [140 mg Q2W ($n = 103$) and 420 mg Q4W ($n = 102$)]	Ezetimibe PCSK9	12 weeks	Yes
LAPLACE-TIMI-57	2012	Phase 2	313	155 Placebo, 158 Evolocumab [140 mg Q2W ($n = 78$) and 420 mg Q4W ($n = 80$)]	Placebo PCSK9	12 weeks	No
LAPLACE 2	2014	Phase 3	1897	558 Placebo, 221 Ezetimibe, 1118 Evolocumab [140 mg Q2W ($n = 556$) and 420 mg Q4W ($n = 562$)]	Placebo Ezetimibe PCSK9	12 weeks	No
McKenney	2012	Phase 2	92	31 Placebo (baseline statin therapy), 61 Alirocumab [150 Q2W ($n = 31$) and 300 Q4W ($n = 30$)	Placebo PCSK9	12 weeks lipids, 20 weeks follow-up	No
MENDEL	2012	Phase 2	225	90 Placebo, 45 Ezetimibe, 90 Evolocumab [140 mg Q2W ($n = 45$) and 420 mg Q4W ($n = 45$)	Placebo Ezetimibe PCSK9	12 weeks	No
MENDEL 2	2014	Phase 3	613	153 Placebo, 154 Ezetimibe, 306 Evolocumab [140 mg Q2W ($n = 153$) and 420 mg Q4W ($n = 153$)]	Placebo Ezetimibe PCSK9	12 weeks	No
ODYSSEY COMBO I	2015	Phase 3	316	107 Placebo, 209 Alirocumab	Placebo PCSK9	24 weeks lipids, 52 weeks follow-up	No
ODYSSEY COMBO II	2015	Phase 3	720	241 Ezetimibe, 479 Alirocumab (started 75 mg Q2W and increased to 150 mg Q2W if not at goal)	Placebo PCSK9	24 weeks lipids, 52 weeks follow-up	No
ODYSSEY LONG TERM	2015	Phase 3	2341	788 Placebo, 1553 Alirocumab (150 mg Q2W)	Placebo PCSK9	24 weeks lipids, 78 weeks follow-up	No
ODYSSEY MONO	2014	Phase 3	103	51 Ezetimibe, 52 Alirocumab (started 75 mg Q2W and increased to 150 mg Q2W if not at goal)	Ezetimibe PCSK9	24 week lipids, 32 week follow-up	No
OSLER 2	2015	Open-label	4465	1489 Placebo, 2976 Evolocumab (420 mg Q4W or 140 mg Q2W)	Placebo PCSK9	48 weeks	Included
RUTHERFORD	2012	Phase 2	112	56 Placebo, 56 Evolocumab (420 mg Q4W)	Placebo PCSK9	12 weeks	No
RUTHERFORD 2	2015	Phase 3	331	110 Placebo, 221 Evolocumab [140 mg Q2W ($n = 111$) and 420 mg Q4W ($n = 110$)]	Placebo PCSK9	12 weeks	No
Stein	2012	Phase 2	46	15 placebo, 31 Alirocumab [300 mg Q4W ($n = 15$) and 150 mg Q2W ($n = 16$)]	Placebo PCSK9	12 weeks lipid, 20 weeks follow-up	No
YUKAWA	2014	Phase 2	207	102 Placebo, 105 Evolocumab [140 mg Q2W ($n = 52$) and 420 mg Q4W]	Placebo PCSK9	12 weeks	No

Q2W, every 2 weeks; Q4W, every 4 weeks.

