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Drug Profile

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Abstract

Introduction: Prescription of statins for low-density lipoprotein cholesterol (LDL-C) reduction is the standard of care in primary and secondary prevention of cardiovascular disease; nevertheless, a large number of patients treated with statins are unable to reach the recommended LDL-C targets. Therefore, there is need for safe and effective novel therapies for the pharmacological management of hypercholesterolaemia, in addition or as alternative to lipid-lowering therapies (LLT) currently in use.

Areas covered: In 2015, the Food and Drug Administration and the European Medicines Agency approved alirocumab (Praluent®; Sanofi), a fully human monoclonal antibody against proprotein convertase subtilisin/kexin type 9 (PCSK9), for the treatment of hypercholesterolaemic patients unable to meet LDL-C targets, as an adjunct to diet in addition/alternative to LLT. The authors review the pharmacological features, clinical efficacy, and safety of alirocumab in lowering LDL-C, and discuss its therapeutic perspectives based on the most recent clinical trials.

Expert commentary: Alirocumab causes a marked reduction in LDL-C, presents good safety and tolerability, and represents a promising approach for LDL-C lowering, particularly in patients with intolerance to statin or elevated LDL-C despite maximal statin therapy; nevertheless, further long-term data on safety and efficacy are necessary, such as data on the improvement of cardiovascular outcomes.

Keywords: alirocumab, LDL-cholesterol, hypercholesterolaemia, PCSK9, monoclonal antibody.

List of abbreviations

CV	cardiovascular
CVD	cardiovascular disease
EGF-A	epidermal growth factor A
HCV	hepatitis C virus
HDL-C	high-density lipoprotein cholesterol
HeFH	heterozygous familial hypercholesterolaemia
LDL-C	low-density lipoprotein cholesterol
LDL-R	LDL-receptor
LLT	lipid-lowering therapies
MACE	major adverse cardiovascular events
non-HDL-C	non-high-density lipoprotein cholesterol
PCSK9	proprotein convertase subtilisin/kexin type 9
Q2W	every 2 weeks
Q4W	every 4 weeks

1. Introduction

Hypercholesterolaemia, particularly elevated serum levels of low-density lipoprotein cholesterol (LDL-C), represents one of the most important risk factor in the pathogenesis of atherosclerotic lesions and for the development of cardiovascular disease (CVD). CVD is the leading cause of death globally, and more than 17.3 million people die of CVD every year. This number is dramatically expected to exceed 23.6 million by 2030[1].

When lovastatin – the first statin developed – had been approved in 1978, the reduction of LDL-C levels has become more achievable and the significant effect of statins in lowering LDL-C and preventing CVD have been well established by evidence from randomized clinical trials [2] and unequivocally shown by a recent meta-analysis [3]. Consequently, in addition to diet and other lifestyle interventions [4], prescription of statins for lowering LDL-C values is the standard of care in primary and secondary prevention of CVD [5,6]. Despite the fact that statins have undoubtedly been proven effective in reducing LDL-C, a significant number of patients is unable to reach the recommended LDL-C treatment targets, resulting in a considerable amount of individuals remaining at high-risk for CVD [7].

Reasons for suboptimal LDL-C levels are different. First, intolerance to statin therapy is not unusual in clinical practice and particularly musculoskeletal related events is one of the main reasons for the discontinuation of therapy or noncompliance [8]. Second, it was observed that increased plasma concentrations of proprotein convertase subtilisin/kexin type 9 (PCSK9) may be related with increases of statin dose and this can lead to statin resistance [9]. Genetic disorders, which cause markedly elevated LDL-C levels, are another condition where the intensive doses of the most potent statins do not allow to achieve the targets LDL-C levels in more than half of individuals [10]. Moreover, meta-analyses reported that high-dose statin therapy compared with moderate-dose

therapy is associated with an increased risk of developing diabetes mellitus [11]. Furthermore, some patients who are already receiving the maximal dose of statin and have reached the targets levels of LDL-C still have a residual cardiovascular (CV) risk [12]. Therefore, there is still need of safe and effective novel therapies for the pharmacological treatment of hypercholesterolaemia and prevention of CVD, in addition or in alternative to lipid-lowering therapies (LLT) currently in use.

