Systematic review protocol

PCSK9-targeting monoclonal antibodies for the management of hypercholesterolemia: a systematic review and meta-analysis

Aris Liakos, MD,[1] Eleni Athanasiadou, MSc,[1] Anna-Bettina Haidich, PhD,[2] Evangelos Rizos, MD PhD,[3] and Apostolos Tsapas, MD PhD MSc[1, 4]

[1] Clinical Research and Evidence-Based Medicine Unit, Second Medical Department, Aristotle University Thessaloniki, Thessaloniki, Greece

[2] Department of Hygiene and Epidemiology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

[3] Second Medical Department, University of Ioannina, Ioannina, Greece

[4] Harris Manchester College, University of Oxford, Oxford, United Kingdom

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BACKGROUND

Cardiovascular disease is a major cause of death associated with several risk factors, including hypercholesterolemia. Statins are currently the mainstay of lipid-lowering management due to their well-established ability of reducing cardiovascular events and all-cause mortality.[1] Beyond statins, there is only weak evidence supporting the use of other available hypolipidemic drugs for cardiovascular protection. Hence, current U.S. guidelines have focused on the safety of non-statin hypolipidaemic agents, stating that these agents should be used whenever evidence for cardiovascular protection is robust.[2] However, some patients may be intolerant to statins, mainly due to muscle-related adverse events.[3] Moreover, subjects at particularly high cardiovascular risk treated with maximal potent statin dose might experience incremental benefits from further reductions in LDL-C.[4] Finally, high intensity treatment with statins cannot address residual cardiovascular risk in patients with heterozygous familial hypercholesterolemia, a relatively common disorder affecting

approximately 1 in 500 people, who have markedly elevated LDL-C levels and require rigorous management of hypercholesterolemia.[5] Statin therapy is also insufficient for the management of the rare homozygous form of this disorder.

Hepatic uptake through the LDL receptor is the principal determinant of circulating LDL-C levels. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a secreted glycoprotein mainly expressed in the liver that plays an important role in cholesterol homeostasis. PCSK9 binds on the LDL receptor in hepatocytes and inhibits its recycling in the cell membrane by accelerating its degradation in the lysosome.[6] Gain-of-function mutations in PCSK9 have been identified in patients with autosomal dominant hypercholesterolemia.[7] Moreover, PCSK9 inhibition using monoclonal antibodies results in higher LDL-C clearance rate, thus lowering LDL-C plasma levels.[6] Several monoclonal antibodies targeting PCSK9 are currently being assessed in extensive phase 3 clinical trials programs with promising preliminary results.[8] We will conduct a systematic review and meta-analysis to assess the efficacy and safety of PCSK9-targeting monoclonal antibodies as monotherapy or add-on treatment in patients with familial and non-familial hypercholesterolemia.

METHODS

This systematic review will be reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses.[9] The protocol will be prospectively registered with the PROSPERO database (Centre for Reviews and Dissemination, University of York).

Data sources and searches

Keywords for searching for relevant studies include drug-category-defining terms and generic and investigational new drug names: PCSK9, proprotein convertase subtilisin/kexin type 9, evolcumab, AMG145, alirocumab, REGN727, SAR236553, RN316, PF04950615, RG7652, MPSK3169A, LGT209.

Electronic databases will be searched, including:

- MEDLINE (via Ovid)
- EMBASE (via Ovid)
- The Cochrane Library

We will use both free-text words and controlled vocabulary (Medical Subject Headings and EMtree terms), combined with a sensitivity-maximizing search filter to restrict our results to randomized controlled trials (RCTs) and observational studies.[10, 11] A detailed search strategy is presented in **Appendix**. Upon re-execution of the initial search, additional or modified search terms may be used in order to accommodate advances in drug nomenclature.

Grey literature sources will also be searched, including:

- Hand-search of abstracts presented at major scientific conferences since 2010 (we will also consider any associated posters, e-posters and oral presentations where available) including:
  - American Heart Association Annual Scientific Sessions
  - American College of Cardiology Annual Meeting
  - European Society of Cardiology Annual Meeting
  - European Atherosclerosis Society Annual Meeting
  - American Association of Clinical Endocrinologists Annual Meeting

  Clinical trials evaluating PCSK9-targeting antibodies were launched only recently, hence extending search of gray literature sources beyond 2010 would probably yield no additional information.
- Publicly available regulatory authorities' reports.
- Search for completed trials with undisclosed results in the International Clinical Trials Registry Platform run by the World Health Organization, which groups records of multiple primary registries (http://apps.who.int/trialsearch/).
- Websites of relevant pharmaceutical companies.

