

# Proprotein convertase subtilisin/kexin type 9: an update on the cardiovascular outcome studies

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## KEYWORDS

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Inhibitors of the 3-hydroxy-3-methylglutaryl coenzyme A reductase enzyme, statins, are powerful cholesterol-lowering medications and have provided outstanding contributions to the primary and secondary prevention of coronary heart disease. Low-density lipoprotein cholesterol (LDL-C) is one of the major modifiable cardiovascular risk factors, indeed, every 1.0 mmol/L (38.7 mg/dL) reduction in LDL cholesterolaemia corresponds to a 21% lowering in the risk of major vascular events. In this context, the pharmacological approach with PCSK9 monoclonal antibodies is considered a promising non-statin therapeutic option for the management of lipid disorders in patients with persistent cardiovascular risk, including patients with diabetes mellitus. Data from two large clinical trials have indisputably demonstrated the efficacy of alirocumab and evolocumab in preventive major adverse cardiovascular events in high risk, secondary-prevention patients with clinical manifestation of atherosclerotic cardiovascular diseases. Finally, PCSK9 monoclonal antibodies did not increase the risk of serious adverse events, neurocognitive events, new-onset of diabetes, muscle-related events, or myalgia.

## Introduction

Low-density lipoproteins (LDLs) represent the most significant biochemical variable associated with atheroma. Indeed, in the process of atherogenesis, changes in endothelial permeability and the composition of the extracellular matrix are associated to the entry and retention of cholesterol-containing LDL particles in the artery wall.<sup>1</sup> The importance of LDL lowering has been clearly demonstrated by numerous meta-analyses showing that lowering low-density lipoprotein cholesterol (LDL-C) by statins reduces the risk of cardiovascular diseases (CVDs). Moreover, prolonged exposure to lower LDL-C beginning early in life, i.e. in the case of polymorphisms associated with lower LDL-C levels, is associated with a greater reduction in the risk of CVD.<sup>2</sup> Based on this evidence, it is worth

to understand how low we can go with LDL safely. Already in 2014, data from statin trials demonstrated that patients achieving very low LDL-C levels had a lower risk to develop major cardiovascular (CV) events vs. those achieving moderately low levels.<sup>3</sup>

Next step in this field was the IMPROVE-IT trial in which ezetimibe added on top of statin led to an incremental fall in LDL-C with a further benefit in the terms of clinical outcomes.<sup>4</sup> Prominent in this area was the approval of two fully human monoclonal antibodies against proprotein convertase subtilisin/kexin type 9 (PCSK9), i.e. alirocumab (IgG1) and evolocumab (IgG2), that have been approved in the USA and in the European Union in August 2015. Heterozygous (HeFH) and homozygous (HoFH) familial hypercholesterolaemic patients can be treated with evolocumab, whereas alirocumab may be prescribed only to HeFH patients. Bococizumab, the third mAbs which underwent a large Phase 3 trial programme, was discontinued

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due to the antidrug antibodies formation leading to an over-time attenuation in the improvement vs. placebo of lipid parameters. Bococizumab was a humanized antibody.<sup>5</sup>

In particular, trials with PCSK9 monoclonal antibodies, e.g. the FOURIER (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk) study, reconfirmed a strong linear relationship between achieved LDL-C and CV events, an effect going down to LDL cholesterolaemia <8 mg/dL.<sup>6</sup> As of now, the best characterized activity of secreted PCSK9 is to post-translationally regulate the number of cell-surface LDL receptors (LDLRs). PCSK9 binds to the LDLR, not allowing the uptake of LDL particles from extracellular milieu into cells; by this mechanism, PCSK9 raises LDL-C levels.<sup>7</sup> Besides this evidence, some other pleiotropic effects have been described: PCSK9 is expressed in endothelial cells, vascular smooth muscle cells and, at low level, in macrophages. Vascular smooth muscle cells produce more PCSK9 than endothelial cells do, especially in response to shear stress.<sup>8</sup> PCSK9<sup>-/-</sup> mice are partially protected from neointimal formation, further supporting the positive effect of PCSK9 on intimal thickening.<sup>9</sup> In humans, serum PCSK9 levels are linearly associated with a higher necrotic core fraction in coronary atherosclerosis<sup>10</sup> and significantly associated with arterial stiffness.<sup>11</sup> Recently, we have added circulating platelets to the list of targets of PCSK9,<sup>12</sup> together with a direct pro-inflammatory effect on macrophages,<sup>13</sup> and a positive effect on arterial and valvular calcification.<sup>14</sup>