Just when it seemed that we knew all about the major molecular players in LDL-C metabolism, another important protein appeared on the scene a few years ago. In 2003, a mutation characterized by gain of function in the PCSK9 gene was discovered in a French family with a form of familial hypercholesterolaemia inherited in an autosomal dominant manner, in absence of the classical mutations in the LDL-receptor (LDL-R) or Apolipoprotein B genes [13].

PCSK9 is one of the nine proteases of the subtilisin family of kexin-like proconvertases, it is produced ~~synthesized~~ most abundantly in the liver [14] and plays an important role in LDL-C homeostasis [15]. PCSK9 is synthesized in endoplasmic reticulum and consists of 692 amino acids. The protein contains three specific regions: a prodomain, a catalytic domain, and a C-terminal domain; the prodomain of the protein is cleaved and remains linked to the catalytic domain, subsequently, the complex between prodomain and catalytic domain is secreted into the plasma [14].

The action of PCSK9 released into the plasma is to regulate the amount of the LDL-R on the hepatocyte cellular surface. Plasmatic PCSK9 binds a specific region of the LDL-R extracellular domain – the epidermal growth factor A (EGF-A) region – and, in particular, the link between catalytic domain of PCSK9 and EGF-A region is formed by hydrophobic residues and polar interactions, and is strongly influenced by pH changes [16]. The complex between LDL-R and PCSK9 on the cellular surface is then internalized by endocytosis and routed to the lysosomes. In the acidic ambient of lysosomes, the affinity between LDL-R and PCSK9 increases for a supplementary interaction between the ligand-binding region of the LDL-R and the C-terminal region of PCSK9 [17]. As a consequence of this interaction, LDL-R is unable to recycle back to the

hepatocyte cellular surface and is degraded by lysosomal proteolysis. By promoting LDL-R degradation, PCSK9 reduces the concentration of LDL-R from hepatocyte surface, resulting in a lower LDL-C clearance rate and elevated levels of plasma LDL-C (Figure 1).

PCSK9 has received growing interest over the last years as an encouraging therapeutic target in the pharmacological treatment of hypercholesterolaemia, and its inhibition by the use of human monoclonal antibodies can represent an innovative therapeutic option for lowering LDL-C. Currently, data from clinical trials are available for three human monoclonal antibodies against PCSK9, two are fully human IgG1 and IgG2 respectively – alirocumab and evolocumab – while bococizumab is a humanized monoclonal antibody; however, the clinical development program for bococizumab was interrupted on November 2016. In fact, for its chemical structure, a significantly high level of immunogenicity – associated with a reduction in LDL-C lowering effect – as well as a high proportion in local injection site reactions has been observed [18].

This review focuses on the pharmacological features, clinical efficacy, safety, and tolerability of alirocumab in lowering LDL-C levels and discusses its therapeutic perspective based on the most recent clinical trials.

2. Introduction to Alirocumab

2.1. Chemistry

Alirocumab is a fully human monoclonal antibody – a G1 isotype immunoglobulin – that targets selectively PCSK9. It is produced by recombinant DNA technology and is composed of two disulfide-linked human heavy chains and two human kappa light chains; each single heavy chain is covalently linked through a disulfide bond to a kappa light chain, and in the constant regions of heavy chain is located a single N-linked glycosylation site. The PCSK9 binding site within the antibody is composed by the variable domains of the heavy and light chains that confer antigenic specificity [19]. The variable domains of the monoclonal antibody interact with a wide number of amino acid residues of the catalytic domain and in part with the prodomain of PCSK9; in particular,

the variable domains of the heavy chains occupy a region of the catalytic domain and form hydrophobic interactions and hydrogen-bonds [20]; as a consequence, when alirocumab is binding to catalytic domain of PCSK9 the interaction with the EGF-A region of LDL-R is hindered.

By binding to PCSK9 and by inhibiting the interaction between PCSK9 and LDL-R, alirocumab increases the amount of LDL-R on cellular surface and, consequently, the uptake of LDL particles in the liver, thereby lowering circulating levels of LDL-C.

