Reviewer Comments

Reviewer 1

We thank the first reviewer for the excellent constructive comments

Major Comments

1. **This reviewer has only the concern that certain information may need to be updated if available. For instance, the results of phase III trials for Bococizumab, the OLE trial, and those of other studies related to PCSK9 inhibition.**

Response: In response to the first comment, we state on page 8, line 12 following new sentence "Recently, the preliminary results of a study of Bococizumab delivery using an auto-injector device (SPIRE-AI) reported successfully meeting co-primary endpoints of percent change from baseline in fasting LDL-C at week 12 and the delivery system success rate, defined as the percent of patients whose attempts to operate the pre-filled pen. SPIRE-AI is a 12-week, double-blind, placebo-controlled, randomized, parallel-group, multicenter, phase III clinical trial in 299 patients with hyperlipidemia or mixed dyslipidemia receiving statin therapy and whose LDL-C ≥70 mg/dL and assessed the efficacy, safety, tolerability and subcutaneous administration of Bococizumab 150 mg and 75 mg with a pre-filled pen".

Page 12, para 2, line 5: "The patients receiving 150 mg Alirocumab every 2 weeks were shown to have a 62% reduction in LDL as opposed to a 1% increase in LDL with placebo at 24 weeks. These results persisted at 78 weeks. In a post-hoc analysis, the reduction in LDL was also associated with reduction in the combined end-point of death from coronary artery disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke or unstable angina requiring hospitalization (1.7% with Alirocumab

versus 3.3% with placebo; HR = 0.52; 95% CI=0.31 - 0.9; p=0.02)”

Page 14, para 2, line 5 “Along similar lines, GAUSS-3 trial evaluated the efficacy of Evolocumab in 218 statin intolerant patients compared to ezetimibe. The initial phase of the study included administration of atorvastatin (20 mg) for 10 weeks and placebo randomized in a 1:1 fashion, followed by a 2-week washout period and crossover to alternate therapy for another 10 weeks. The patients who experienced muscle related adverse effects while on statin therapy and not on placebo were further enrolled in the second phase of the study, which was a 24 week double blinded randomized controlled trial to compare Evolocumab (420 mg/month divided in 3 doses) with ezetimibe (10 mg/day). At 24 weeks, LDL-C was reduced by 53% with Evolocumab compared to 17% with ezetimibe. Muscle-related side effects were reported in 21% patients on Evolocumab compared to 29% with ezetimibe with stoppage of drug administration due to muscle symptoms in 1% of patients in Evolocumab and 7% of patients on ezetimibe.”

Page 15, para 2: “In the Open-Label Study of Long-Term Evaluation Against LDL-C (OSLER) 1 and 2 trials 4465 patients were enrolled who had completed 1 of the phase 2 or phase 3 studies of Evolocumab (MENDEL-1, LAPLACE TIMI 57, GAUSS-1, RUTHERFORD-1, YUKAWA-1, MENDEL-2, LAPLACE-2, GAUSS-2, RUTHERFORD-2, DESCARTES, THOMAS-1 or THOMAS-2) and randomized to receive either Evolocumab (420 mg/month in OSLER-1 and 140 mg every 2 weeks or 420 mg once a month in OSLER-2 trial) plus standard therapy (n=2,976) or standard therapy (n=1,489). The median follow-up was 11.1 months. This study showed a 61% reduction in LDL-C with Evolocumab compared to standard therapy (95% CI 59% to 63%; p<0.001). Overall adverse events were in 69% of patients in Evolocumab group compared to 65% in standard therapy group. Of note, the

neurocognitive adverse events were low, but were more frequent in Evolocumab group and appeared to be unrelated to LDL level at the time of treatment. Composite adverse cardiovascular events (all –cause death, coronary events including myocardial infarction, unstable angina requiring hospitalization, or coronary revascularization, cerebrovascular events including stroke or transient ischemic attack, and heart failure requiring hospitalization) were significantly lower in patients with Evolocumab compared to standard therapy (HR=0.47; 95% CI 0.28 to 0.78; p=0.003).

The TAUSSIG trial (NCT01624142) is evaluating Evolocumab therapy in 300 patients with severe familial hypercholesterolemia to determine its efficacy and side effect profile. The results of this study are anticipated by March 2020. Preliminary results reported by Stein et al on 8 patients with LDLR-negative or LDLR defective homozygous familial hypercholesterolemia on stable drug therapy when treated with Evolocumab at 420 mg monthly for ≥ 12 weeks, followed by 420 mg every 2 weeks for another 12 weeks showed LDL reduction by 14% to 16% at 12 weeks with 2 week and 4 weeks dosing regimens respectively with no serious adverse events reported. Finally, the preliminary results of GLAGOV study (NCT01813422) evaluating 950 patients with coronary artery disease on lipid lowering therapy undergoing cardiac catheterization for changes in percentage atheroma volume after 78 weeks of Evolocumab therapy met primary and secondary endpoints and final results are to be reported in American Heart Association (AHA) conference in November, 2016."