Finally, we will subject eligible studies and relevant reviews to a forward citation search to identify any additional eligible studies.

Eligibility criteria and study selection

For our systematic review we will consider data from studies that assess any anti-PCSK9 monoclonal antibody in adult patients with familial or non-familial hypercholesterolemia. Hypolipidemic treatment is recommended at different LDL-C levels based on cardiovascular risk stratification. We will include all eligible studies irrespective of definition of

hypercholesterolemia utilized, year of publication or publication status. We will impose no language restrictions. For our meta-analysis, we will consider only RCTs that compare an anti-PCSK9 monoclonal antibody with placebo or any other lipid-lowering agent. In our systematic review we will also include any identified observational studies to fully elucidate the safety profile. Studies assessing other pertinent RNA-based technologies targeting PCSK9, such as RNA interference, monobodies or active immunization strategies (vaccines) will be excluded. All references will be imported in a reference management software (EndNote X7, Thomson Reuters). Study selection will be carried out by two reviewers with expertise in systematic reviews working independently (A.L. and E.A.). We will resolve differences in opinion at any stage by consensus, after consulting with a senior reviewer (A.T.).

Data extraction

Data for each eligible study will be extracted in duplicate (A.L. and E.A.) using a predesigned data collection form in Microsoft Excel 2011 (Microsoft Corporation). Any discrepancies will be arbitrated by a third reviewer (A.T.) and resolved by consensus. The data extraction form will be pilot tested in a subset of eligible studies and subsequently finalized. From each eligible study we aim to extract the following information (non-exhaustive list):

- Study characteristics: relevant identifiers, PCSK9 inhibitor and comparator(s) used (including dose and dosing interval) and duration of the intervention.
- Participants' baseline characteristics: background antihyperlipidemic medication, type of hypercholesterolemia (familial or non-familial), demographic and somatometric data, and lipid profile. Where available, we will also extract number of participants with established cardiovascular disease and number of participants with risk factors associated with cardiovascular disease (including diabetes mellitus, smoking and arterial hypertension).
- Percentage of change (%) and absolute change (mg/dL) in fasting LDL-C concentration from baseline to the end of active treatment period **(primary outcome)**. If LDL-C is both measured by means of ultracentrifugation and calculated using the Friedewald formula, we will utilize data from direct measurements.

- To fully assess the effect of PCSK9 inhibitors on lipid profile, **secondary outcomes** include the percentage of change (%) from baseline to the end of active treatment period in fasting state for the following parameters:
  - Total cholesterol (TC)
  - High Density Lipoprotein Cholesterol (HDL-C)
  - Non-HDL-C
  - Triglycerides
  - Apolipoprotein B
  - Apolipoprotein A1
  - Lipoprotein(a)
  - Free PCSK9
- Safety outcomes: we will utilize a broad focus approach to collate a wide range of adverse events reported in eligible studies (including but not limited to injection-site reactions, upper respiratory tract infections, creatine kinase and aminotranferases elevations). We will seek to synthesize data from RCTs for the five most frequent adverse events and all other adverse events that will be regarded as serious.
- Hard endpoints including cardiovascular events (composite endpoint defined as acute coronary syndrome, coronary revascularization, stroke, transient ischemic attack, congestive heart failure requiring hospital admission, or death due to cardiovascular disease) and all-cause mortality.

We will convert all cholesterol and triglycerides concentration values (mmol/L) to mg/dL by multiplying with a factor of 38.66976 and 88.57396 respectively.

Where available, we will collate multiple reports for the same study population to maximize information yield. In case of studies with extension periods we will use the report with the longest duration of intervention. Moreover, we will request missing data via e-mail to authors of original reports. We will also send a reminder e-mail, if no reply is received within four weeks from the initial correspondence. In case that standard deviation values necessary for synthesis of results are not available, even after contacting the corresponding authors, we will impute them borrowing values from similar studies.[12]

Risk of bias assessment

We will assess risk of bias for change in LDL-C, TC, triglycerides and HDL-C, and select safety outcomes, according to the criteria set forth by the Cochrane Collaboration.[13] We will address the following domains at the study level: random sequence generation, allocation concealment and sponsorship. Blinding of participants and personnel, blinding of outcome assessment (if applicable), selective outcome reporting and incomplete outcome data will be assessed separately for every different outcome. Regarding incomplete outcome data in particular, relatively low (< 20%) and balanced attritions rates between treatment arms, intention-to-treat analyses and use of appropriate imputation methods to handle missing data will indicate low risk of bias for this domain.