The approximate molecular weight of alirocumab is 146 kDa [19].

Intriguingly, it should be considered that alirocumab is a fully human IgG1 while the second PCSK9 inhibitor approved in the United States and in the European Union – evolocumab – is a fully human IgG2. The human IgG1 presents high affinity for the Fcγ receptor expressed in many cells and is able to interact with the complement factor C1 – so this IgG isotype presents a good antibody-dependent cell-mediated cytotoxicity activity that may be optimal in the area of oncology – while the human IgG2 presents an extremely low affinity for the Fcγ receptor and is unable to interact with C1. However, when the neutralizing effect of IgG1/IgG2 against a plasma protein – such as PCSK9 – is the therapeutic objective, the two isotypes have the same efficacy and can successfully be used [21].

2.2. Pharmacodynamics

When alirocumab was administered in a single subcutaneous injection of 75 mg or 150 mg, the maximal suppression of plasma PCSK9 occurred in few hours – within four to eight hours – and it is strictly related to dose administered [22]; the maximal reduction in plasma LDL-C was obtained on day 15 [23].

2.3. Pharmacokinetics and metabolism

After a single-dose subcutaneous administration of 75 mg or 150 mg, alirocumab is absorbed into the bloodstream, and the maximum concentrations at a median time of relatively 3-7 days is

observed [19, 24]; similar pharmacokinetics were observed after its administration into the abdomen, upper arm, or thigh [23]. After subcutaneous administration alirocumab presented an absolute bioavailability of about 85% and steady state was achieved after 2 to 3 doses. In patients receiving alirocumab 75 mg or 150 mg subcutaneous every 2 weeks (Q2W), in absence of other LLT, the median apparent half-life of the monoclonal antibody at steady state was 17-20 days [19, 25]. Pharmacokinetics of alirocumab was not significantly influenced by age, gender, race, body weight, creatinine clearance and consequently, no specific dose adjustments was needed. So far, no data are available for patients with severe impairment of liver or renal functions [24].

3. Clinical efficacy

3.1. Phase I trials

The first studies on the effects of alirocumab in humans have been conducted by Stein and colleagues [25] (Table 1). They performed two randomized, single sequential-dose trials of alirocumab administered either intravenous or subcutaneous, in 72 healthy volunteers. The lowering in LDL-C ranged from 28% to 65% versus placebo when alirocumab was administered intravenous, while it was observed a reduction in LDL-C ranged from 32% to 45% versus placebo with subcutaneous injection. These short-term studies were followed by a randomized, sequential-dose, placebo-controlled trial in three different cohorts of subjects: adults with heterozygous familial hypercholesterolaemia (HeFH) who were taking atorvastatin, hypercholesterolaemic adults with non HeFH who were taking atorvastatin, and the last group consisted of subjects with non HeFH who were treated with diet only. Alirocumab given subcutaneously significantly reduced LDL-C by 39.2% to 61.0% versus placebo, in dose dependent manner [25].

3.2. Phase II trials

The efficacy of various doses of alirocumab has been evaluated in three Phase II randomized, controlled and double-blind trials in subjects with LDL-C values ≥100 mg/dL and stable statins therapy with a treatment duration ranged from 8 to 12 weeks (Table 1). In the first study, alirocumab in a dose dependently manner decreased LDL-C by 57.3% with 150 mg Q2W and by 18.2%, 20.9%, and 31.9% with 150 mg, 200 mg, and 300 mg every 4 weeks (Q4W) versus placebo [26]. In the second study, alirocumab induced a significant dose dependent effect in LDL-C reduction versus placebo equal to 34%, 59%, and 67% with the administration of 50 mg, 100 mg, and 150 mg Q2W, respectively, 38% and 43% with 200 mg and 300 mg Q4W [27].