Minor Comments

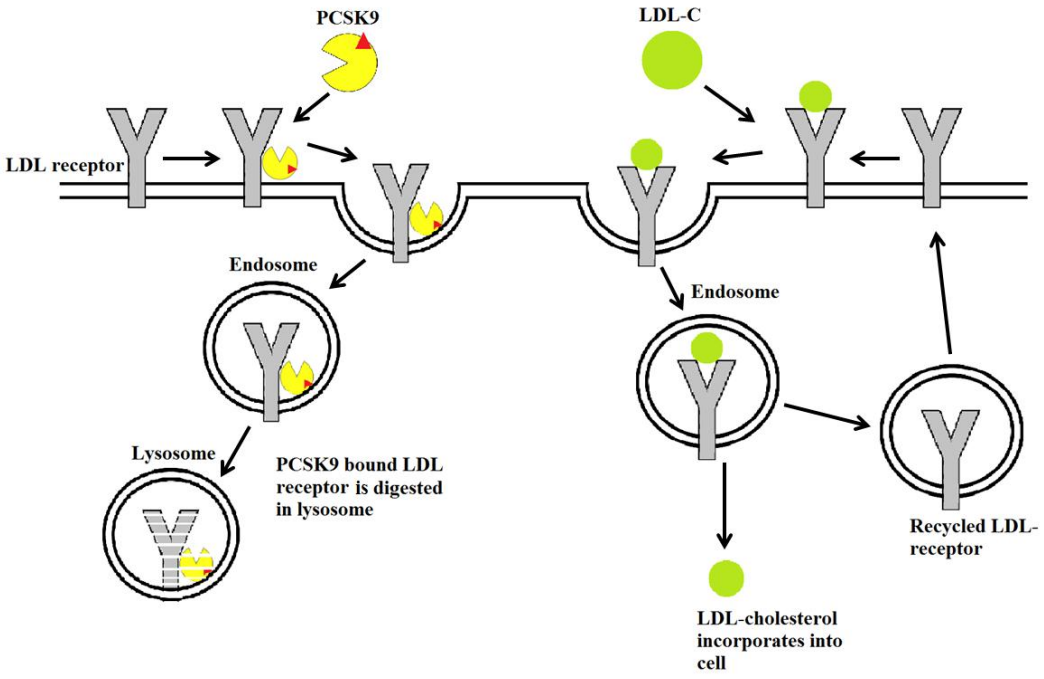
1. **If possible, on describing the role of PCSK9 in lipid metabolism, it would be excellent if a schematic illustration is available to facilitate the understanding of the detailed and yet complicated process.**

Response: In response to the first minor comment, we have added on page 6, paragraph 1, a figure illustrating PCSK9 metabolism

Changes in manuscript:

"PCSK9 binding inhibits this change and locks the LDLR in an open conformation which prevents its recycling. The LDLR is then routed to lysosomes for degradation (Figure 1)."

Figure 1: Mechanism and role of PCK9 in LDL-cholesterol metabolism



2. **Typos and minor grammatical mistakes may be minimized by careful proof-reading.**

Response: In response to the second minor comment we have made suggested changes in the manuscript.

Reviewer 2:

We thank the second reviewer for the excellent comments

Minor Comments

1. **Some dates need to be updated, which may stem from delays in the submission process: p8.: ....April 2016, should be available?; p.17.: July 2016, results available?**

Response: In response to the first minor comment, we have updated all the dates and results of the studies which were previously unpublished at the time of submission and are now published.

Changes in manuscript:

Response: In response to the first comment, we state on page 8, lines 12 following new sentence "Recently, the preliminary results of a study of Bococizumab delivery using an auto-injector device (SPIRE-AI) reported successfully meeting co-primary endpoints of percent change from baseline in fasting LDL-C at week 12 and the delivery system success rate, defined as the percent of patients whose attempts to operate the pre-filled pen. SPIRE-AI is a 12-week, double-blind, placebo-controlled, randomized, parallel-group, multicenter, phase III clinical trial in 299 patients with hyperlipidemia or mixed dyslipidemia receiving statin therapy and whose LDL-C ≥70 mg/dL and assessed the efficacy, safety, tolerability and subcutaneous administration of Bococizumab 150 mg and 75 mg with a pre-filled pen".