Table 2 Patient characteristics of included randomized controlled trials

	Age (year)	Male gender	Caucasian	Black	Known CAD	Hypertension	Diabetes mellitus	Smoking	Family history of CAD	Baseline statins
DESCARTES	56 ± 11	48%	80.4%	8.4%	15%	49%	12%	15%	23%	88%
GAUSS	61 ± 8	35%	87.3%	4.3%	20%	49%	12%	14%	22%	16%
GAUSS 2	62 ± 10	54%	93.5%	2.3%	29%	59%	20%	8%	32%	18%
LAPLACE-TIMI-57	63 ± 8	45%	90.5%	NR	32%	70%	17%	16%	31%	99%
LAPLACE 2	60 ± 10	54%	94%	4%	23%	NR	15%	NR	NR	100%
McKenney	56 ± 10	43%	87%	12%	5%	41%	14%	27%	NR	100%
MENDEL	51 ± 12	36%	79.2%	17%	0%	33%	0%	14%	9%	0%
MENDEL 2	53 ± 12	31%	83.1%	6%	0%	29%	0.2%	12%	9%	0%
ODYSSEY COMBO I	63 ± 9	66%	81.6%	16.1%	78%	NR	43%	NR	NR	100%
ODYSSEY COMBO II	62 ± 9	74%	84.7%	3.9%	90%	NR	31%	NR	NR	100%
ODYSSEY LONG TERM	61 ± 10	62%	92.7%	NR	69%	NR	35%	21%	NR	100%
ODYSSEY MONO	60 ± 5	53%	90.3%	9.7%	NR	NR	4%	NR	NR	0%
OSLER 2	58 ± 11	51%	85.7%	NR	20%	52%	13%	15%	24%	70%
RUTHERFORD	51 ± 12	53%	89.3%	0.9%	21%	NR	NR	17%	62%	100%
RUTHERFORD 2	51 ± 12	42%	89%	NR	31%	NR	NR	NR	NR	100%
Stein	54 ± 10	63%	95.6%	NR	39%	NR	0%	NR	NR	100%
YUKAWA	61 ± 10	68%	0%	0%	27%	73%	35%	26%	NR	100%

Data presented as mean ± SD. CAD, coronary artery disease; NR, not reported.

Table 3 Baseline lipid levels of for included patients from the included randomized controlled trials

	LDL (mg/dL)	Total cholesterol (mg/dL)	HDL (mg/dL)	ApoB (mg/dL)	ApoA1 (mg/dL)	PCSK9 (ng/mL)	Lipoprotein(a) (nmol/L)
DESCARTES	104 ± 22	178 ± 27	53 ± 16	87 ± 16	153 ± 28	479 ± 160	86 ± 102
GAUSS	194 ± 53	282 ± 55	57 ± 19	143 ± 30	174 ± 32	380 ± 97	NR
GAUSS 2	193 ± 59	NR	52 ± 16	138 ± 33	150 ± 27	286 ± 97	NR
LAPLACE-TIMI-57	122 ± 27	201 ± 33	54 ± 17	100 ± 20	160 ± 30	241 ± 66	NR
LAPLACE 2	109 ± 41	189 ± 46	54 ± 16	89 ± 26	NR	354 ± 112	NR
McKenney	129 ± 26	207 ± 29	50 ± 14	105 ± 22	143 ± 20	NR	NR
MENDEL	142 ± 22	221 ± 32	53 ± 17	42 ± 8	154 ± 30	190 ± 47	71 ± 76
MENDEL 2	143 ± 23	NR	55 ± 15	106 ± 18	158 ± 30	272 ± 86	NR
ODYSSEY COMBO I	102 ± 32	NR	48 ± 14	91 ± 22	NR	NR	NR
ODYSSEY COMBO II	107 ± 35	185 ± 42	46 ± 13	90 ± 20	NR	NR	NR
ODYSSEY LONG TERM	123 ± 442	NR	50 ± 12	102 ± 28	147 ± 26	NR	NR
ODYSSEY MONO	140 ± 26	223 ± 32	57 ± 18	104 ± 19	158 ± 31	NR	NR
OSLER 2	120 (97,150)	203 (175,234)	51 (42,62)	NR	NR	NR	NR
RUTHERFORD	156 ± 39	228 ± 46	50 ± 14	125 ± 30	145 ± 20	601 ± 184	NR
RUTHERFORD 2	151 ± 42	NR	51 ± 15	113 ± 29	140 ± 29	446 ± 138	NR
Stein	146 ± 31	225 ± 38	52 ± 14	125 ± 27	152 ± 26	NR	NR
YUKAWA	139 ± 21	222 ± 24	54 ± 12	110 ± 20	160 ± 20	402 ± 124	NR