In the last Phase II trial, the LDL-C reduction from baseline was 73.2% with alirocumab 150 mg Q2W plus 80 mg of atorvastatin, 66.2% with alirocumab 150 mg Q2W plus 10 mg of atorvastatin and 17.3% with placebo Q2W and 80 mg of atorvastatin [28]. Interestingly, researchers observed that the effect of alirocumab in LDL-C lowering was not significantly different with 80 mg or 10 mg of atorvastatin.

3.3. Phase III trials

The effect of alirocumab in lowering LDL-C has been evaluated in a large Phase III program (called ODYSSEY) comprising eighteen randomized, parallel-group, controlled, double-blind clinical trials. The percentage change in LDL-C at week 24 was the primary end point of ODYSSEY Phase III program (in one study only it was evaluated LDL-C change at week 6). The alirocumab dosing regimen was 75 mg Q2W starting dose increased to 150 mg Q2W at week 12 if participants did not achieved LDL-C targets, depending on their baseline CV risk. In four studies the starting dose of alirocumab was 150 mg; in two trials was used 150 mg and 300 mg Q4W, respectively.

Three studies investigated the effect of alirocumab in hypercholesterolemic patients at moderate to very-high CV risk not receiving statins (Table 2). In ODYSSEY MONO LDL-C was decreased by 31.6% with alirocumab 75 mg Q2W versus ezetimibe 10 mg/day [29], while in ODYSSEY ALTERNATIVE, alirocumab 150 mg Q2W decreased LDL-C by 30.4% versus ezetimibe [30]. In

ODYSSEY CHOICE II, patients were randomly assigned to placebo, alirocumab 150 mg Q4W or 75 mg Q2W. LDL-C was significantly reduced from baseline in both alirocumab treatment groups by 56.4% and 58.2%, respectively, versus placebo [31].

Seven trials evaluated the efficacy of add-on alirocumab in combination with stable doses of statin treatment in hypercholesterolemic patients with moderate to very-high CV risk (Table 3).

In ODYSSEY COMBO I, alirocumab 75 mg Q2W reduced LDL-C by 45.9% versus placebo [32], while in ODYSSEY COMBO II was observed a reduction in LDL-C by 29.9% versus ezetimibe [33]. In ODYSSEY OPTIONS I, patients with baseline atorvastatin 20 mg or 40 mg were randomized to add-on alirocumab 75 mg Q2W, or ezetimibe 10 mg/day; or double atorvastatin dose; or switch for atorvastatin 40 mg/day to rosuvastatin 40 mg/day. At week 24, it was demonstrated that among patients treated with atorvastatin 20 mg and 40 mg, respectively, add-on alirocumab provided a reduction in LDL-C levels by 44.1% and 54.0%; add-on ezetimibe provided a reduction in LDL-C levels by 20.5% and 22.6%; doubling of atorvastatin dose provided a reduction in LDL-C levels by 5.0% and 4.8%; and switching atorvastatin 40 mg/day to rosuvastatin 40 mg/day provided a reduction in LDL-C levels by 21.4% [34]. Similarly, in ODYSSEY OPTIONS II alirocumab 75 mg Q2W added to stable doses of rosuvastatin (10 or 20 mg/day) provided a reduction in LDL-C levels by 50.6% and 36.3%, respectively; add-on ezetimibe 10 mg/day provided a reduction in LDL-C levels by 14.4% and 11 %; and doubling of rosuvastatin dose (20 or 40 mg/day) provided a reduction in LDL-C levels by 16.3% and 15.9% [35]. In ODYSSEY JAPAN was observed a reduction in LDL-C by 64.1% with alirocumab 75 mg Q2W versus placebo [36]. In ODYSSEY LONG TERM alirocumab 150 mg Q2W reduced LDL-C levels at week 24 and 78 by 61.8% and 52%, respectively, versus placebo. [37]. In ODYSSEY CHOICE I patients receiving a LLT different from statin showed significant LDL-C reductions with values 52.7% and 50.2% lower than at baseline with alirocumab 300 mg Q4W and 75 mg Q2W compared to placebo, respectively, and by 58.8% and 51.6% in patients receiving concomitant statin [38].