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versus 3.3% with placebo; HR = 0.52; 95% CI=0.31 - 0.9; p=0.02)”

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and appeared to be unrelated to LDL level at the time of treatment. Composite adverse cardiovascular events (all –cause death, coronary events including myocardial infarction, unstable angina requiring hospitalization, or coronary revascularization, cerebrovascular events including stroke or transient ischemic attack, and heart failure requiring hospitalization) were significantly lower in patients with Evolocumab compared to standard therapy (HR=0.47; 95% CI 0.28 to 0.78; p=0.003).

The TAUSSIG trial (NCT01624142) is evaluating Evolocumab therapy in 300 patients with severe familial hypercholesterolemia to determine its efficacy and side effect profile. The results of this study are anticipated by March 2020. Preliminary results reported by Stein et al on 8 patients with LDLR-negative or LDLR defective homozygous familial hypercholesterolemia on stable drug therapy when treated with Evolocumab at 420 mg monthly for ≥ 12 weeks, followed by 420 mg every 2 weeks for another 12 weeks showed LDL reduction by 14% to 16% at 12 weeks with 2 week and 4 weeks dosing regimens respectively with no serious adverse events reported. Finally, the preliminary results of GLAGOV study (NCT01813422) evaluating 950 patients with coronary artery disease on lipid lowering therapy undergoing cardiac catheterization for changes in percentage atheroma volume after 78 weeks of Evolocumab therapy met primary and secondary endpoints and final results are to be reported in American Heart Association (AHA) conference in November, 2016."

2. **p.10, last sentence, first paragraph: It is not possible to induce PCSK9 by statins in patients not taking statins.**

Response: In response to the second minor comment we have deleted that line from manuscript.

Changes in manuscript:

"It was observed that Alirocumab remained effective for a longer period of time in patients not on statins."

3. **In the last chapter (p20 ff.) some "left overs" are found and should be corrected.**

    a. **"goal" should be removed (line 5 from bottom)**

Response: In response to the third minor comment we have removed it from the manuscript

    b. **The following sentence is hard to follow.**

Response: In response to the third minor comment we have made suggested changes in the manuscript.

Changes in manuscript:

Page 19, para 1, line 2 "Subsequently, the IMPROVE-IT trial demonstrated that addition of ezetimibe to simvastatin lowers LDL-C more than that achieved by simvastatin alone and that this reduction in LDL-C was associated with a greater reduction in CVD events compared with simvastatin alone. This study raises the issue of LDL-C treatment targets with a lower level of LDL-C corresponding to a lower risk of CVD events."

    c. **p21 top: PCSK9 inhibitors....This sentence does not make sense**.

Response: In response to the third minor comment, we have made suggested changes in the manuscript.

Changes in manuscript:

Page 19, para 2 "PCSK9 inhibitors are especially beneficial in the treatment of familial hypercholesterolemic patients who are intolerant to statins or have an elevated LDL-C level despite being on maximally tolerated statin therapy. Intuitively, addition of a PCSK9 inhibitor to low dose statin therapy will be more effective in lowering LDL and avoiding the side effects of statins, since low dose and high dose statin regimens have

yielded similar efficacy when combined with PCSK9 inhibitors.

d. **The costs for PCSK9 antibodies is estimated from NNT5 is 28. However, until now we have only surrogate parameters which are improved, but no hard end data like OS or CVE. Thus, a not should be added that costs may be reevaluated in the near future, considering the new endpoints.**

Response: In response to the third minor comment, we have made suggested changes in the manuscript.

Changes in manuscript:

Page 20, para 1, line 2: It should be noted that since there are limited data on clinical adverse cardiovascular events, cost effectiveness data might change once results from ongoing CVD endpoint studies are available.

There is some concern that cognitive impairments may be induced by PCSK9 Inhibitors. A note on this aspect should be added.

Response: In response to the third minor comment, we have made suggested changes in the manuscript

Changes in manuscript:

Page 18, para 1"In addition, data from trials evaluating Evolocumab and Alirocumab have shown a higher incidence of cognitive adverse events in patients treated with PCSK9 inhibitors (0.9% versus 0.3% for Evolocumab compared to standard care and 1.2% versus 0.5% for Alirocumab compared to placebo). It has been suggested that responder and ascertainment bias might have played a role in reporting of adverse cognitive events in the OSLER trial since the adverse events were not related to the degree of LDL-C lowering with no clustering in the LDL-C <25 mg/dL group relative to the 25-50 mg/dL or >50 mg/dL groups. However, patients in ODYSSEY LONG

TERM trial were blinded to treatment and followed for nearly 18 months. Also, the neurocognitive adverse events were measured subjectively and not verified by neurocognitive testing. A dedicated study evaluating neurocognitive adverse events with PCSK9 inhibitors is underway: Evaluating PCSK9 Binding antiBody Influence oN coGnitive HeAlth in High cardiovascUlar Risk Subjects (EBBINGHAUS) (NCT02207634). It is enrolling individuals without dementia or mild cognitive impairment at baseline randomized in a double-blind, placebo-controlled fashion to evaluate Evolocumab on background statin therapy versus statin therapy alone. The primary outcome being measured is Spatial Working Memory test; an assessment of executive function and the results are expected in September 2017.”