Data presented as mean ± SD while OSLER 2 provides data as median with (interquartile range). Lipoprotein(a) levels were provided as medians in the majority of studies and could not be accurately combined, though we could assess the change with therapy as this was reported.
ApoB, apolipoprotein B; ApoA1, apolipoprotein A1; NR, not reported.

studies with high baseline statin use, PCSK9 inhibitors led to a 57.8% decrease in LDL compared with placebo and a 33.8% decrease in LDL compared with ezetimibe. In studies with low baseline statin use, PCSK9 inhibitors led to a 53.8% decrease in LDL compared with placebo and a 36.7% decrease in LDL compared with ezetimibe.

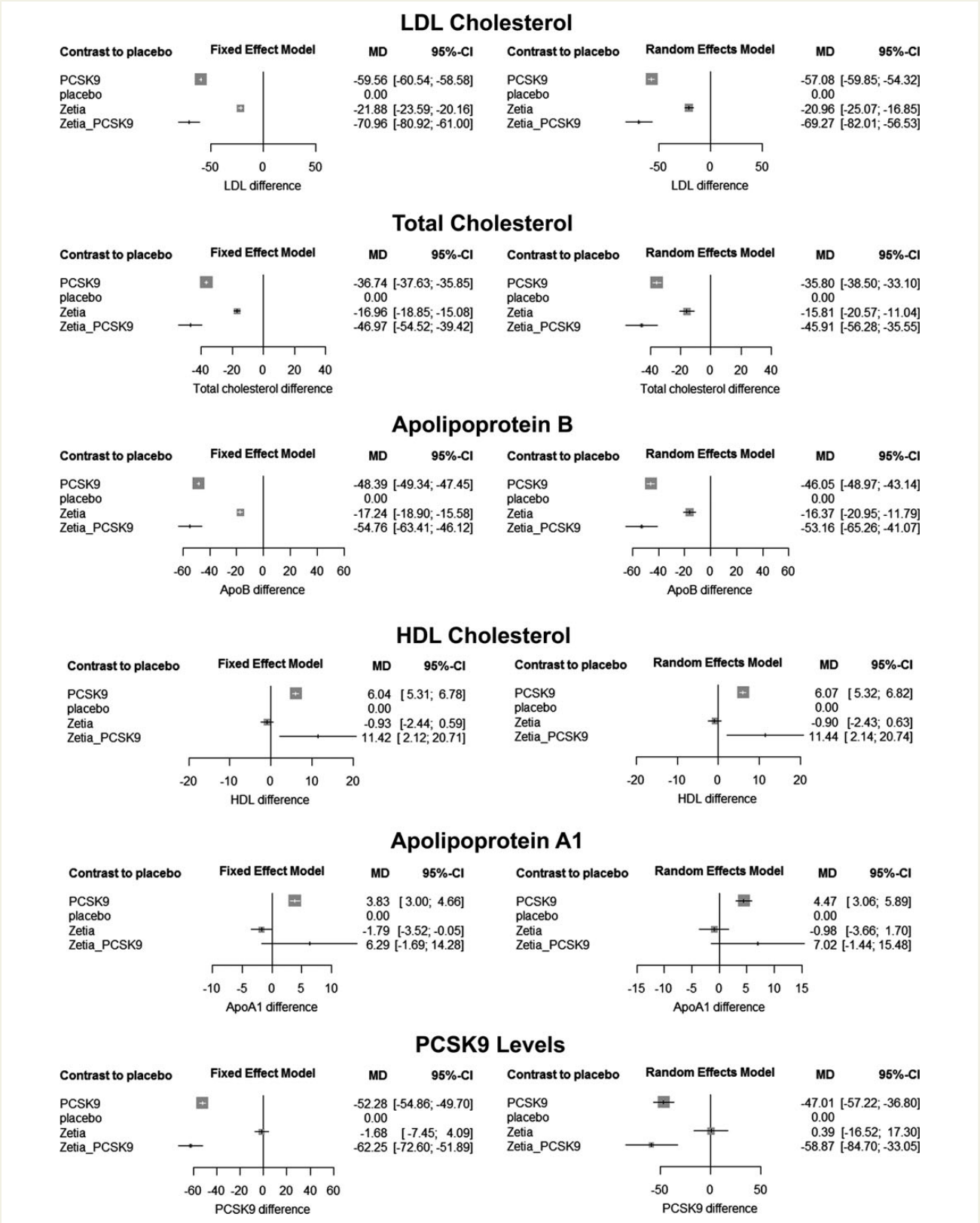

Figure 2 Forest plots generated using a frequentist approach providing both fixed-effects and random-effects models to assess the impact of proprotein convertase subtilisin-kexin type 9 serine protease inhibitors, ezetemibe (Zetia), and the combination of proprotein convertase subtilisin-kexin type 9 serine protease inhibitors and ezetemibe on lipid and proprotein convertase subtilisin-kexin type 9 serine protease levels relative to placebo.

Table 4 Rank scores providing probability rank of therapy providing the greatest reduction in lipid and proprotein convertase subtilisin-kexin type 9 serine protease levels following therapy with the different treatment groups where the closer to one equates to the probability the therapy leads to the greatest reduction

	Ezetimibe + PCSK9 inhibitor	PCSK9 inhibitor	Ezetimibe	Placebo
LDL cholesterol	0.991	0.676	0.333	0.0
Apoliporotein B	0.960	0.706	0.333	0.0
Total cholesterol	0.992	0.675	0.333	0.0
HDL cholesterol	0.047	0.291	0.957	0.706
Apolipoprotein A1	0.119	0.242	0.911	0.729
PCSK9 level	0.944	0.723	0.161	0.173

Proprotein convertase subtilisin-kexin type 9 serine protease inhibition and outcomes