Random sequence generation and incomplete outcome data will be used as key domains for efficacy and safety outcomes. We will use blinding as an additional key domain for safety outcomes. We will summarize risk of bias for every different efficacy and safety outcome based on an a priori formulated rule that overall risk will be considered high in the presence of high bias in any key domain, or low when low for all key domains. In all other cases overall risk of bias will be deemed unclear.

We will also conduct a meta-regression analysis to explore the impact of risk of bias assessments on meta-analytic estimates for our primary outcome, comparing results of studies at high risk or unclear of bias with those of studies at low risk of bias.

Data synthesis and statistical analysis

We will synthesize results if data from at least three studies are available. For studies testing multiple drug doses, we will analyze and present efficacy and dose-related safety outcomes for the highest available approved dose. For agents that have not received approval, we will utilize the highest most common dose. If different dosing schemas are available, we will conduct separate analyses per schema. For the remaining safety outcomes and hard endpoints, we will pool data for all treatment groups irrespective of dose and dosing schema of PCSK9 inhibitor.

Regarding efficacy outcomes, we will synthesize only data from trials with at least six weeks duration of treatment. We also plan to perform separate analyses for comparisons with

placebo and active controls. Finally, we will perform subgroup analyses for studies using anti-PCSK9 monoclonal antibodies as monotherapy or as add-on treatment, and for studies or subsets of studies enrolling patients with familial and non-familial hypercholesterolemia.

We will calculate weighted mean differences for continuous outcomes and odds ratios for dichotomous outcomes, with 95% confidence intervals. We will synthesize results using an inverse-variance weighted random effects model, to account for heterogeneity between studies beyond that attributed to sampling variability.[14] For safety outcomes with rare events, we will verify robustness of our analyses across a set of different pooling methods.[15]

We will assess presence of statistical heterogeneity by means of the chi-square-based Cochran Q test and the magnitude of heterogeneity by means of the I^2 statistic,[16] with P values < 0.10 and I^2 > 50% respectively representing high heterogeneity. All analyses will be undertaken in Stata 12.1 (StataCorp, Texas, USA).

Finally, we will explore publication bias both visually, by inspecting funnel plots for asymmetry, and formally, by applying the Egger's test,[17] if an adequate number of studies (> 10) is available and heterogeneity is minimal.[18]

Grading of evidence

We will summarize the evidence profile regarding use of PCSK9-targeting antibodies in patients with hypercholesterolemia using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.[19] Outcomes to be considered include change in LDL-C, TC, HDL-C and tryglicerides, incidence of cardiovascular events and incidence of select adverse events. Two reviewers working independently (A.L. and E.A.) will rate the quality of evidence across studies in terms of risk of bias, publication bias, imprecision, inconsistency and indirectness. We will resolve any disagreements upon discussion with a senior reviewer (A.T.). We will use GRADEpro 3.6 (GRADE Working Group) to produce a summary of findings table.

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Lifelong Learning", European Social Fund, National Strategic Reference Framework 2007-2013.

Conflicts of interest

E.C. has received speaker honoraria, consulting fees, and has been taking part in clinical trials with Novartis, Sanofi, AstraZeneca/BMS, MSD, Vianex, Amgen, Boehringer Ingelheim and Plus Pharmaceutical. The remaining reviewers declare no conflict of interest.

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Appendix. Search strategy for electronic databases.

MEDLINE (via Ovid)

#1 pcsk.mp.

#2 pcsk9.mp.

#3 pcsk-9.mp.

#4 proprotein convertase subtilisin.mp.

#5 protein convertase subtilisin.mp.

#6 kexin type.mp.

#7 evolcumab.mp.

#8 amg145.mp.

#9 amg-145.mp.

#10 alirocumab.mp.

#11 regn727.mp.

#12 regn-727.mp.