Since PCSK9 inhibitors reduced major adverse cardiac events (MACEs) {RR 0.83 [95% confidence interval (CI): 0.78-0.88]}, but did not clearly reduce mortality [RR 0.93 (95% CI: 0.85-1.02)],<sup>15</sup> the aim of this review article is to briefly summarize the CV preventive activity of PCSK9 inhibition which was evaluated in the ODYSSEY OUTCOMES (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) and the FOURIER Phase 3 trials.

## Alirocumab

The ODYSSEY OUTCOMES (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) trial, with a median follow-up of 2.8 years, enrolled patients with an acute coronary syndrome, already on intensive or maximum-tolerated statin therapy. In order to achieve and maintain an LDL cholesterolaemia between 25 and 50 mg/dL, two different doses of alirocumab were used, i.e. 75 and 150 mg. LDL-C fell by 53 mg/dL which corresponds to a 55% decrement if compared to placebo. The Kaplan-Meier rates for the primary endpoint at 4 years showed that alirocumab taken every other week led to a 15% reduction [hazard ratio (HR) 0.85; 95% CI: 0.78-0.93] in the MACEs, namely the composite of coronary heart disease death, non-fatal myocardial infarction (MI), fatal or non-fatal ischaemic stroke, or unstable angina requiring hospitalization. Specifically, the HRs were 0.92 (0.76, 1.11) for coronary heart disease death ( $P=0.38$ ), 0.86 (0.77-0.96) for non-fatal MI ( $P=0.006$ ), 0.73 (0.57-0.93) for ischaemic stroke ( $P=0.01$ ), and 0.61

(0.41-0.92) for unstable angina.<sup>16</sup> Interestingly, patients given alirocumab experienced fewer non-fatal CV events and were less likely to die from a non-CV event, being these two findings statistically associated: HR 2.35 (95% CI: 1.98-2.73).<sup>17</sup> Pre-specified subgroup analyses for the primary endpoint showed that the benefit was driven by the effect seen in 5629 patients with baseline LDL-C >100 mg/dL. No significant differences between groups were found for the secondary endpoints of death from coronary heart disease and from CV causes. No statistical differences were seen in the incidence of adverse events, but local injection-site reactions (3.8% with alirocumab vs. 2.1% in the placebo).<sup>16</sup>

## Evolocumab

The efficacy of evolocumab in preventing MACEs, namely the composite of CV death, MI, stroke, was tested in atherosclerotic cardiovascular disease patients (ASCVD). From February 2013 to June 2015, a total of 27 564 patients were randomly assigned to receive either evolocumab (13 784) or placebo (13 780), 24% of whom were women. After 48 weeks, the primary endpoint occurred in 9.8% of patients at evolocumab and in 11.3% of those at placebo; the HR was 0.85 (95% CI 0.79-0.92), an effect primarily ascribable to MI (-27%), stroke (-21%), and coronary revascularization (-22%) reductions. A step-wise risk reduction was found: 12% during the first year and 19% beyond the first year. Secondary endpoints, namely the composite of CV death, MI, or stroke were also reduced, HR 0.80 (95% CI 0.73-0.88). A 16% reduction was seen during the first year reaching a 25% beyond the first year. LDL cholesterolaemia whose basal levels were 92 mg/dL dropped by 59% vs. placebo, i.e. -56 mg/dL. Interestingly, 87% of patients on evolocumab reached an LDL cholesterolaemia ≤70 mg/dL, 67% reached levels ≤40 mg, and 42% levels ≤25 mg/dL.