As the majority of studies in our meta-analysis provided comparison of PCSK9 inhibitors alone and placebo, we generated forest plots with random-effects modelling to compare outcomes between the two groups. As seen in *Figure 3*, PCSK9 inhibitors significantly reduced the incidence of all-cause mortality (OR 0.43 [95% CI 0.22–0.82], I^2 0%, $P = 0.01$) with a trend toward reduced CV death (OR 0.50 [95% CI 0.22–1.13], $I^2 = 0\%$, $P = 0.10$) and CV events (OR 0.67 [95% CI 0.43–1.04], $I^2 = 20\%$, $P = 0.07$) when compared with placebo. However, neurocognitive adverse events were significantly increased with PCSK9 inhibitors compared with placebo (OR 2.34 [95% CI 1.11–4.93], $I^2 = 4\%$, $P = 0.02$). These findings appear to be largely driven by the findings of the ODYSSEY LONG TERM and OSLER 2 trials.[38,40] When limiting the analysis to studies with follow-up of 6 months or greater, there were significantly fewer CV events in the PCSK9 inhibitor group than in patients randomized to placebo (OR 0.54 [95% CI 0.38–0.77, $P = 0.0006$]). We next compared outcomes in patients randomized to PCSK9 inhibitors or ezetimibe and found no significant difference in all-cause mortality or CV events (*Figure 4*), though the number of patients and duration of follow-up is limited. Interestingly, patients on PCSK9 therapy had a trend toward less five-fold elevation of creatine kinase compared with placebo (OR 0.69 [95% CI 0.46–1.02], $I^2 = 6\%$, $P = 0.06$) and less three-fold elevation of LFTs (OR 0.72 [0.50–1.04], $P = 0.08$) (see Supplementary material online, *Figure S3*). However, there was a trend towards a greater number of any adverse events in the PCSK9 inhibitor group compared with placebo (OR 1.14 [95% CI 0.99–1.31], $P = 0.06$) but no difference in serious adverse events (OR 0.99 [95% CI 0.85–1.14], $P = 0.87$) or events leading to premature drug discontinuation (OR 1.12 [95% CI 0.85–1.48], $P = 0.40$) (see Supplementary material online, *Figure S4*). Visual assessment of funnel plots did not suggest evidence of publication bias. Additionally, there was no evidence of publication bias as assessed by Peters' test for all-cause mortality ($P = 0.39$), CV death ($P = 0.47$), or CV events ($P = 0.86$) when comparing PCSK9 inhibitors with placebo.