#13 sar236553.mp.

#14 sar-236553.mp.

#15 rn316.mp.

#16 rn-316.mp.

#17 pf04950615.mp.

#18 pf-04950615.mp.

#19 rg7652.mp.

#20 rg-7652.mp.

#21 MPSK3169A.mp.

#22 MPSK-3169A.mp.

#23 lgt209.mp.

#24 lgt-209.mp.

#25 or/1-24

#26 randomized controlled trial.pt.

#27 controlled clinical trial.pt.

#28 randomized.ab.

#29 placebo.ab.

#30 drug therapy.fs.

#31 randomly.ab.

#32 trial.ab.

#33 groups.ab.

#34 or/26-33

#35 exp animals/ not humans.sh.

#36 34 not 35

#37 Epidemiologic studies/

#38 Exp case control studies/

#39 Exp cohort studies/

#40 Case control.tw.

#41 (cohort adj (study or studies)).tw.

#42 Cohort analy$.tw.

#43 (Follow up adj (study or studies)).tw.

#44 (observational adj (study or studies)).tw.

#45 Longitudinal.tw.

#46 Retrospective.tw.

#47 Cross sectional.tw.

#48 Cross-sectional studies/

#49 or/37-48

#50 25 and 36

#51 25 and 49

#52 50 or 51

THE COCHRANE LIBRARY

#1 pcsk

#2 pcsk9

#3 pcsk NEXT 9

#4 *protein convertase subtilisin NEAR/2 kexin type 9

#5 *protein convertase subtilisin

#6 *protein convertase NEAR 9

#7 kexin NEAR 9

#8 evolcumab

#9 amg145

#10 amg NEXT 145

#11 alirocumab

#12 regn727

#13 regn NEXT 727

#14 sar236553

#15 sar NEXT 236553

#16 rn316

#17 rn NEXT 316

#18 pf04950615

#19 pf NEXT 04950615

#20 rg7652

#21 rg NEXT 7652

#22 mpsk3169a

#23 mpsk NEXT 3169a

#24 lgt209

#25 lgt NEXT 209

#26 or/1-25

EMBASE (via Ovid)

#1 pcsk.mp.

#2 pcsk9.mp.

#3 pcsk-9.mp.

#4 proprotein convertase subtilisin.mp.

#5 protein convertase subtilisin.mp.

#6 kexin type.mp.

#7 evolcumab

#8 amg145.mp.

#9 amg-145.mp.

#10 exp alirocumab/

#11 alirocumab.mp.

#12 regn727.mp.

#13 regn-727.mp.

#14 sar236553.mp.

#15 sar-236553.mp.

#16 rn316.mp.

#17 rn-316.mp.

#18 pf04950615.mp.

#19 pf-04950615.mp.

#20 rg7652.mp.

#21 rg-7652.mp.

#22 mpsk3169a.mp.

#23 mpsk-3169a.mp.

#24 lgt209.mp.

#25 lgt-209.mp.

#26 or/1-25

#27 Clinical trial/

#28 Randomized controlled trial/

#29 Randomization/

#30 Single blind procedure/

#31 Double blind procedure/

#32 Crossover procedure/

#33 Placebo/

#34 Randomi?ed controlled trial$.tw.

#35 Rct.tw.

#36 Random allocation.tw.

#37 Randomly allocated.tw.

#38 Allocated randomly.tw.

#39 (allocated adj2 random).tw.

#40 Single blind$.tw.

#41 Double blind$.tw.

#42 ((treble or triple) adj blind$).tw.

#43 Placebo$.tw.

#44 Prospective study/

#45 or/27-44

#46 Case study/

#47 Case report.tw.

#48 Abstract report/or letter/

#49 or/46-48

#50 46 not 49

#51 Clinical study/

#52 Case control study

#53 Family study/

#54 Longitudinal study/

#55 Retrospective study/

#56 Prospective study/

#57 Cohort analysis/

#58 (Cohort adj (study or studies)).mp.

#59 (Case control adj (study or studies)).tw.

#60 (follow up adj (study or studies)).tw.

#61 (observational adj (study or studies)).tw.

#62 (epidemiologic$ adj (study or studies)).tw.

#63 (cross sectional adj (study or studies)).tw.

#64 or/51-63

#65 26 and 50

#66 26 and 64

#67 65 or 66