Four studies investigated the efficacy of alirocumab Q2W in the HeFH-only population with elevated LDL-C despite the highest dose of statin therapy tolerated (Table 4). In ODYSSEY FH I and FH II alirocumab 75 mg decreased mean LDL-C values by 57.9% in FH I and by 51.5% in FH II, respectively, versus placebo [39]. In ODISSEY HIGH FH, performed in patients with LDL-C ≥160 mg/dL, alirocumab 150 mg decreased LDL-C levels by 39.1% versus placebo [40]. In ODYSSEY ESCAPE was investigated the effect of alirocumab in HeFH patients treated regularly with lipoprotein apheresis. At week 6 alirocumab 150 mg reduced preapheresis baseline LDL-C levels by 55.3% versus placebo with a drastic reduction in the rate of apheresis treatments [41].

At present, four of the eighteen ODYSSEY trials are still ongoing: in ODYSSEY OUTCOMES, about 18.000 patients with a history of acute coronary syndrome will be enrolled aiming to evaluate whether the adjunct of alirocumab versus placebo to maximal statin therapy reduces the incidence of major adverse cardiovascular events (MACE) [42]. In ODYSSEY OLE, an extension study for HeFH patients, the long-term safety, efficacy, and immunogenicity of alirocumab will be assessed [43]. In the last two studies ongoing, the efficacy and safety of alirocumab will be evaluated in high CV risk diabetic patients with mixed dyslipidemia and non-HDL-C ≥100 mg/dL (ODYSSEY DM-DYSLIPIDEMIA) or with LDL-C ≥70 mg/dL (ODYSSEY DM-INSULIN) on maximal LLT, respectively [44, 45].

In conclusion, the ODYSSEY trials clearly show that alirocumab, administered in different combinations, significantly reduces LDL-C. When we consider its efficacy as monotherapy, alirocumab reduced LDL-C by 56.4–58.2% versus placebo and by 30.4–31.6% versus ezetimibe [29-31]; in combination with stable statin doses, it reduced LDL-C from 45.9% to 64.1% versus placebo and from 27.5% to 30.8% versus ezetimibe [32-38]. Finally, in the HeFH population, alirocumab decreased LDL-C by 39.1–57.9% versus placebo [39-41]. Noteworthy it also the proportion of patients reaching risk-based LDL-C targets with alirocumab: in monotherapy the LDL-C targets were achieved by 41.9 –70.3% of patients [29-31]. In combination with stable doses

of statins, about 66.7–97.6% of patients achieved the LDL-C targets [32-38]; moreover, about 41.0–81.4% of patients with HeFH achieved their LDL-C targets [39-41].

In the ODYSSEY trials, a beneficial effect of alirocumab on several lipid variables has also been observed. Based on the different dose of alirocumab, comparator arm, background therapy and population studied, non-high-density lipoprotein cholesterol (non-HDL-C) decreased by 22.9–52.4%; high-density lipoprotein cholesterol (HDL-C) increased by 4.4–10% and apolipoprotein A-1 modestly increased by 2.9–7.2%; apolipoprotein B decreased by 21.4–54% and lipoprotein (a) consistently decreased by 14.6–41.4%. The effect of alirocumab on fasting triglycerides was variable, with a reduction versus placebo when combined with statins, but no effect when compared with ezetimibe [46, 47]. Interestingly, in the subgroups of individuals with diabetes, in whom an atherogenic lipid profile is more frequent, alirocumab improved LDL-C and non-HDL-C values versus placebo, with similar results observed in individuals with and without mixed dyslipidemia. Moderate improvements in triglycerides and HDL-C levels were also observed [48].

3.4. Cardiovascular outcomes

Based on the relevant effect in lowering LDL-C and other classical lipid variables, alirocumab, as well as other LLT [12, 49], may be very useful in reducing CV events. To this respect, in a post hoc analysis from ten ODYSSEY trials, for every additional reduction in 39 mg/dL of LDL-C, it was observed a further 24% lower risk of MACE [50]; in another post hoc analysis, the rate of MACE was 48% lower among hypercholesterolaemic subjects who received alirocumab versus placebo [37]. Currently, the results of a very recent trial on CV outcomes show that the adjunct of evolocumab to statin therapy provided a further significant 15% reduction in the risk of MACE [51]. Of course, definitive evidence of the clinical efficacy of alirocumab on CV events reduction will be available upon completion of the ongoing ODYSSEY OUTCOMES.