Meta-regression analysis did not show a significant association between study-level variables such as age, duration of follow-up, baseline statin therapy, or percentage of patients with CAD and the impact of PCSK9 inhibition on all-cause mortality, CV death, or CV events. Furthermore, there was no correlation with all-cause mortality or CV events and baseline lipid values such as LDL cholesterol, ApoB, or total cholesterol on meta-regression analysis. We further sought to assess whether there was a study-level correlation between the impact of PCSK9 therapy on all-cause mortality, CV death, or CV events and between baseline LDL, percent decrease in LDL for the PCSK9 group, absolute decrease in LDL for the PCSK9 group, or the post-therapy LDL. As seen in *Table 5*, there was no significant study-level association between the LDL parameters and outcomes.

Discussion

The findings of our meta-analysis of 17 RCTs with 13 083 patients suggest that PCSK9 inhibitors reduce LDL cholesterol, total cholesterol, ApoB, Lp(a), and PCSK9 levels compared with both placebo and ezetimibe groups when on baseline medical therapy. The reduction in LDL cholesterol is on top of that seen with statin therapy, as shown in our subgroup analysis. Following therapy with PCSK9, the average baseline LDL of 122 mg/dL dropped to 51 mg/dL post-therapy with an absolute reduction of 71 mg/dL. There was a significant increase in HDL cholesterol levels with PCSK9 inhibition compared with placebo. Furthermore, PCSK9 therapy was associated with a significant reduction in all-cause mortality and a trend toward reduction in CV death and CV events during follow-up compared with placebo. It is important to note that the improvement in all-cause mortality is largely derived from the findings of ODYSSEY LONG TERM[38] and OSLER 2,[40] which had a longer follow-up and included a very large number of patients. These findings are very encouraging and suggest that ongoing large RCTs of PCSK9 therapy focusing on reduction of CV events may demonstrate conclusive evidence in favour of PCSK9 therapies for these outcomes without increasing the risk of adverse events. However, PCSK9 therapy was associated with a significant increase in neurocognitive adverse events compared with placebo. Proprotein convertase subtilisin-kexin type 9 serine protease inhibitors were not associated with an increased incidence in LFT and creatine kinase elevation compared with placebo. Thus, PCSK9 inhibitor therapy may serve as an excellent alternative for patients with statin intolerance. Further studies are needed to compare the relative impact of PCSK9