Eleven thousand and thirty-one patients had diabetes vs. 16 533 who did not, evolocumab reduced primary endpoint by a 17% vs. 13%, respectively. Same efficacy was seen for the secondary key points with a percentage reduction of 18% and 22%, respectively. Evolocumab did not raise the risk of new-onset diabetes in non-diabetics at baseline (HR: 1.05; 95% CI: 0.94-1.17) with no changes in the levels of glycated haemoglobin and fasting plasma glucose.<sup>18</sup>

In an effort to comply with a cost-efficient medicine, a *post hoc* analysis of FOURIER tested the hypothesis whether or not the timing from the most recent MI, the number of prior MIs, and the presence of residual multivessels CAD could identify the most suitable subgroup benefitting the most from the therapy. Among a total of 22 351 patients, representing 81% of the overall FOURIER trial, evolocumab lowers both primary and secondary endpoints by 11% and 18%, respectively. When patients were stratified for timing of qualifying MI (<2 years), number of prior MIs (≥2), and residual multivessel coronary diseases (presence) the relative risk reduction was 20%, 18%, and 21% in the high-risk subgroup vs. 5%, 8%, and 7% in those at lower risk, i.e. qualifying MI >2 years, one prior MI with no multivessel coronary diseases.<sup>19</sup>

A further *sub-analysis* was the stratification of the FOURIER trials in peripheral artery disease (PAD) symptomatic patients vs those without. In patients with PAD, the absolute risk reduction for the primary endpoint was 3.5% (NNT = 29), a change rate superior to the 1.6% (NNT = 63) found in patients without PAD. Consistent with these data, evolocumab was superior vs. placebo in reducing major adverse limb events by 42% (HR 0.58; 95% CI: 0.38-0.88). This benefit followed a linear relationship with LDL-C reduction, at least for values <10 mg/dL.<sup>20</sup>

Finally, when the role of the inflammatory component, i.e. baseline high sensitivity C-reactive protein (hsCRP) levels, was considered, higher levels of C-reactive protein (CRP) were associated with a higher absolute risk reduction possibly due to an increased baseline ASCVD risk. Stratification according to baseline hsCRP, i.e. <1, 1-3, and >3 mg/dL, led to an absolute reduction of 1.6%, 1.8%, and 2.6% for the primary endpoint.<sup>21</sup>

## Conclusions

PCSK9 pharmacological therapy represents a new and valid approach for controlling hypercholesterolaemia with confirmed CVD protective effect. However, considering the effect of evolocumab on mmol/L reduction in LDL-C, treatment with evolocumab, in the FOURIER trial, reduced the risk of the primary outcome by 11.0% per mmol/L reduction in LDL-C.<sup>22</sup> The magnitude of this effect appears to be slightly <22% reduction in risk per mmol/L reduction in LDL-C during treatment with a statin as reported by CTT collaboration for primary outcome.<sup>23,24</sup> These apparently discrepant results have been attributed to a shorter follow-up of FOURIER (2.2 years) compared to trials conducted with statins (3-4 years).<sup>25</sup> However, it is still possible that therapies anti PCSK9 produce an anti-atherosclerotic activity which is mainly based on the reduction of the LDL-C levels, while statins may possess the so-called 'pleiotropic effects' beyond their lipid-lowering properties. This hypothesis is mainly based on the evidence that statins significantly reduced CRP levels, while evolocumab and alirocumab did not. In addition, differences in terms of lipid-lowering properties have been observed between statins and PCSK9 inhibitors. Inhibition of PCSK9 seems to have weaker effects on very-low-density lipoprotein lipids compared with statins for an equivalent lowering of LDL-C, which potentially translate into smaller reductions in CVD risk. Finally, while statin therapy is associated to muscle-related side effects, i.e. myalgia and myopathy, as well as increased risk of new onset of Type 2 diabetes, PCSK9 inhibitor therapy does not seem to be associated to these side effects.<sup>26</sup> Thus, in conclusion, both statins and PCSK9 inhibitors elicit an effective LDL-C lowering effect; however, significant differences can be observed between the two pharmacological approaches. Further clinical experience will better define the final outcomes between the two approaches.

**Conflict of interest:** none declared.

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