Reviewer 3:

We thank the third reviewer for the excellent constructive comments

1. **Prior studies have reported that lipid-lowering effects of statins were different between Caucasians and Asians. If data were available, authors should add a description regarding an effect of race and/or genetic factors on effectiveness and adverse effects of PCSK9 inhibitors.**

Response: We reviewed the existing literature but unfortunately, we were unable to find any available data regarding the effect of race or genetics on effectiveness and adverse effects of PCSK9 inhibitors.

2. **Authors are encouraged to add the outcome data of ODYSSEY LONG TERM and OSLER trials.**

Response: In response to the second comment, we have made suggested changes in the manuscript.

Changes in manuscript:

Response: In response to the second comment, we state on page 8, line 12 following new sentence "Recently, the preliminary results of a study of Bococizumab delivery using an auto-injector device (SPIRE-AI) reported successfully meeting co-primary endpoints of percent change from baseline in fasting LDL-C at week 12 and the delivery system success rate, defined as the percent of patients whose attempts to operate the pre-filled pen. SPIRE-AI is a 12-week, double-blind, placebo-controlled, randomized, parallel-group, multicenter, phase III clinical trial in 299 patients with hyperlipidemia or mixed dyslipidemia receiving statin therapy and whose LDL-C ≥70 mg/dL and assessed the efficacy, safety, tolerability and subcutaneous administration of Bococizumab 150 mg and 75 mg with a pre-filled pen".

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Page 14, para 2, line 5 "Along similar lines, GAUSS-3 trial evaluated the efficacy of Evolocumab in 218 statin intolerant patients compared to ezetimibe. The initial phase of the study included administration of atorvastatin (20 mg) for 10 weeks and placebo randomized in a 1:1 fashion, followed by a 2-week washout period and crossover to alternate therapy for another 10 weeks. The patients who experienced muscle related adverse effects while on statin therapy and not on placebo were further enrolled in the second phase of the study, which was a 24 week double blinded randomized controlled trial to compare Evolocumab (420 mg/month divided in 3 doses) with ezetimibe (10 mg/day). At 24 weeks, LDL-C was reduced by 53% with Evolocumab compared to 17% with ezetimibe. Muscle-related side effects were reported in 21% patients on Evolocumab compared to 29% with ezetimibe with stoppage of drug administration due to muscle symptoms in 1% of patients in Evolocumab and 7% of patients on ezetimibe."

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receive either Evolocumab (420 mg/month in OSLER-1 and 140 mg every 2 weeks or 420 mg once a month in OSLER-2 trial) plus standard therapy (n=2,976) or standard therapy (n=1,489). The median follow-up was 11.1 months. This study showed a 61% reduction in LDL-C with Evolocumab compared to standard therapy (95% CI 59% to 63%; p<0.001). Overall adverse events were in 69% of patients in Evolocumab group compared to 65% in standard therapy group. Of note, the neurocognitive adverse events were low, but were more frequent in Evolocumab group and appeared to be unrelated to LDL level at the time of treatment. Composite adverse cardiovascular events (all –cause death, coronary events including myocardial infarction, unstable angina requiring hospitalization, or coronary revascularization, cerebrovascular events including stroke or transient ischemic attack, and heart failure requiring hospitalization) were significantly lower in patients with Evolocumab compared to standard therapy (HR=0.47; 95% CI 0.28 to 0.78; p=0.003).

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3. **In page 6, the paragraph describing PCSK9 and melanoma metastasis is quite vague. If the circulating cholesterol level per se is associated with metastasis as written, this paragraph could be deleted, because the relationship between PCSK9 and metastasis is not established.**

Response: In response to the third comment, we have deleted this from the manuscript**.**

4. **There are several grammatical errors and misspelling so that proofreading should be done**

Response: In response to the forth comment, we have made suggested changes in the manuscript.

Reviewer 4

We thank the forth reviewer for the excellent constructive comments

1. **Before publishing, if possible, the authors should update their data from clinical trials such as (NCT01968980, NCT01813422 etc..).**

Response: In response to first comment, we have made suggested changes in the manuscript.

Changes in manuscript:

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