4. Safety and tolerability

Safety and tolerability of alirocumab was assessed by a large pooled safety analysis data from 14 randomized, controlled, double-blind phase 2 and phase 3 trials [52]. The safety data were divided according to the type of control (placebo/ezetimibe) and involved 3.340 patients treated with alirocumab, 1.276 treated with placebo, and 618 treated with ezetimibe. Overall the proportion of treatment-emergent adverse events, serious adverse events, discontinuations, and deaths was not significantly different between alirocumab and control groups.

In terms of adverse events of special interest (adverse events related to alirocumab chemical structure and mode of action, and frequently observed among other LLT and injectable monoclonal antibodies), a significantly higher proportion in local injection site reactions – graded as mild in severity and transient – were reported in patients treated with alirocumab (7.4%) versus placebo (5.3%). It was also reported a significantly higher incidence of pruritus (1.3%).

The incidence of musculoskeletal related events, nervous system disorders – neurologic, neurocognitive and ophthalmologic events – and abnormalities in liver function was similar in alirocumab and control groups.

Particular attention was paid to possible adverse events on glucose metabolism; to this regard no difference was observed in fasting plasma glucose and glycated hemoglobin over time between alirocumab and control groups; furthermore, based on phase 3 data, alirocumab did not increase the incidence of new-onset diabetes [53].

Adverse events of no special interest were generally well balanced between treatment groups, aside from upper respiratory tract infections, such as oropharyngeal pain, rhinorrhea, and sneezing, which were reported at a statistically higher rate with alirocumab (2.1%) versus placebo (1.1%).

Similar rates of treatment-emergent adverse events were also observed in the 14 ODYSSEY Phase III trials discussed above in which were included 4.112 patients treated with alirocumab, 1.554 treated with placebo, and 618 treated with ezetimibe (Table 5).

In terms of immunogenicity, 4.8% of patients had newly detected antidrug antibodies after receiving alirocumab treatment versus 0.6% of those receiving placebo; among subjects treated with alirocumab, only 1.2% exhibited neutralizing antibodies [24].

In conclusion, alirocumab is generally well tolerated and presents a favorable profile in terms of safety; the most frequent adverse events are local injection site reactions, pruritus, and clinical signs of upper respiratory tract infection.

5. Conclusions

Data of ODYSSEY trials show that alirocumab reduced LDL-C by 31.6–64.1% compared with control, and it allows to achieve LDL-C targets in several patient categories – including HeFH, moderate to very-high CV risk, and patients unable to tolerate statin therapy – with good safety and tolerability.

Based on the encouraging efficacy and safety data from the Phase III program, on July 24, 2015, the Food and Drug Administration approved alirocumab (Praluent®; Sanofi), for the pharmacological management of hypercholesterolaemia as an adjunct to diet and maximal tolerated statin therapy in adults with HeFH or clinical atherosclerotic CVD unable to achieve LDL-C targets. Two months later, the European Medicines Agency approved alirocumab in Europe for the pharmacological management of adult patients with primary hypercholesterolaemia or mixed dyslipidaemia, as an adjunct to diet: (a) in combination with a statin – with or without other LLT – in patients unable to achieve LDL-C targets despite the maximal tolerated statin therapy, or (b) alone, or (c) in combination with other LLT in patients intolerant to statin or for whom a statin is contraindicated. Recently, Glueck et al. have estimated that about 24 million of hypercholesterolaemic adults in the American population could be eligible for PCSK9 inhibitors prescription [54]; however, according to Intercontinental Marketing Services Health – an American company that provides information for the healthcare industry – nearly 9.500 prescriptions for alirocumab were written in the first six

months (August 2015 - February 2016) after Food and Drug Administration approval in United States [55].