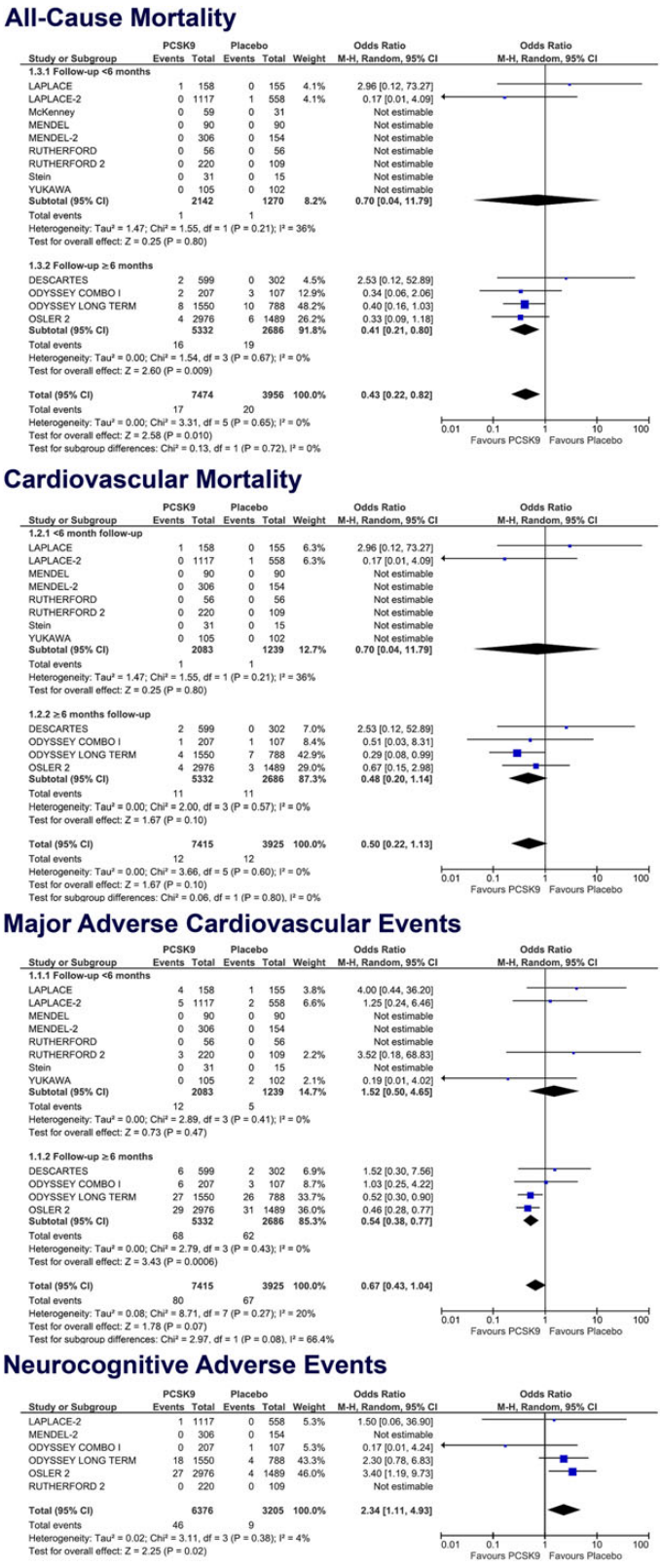

Figure 3 Forrest plots comparing the incidence of all-cause mortality, cardiovascular death, cardiovascular events, and neurocognitive adverse events for patients randomized to proprotein convertase subtilisin-kexin type 9 serine protease inhibitors or placebo. Data are presented with odds ratios and 95% confidence intervals.

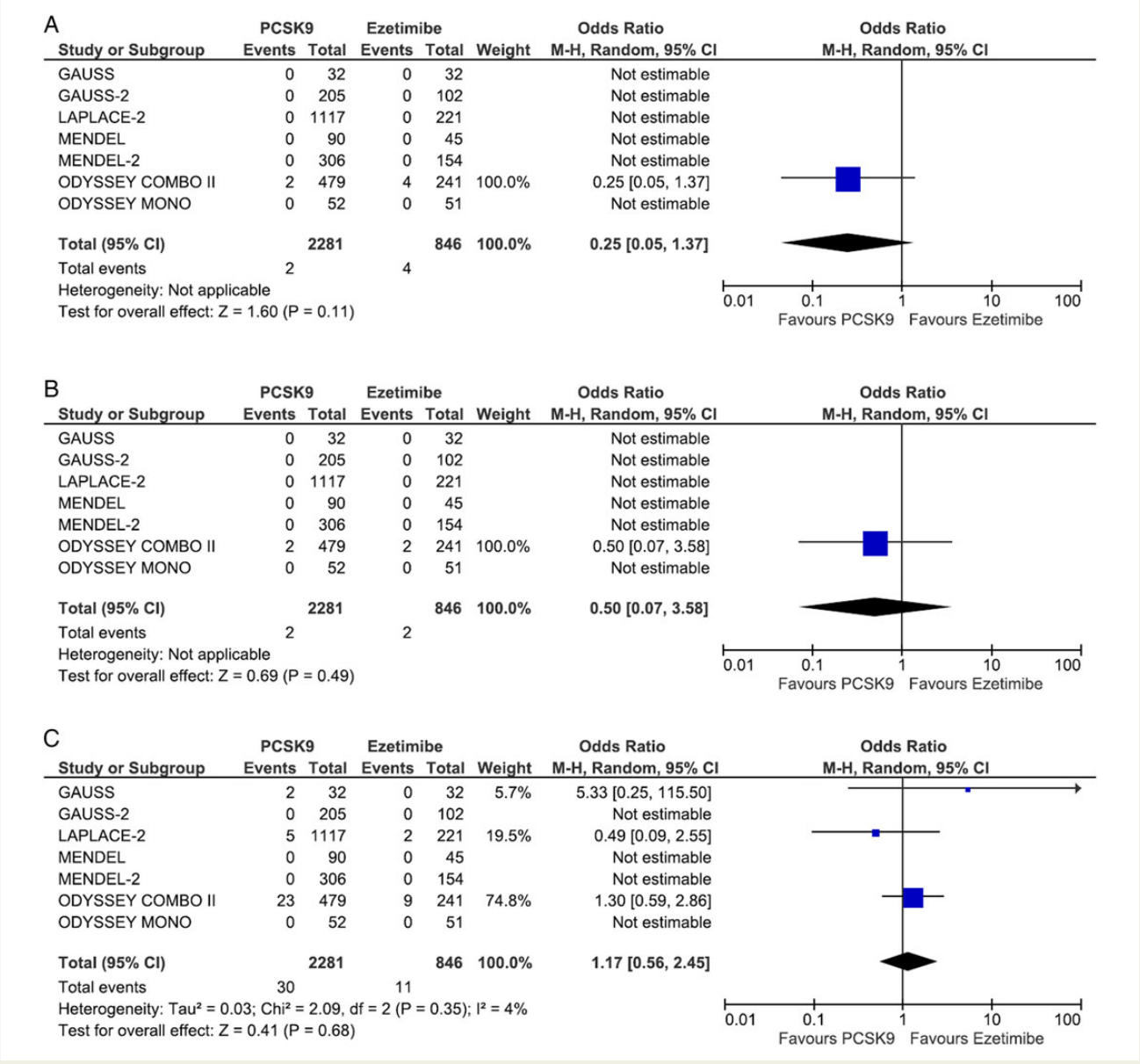

Figure 4 Forrest plots comparing the incidence of all-cause mortality (*A*), cardiovascular death (*B*), and cardiovascular events (*C*) for patients randomized to proprotein convertase subtilisin-kexin type 9 serine protease inhibitors or ezetimibe. Data are presented with odds ratios and 95% confidence intervals.

inhibition with other lipid-lowering therapies such as statins and ezetimibe on CV events. Interestingly, meta-regression analysis did not demonstrate a study-level association between outcomes and specific LDL parameters such as baseline LDL, post-therapy LDL, the percent change in LDL, or the absolute reduction in LDL. Thus, further large patient-level analysis will be necessary to determine whether the benefits in mortality with PCSK9 inhibitors have greater association with the degree of LDL reduction or the LDL level achieved following therapy.

Despite setbacks with torcetrapib[3] and niacin,[4] which were likely a result of adverse 'off-target' effects, the recent IMPROVE-IT trial suggested that further reduction in LDL cholesterol beyond that provided with statin therapy resulted in a significant reduction in CV events.[5] The results of IMPROVE-IT appear to support the findings of the meta-analysis of the Cholesterol Treatment Trialists' Collaboration who showed continued incremental benefit with LDL cholesterol reduction to 50 mg/dL.[2] While a meta-analysis was recently performed comparing lipid levels and outcomes with PCSK9 inhibitors with no PCSK9 inhibitor therapy,[45] our data provide comparison with ezetimibe to enable clinicians to determine the degree of additional change in lipid levels with PCSK9 inhibitors beyond that provided by ezetimibe. Given the improvement in CV outcomes seen with IMPROVE-IT and our meta-analysis of PCSK9 inhibitors, the question remains: Are target LDL cholesterol goals appropriate, or should we attempt to achieve the greatest tolerated LDL reduction possible? Another important question raised by these data are

Table 5 Meta-regression analysis demonstrating the study-level association between baseline LDL, percent decrease in LDL for the proprotein convertase subtilisin-kexin type 9 serine protease group, absolute decrease in LDL for the proprotein convertase subtilisin-kexin type 9 serine protease group, or the post-therapy LDL and the outcomes of all-cause death, cardiovascular death, or cardiovascular events

Variable	All-cause death Regression	*P*-value	CV death Regression	*P*-value	CV events Regression	*P*-value
Baseline LDL	−0.025 (95% CI −0.130 to 0.08)	0.65	−0.029 (−0.14 to 0.081)	0.60	−0.018 (−0.067 to 0.031)	0.473
Percent decrease in LDL	0.076 (−0.083 to 0.238	0.36	0.078 (−0.088 to 0.243)	0.358	0.054 (−0.034 to 0.141)	0.22
Absolute decrease in LDL	0.036 (−0.06 to 0.13)	0.47	0.043 (−0.058 to 0.144)	0.404	0.021 (−0.025 to 0.067)	0.38
Post-LDL	0.200 (−0.169 to 0.569)	0.29	0.253 (−0.121 to 0.627)	0.18	0.065 (−0.055 to 0.184	0.29

whether there is an LDL threshold at which lowering LDL beyond a certain value does not lead to further reductions in CV events. We eagerly await the large outcome studies with PCSK9 inhibitors as they may help answer the above questions. Furthermore, PCSK9 inhibitors may serve as a value therapy for those patients with statin intolerance and patients with heterozygosity for familial hypercholesterolemia who remain above goal LDL despite maximal cholesterol-lowering therapy.

The development of PCSK9 inhibitors highlights the importance of translating findings in basic science to patient care. Our basic understanding of hypercholesterolaemia continues to grow as it has not been long since mutations in the PCSK9 gene were shown to result in a gain of function for PCSK9,[46] leading to greater binding of the LDL receptor by PCSK9 and therefore reduced hepatic intracellular uptake of LDL cholesterol by the LDL receptor.[47] While PCSK9 inhibitors significantly reduce LDL cholesterol in all patient groups, there was a significant increase in neurocognitive events with PCSK9 inhibitors compared with placebo. Ongoing, large, adequately powered RCTs with long-term follow-up will help determine whether PCSK9 therapy significantly reduces all-cause mortality and CV events and whether this increases the risk of neurocognitive adverse events.

This analysis has several limitation inherent to meta-analysis, including the lack of raw or uniform data. Network meta-analysis has theoretical limitations given the incorporation of data from direct and indirect comparisons of different drugs and dosages. Though random-effects pooling reduces heterogeneity, another limitation is the heterogeneity observed among studies with different baseline CV risk and baseline therapy. However, heterogeneity in our analysis appeared to be low and there did not appear to be publication bias. Studies included in this meta-analysis were not powered to assess outcomes and therefore the results of the meta-analysis should be viewed as hypothesis generating. We only included neurocognitive adverse event data from studies that specifically defined an outcome as an adverse neurocognitive event. Another limitation of the analysis is the non-uniform definition of CV events between the studies. Furthermore, we did not include data in abstract form in our meta-analysis which limits the data included in the meta-analysis but resulted in more thorough and detailed data on lipid levels and outcomes. Finally, meta-regression techniques are limited given the lack of raw patient information and should therefore be viewed with caution and as hypothesis generating.

Conclusion

Proprotein convertase subtilisin-kexin type 9 serine protease inhibitors were associated with significant reduction in LDL cholesterol, total cholesterol, apolipoprotein B, lipoprotein (a), and PCSK9 levels compared with placebo and ezetimibe. Furthermore, PCSK9 inhibitors were associated with a reduction in all-cause mortality compared with placebo in patients with primary hypercholesterolaemia, though there was an increased risk of neurocognitive adverse events. Once approved for marketing, PCSK9 inhibitors may serve as an excellent alternative for patients with statin intolerance and for those who do not respond to conventional lipid reduction therapy.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Authors' contributions

M.J.L., U.B., R.O.E., and G.B.-Z..: performed statistical analysis. R.T., H.B.B., and R.W.: handled funding and supervision. M.J.L., U.B., and R.O.E.: acquired the data. M.J.L., U.B., G.B.-Z., R.T., H.B.B. Jr, and R.W.: conceived and designed the research. M.J.L., U.B., R.O.E., G.B.-Z., T.L., and N.C.B.: drafted the manuscript. M.J.L., U.B., R.O.E., G.B.-Z., T.L., N.C.B., R.T., H.B.B., Jr, and R.W.: made critical revision of the manuscript for key intellectual content.

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