The era of the human monoclonal antibodies against PCSK9 will significantly influence the future of hypercholesterolaemia management, particularly for patients at high CV risk unable to obtain LDL-C targets despite the maximal statin dose or unable to tolerate statins. However, longer term safety and efficacy data are needed, including further investigations on the possible improvement in CV events incidence.

6. Expert commentary

Prescription of statins for LDL-C reduction is the standard of care in primary and secondary prevention of CVD. Nevertheless, a large amount of patients treated with statins is unable to achieve the recommended LDL-C targets in routine clinical practice. The comprehensive review of Mitchell – in which 42 observational studies are identified – clearly shows that the majority of high CV risk patients do not reach the LDL-C levels recommended by the published guidelines. In particular, about 68–96% of very high CV risk patients did not reach an LDL-C target <70 mg/dL and about 62–94% of high CV risk patients did not reach an LDL-C target <100 mg/dL [56].

It should also be considered that some patients who are adequately controlled with the maximal recommended dose of a statin still have a residual CV risk [12], and some present statin-intolerance. Alirocumab causes a more marked reduction in LDL-C values than that obtained with statins or ezetimibe, and can offer an additional therapeutic option for the management of hypercolesterolaemia and, therefore, for the reduction in CV events. However, despite the significant efficacy and its apparent safety, some aspects still need to be investigated and clarified.

Firstly, the potential adverse effects of long-term extremely low LDL-C levels, so far never obtained with current LLT, are not known.

Cholesterol plays a key role in many biological functions: cellular membrane plasticity, brain and nerve function, steroid hormones synthesis [57]. An inverse relationship between serum cholesterol

level and nervous system disorders – in particular hemorrhagic stroke or neurocognitive impairment – has been observed [58]; furthermore, studies in rodents have shown that adrenal cells can reduce hormone production in the presence of extremely low cholesterol levels [59]. What is the current knowledge on the effect of extremely low LDL-C obtained with alirocumab on these aspects?

In ODYSSEY LONG TERM, neurocognitive impairments including amnesia, memory impairment, and confusional state were more common with alirocumab than placebo, but not statistically significant, in 78 weeks of follow-up [37]; however, the formal assessment of neurocognitive function as part of the study design was not performed. In the same study, gonadal and adrenal hormones plasma concentrations were also measured and no relevant change has been observed. Among alirocumab-treated patients, there was no increased incidence in adverse events for patients who achieved LDL-C values <25 mg/dL compared with those >25 mg/dL, including neurocognitive impairment [52]. However, in a very recent pooled safety analysis data from 14 phase 2 and phase 3 trials, an increase in incidence of cataract was found in subjects with LDL-C values <25 mg/dL, even though the incidence of this ophthalmological adverse event was not significantly different between alirocumab and control groups [60]; furthermore, a metanalysis of studies with alirocumab and evolocumab showed in the subgroup of the two larger and longer duration outcome studies an elevated incidence of neurocognitive adverse events (OR, 2.85; 95% CI, 1.34−6.06) in patients treated with monoclonal antibodies against PCSK9, although the incidence was rather low (<1%) [61]. Furthermore, it should be considered that elderly subjects with genetic loss of function in PCSK9 and very low LDL-C have a very low risk of CVD and do not exhibit neurocognitive impairments [62, 63]. However, it is also true that extremely low levels of LDL-C obtained with a pharmacological treatment are a condition completely different from to have genetically low levels of LDL-C.

The second important concern regards the long-term inhibition of the other functions of PCSK9. In fact, if the role of PCSK9 in cholesterol metabolism is rather clear, all its other physiological functions need to be further explored. Experimental data show that PCSK9 exhibits pleiotropic

effects: it may directly act as a trigger of inflammatory response, promoting the inflammatory response and the accumulation of oxidized-LDL in the subendothelial space [64]. Therefore, PCSK9-inhibition could reduce CVD, beyond lowering LDL-C alone. Nevertheless, with respect to a possible anti-inflammatory role, a meta-analysis of randomized controlled trial showed that PCSK9 inhibitors have not significant effect in lowering high-sensitivity C-reactive protein levels [65]. However, the meta-analysis presents some limitations: the baseline levels of high-sensitivity C-reactive protein were not elevated and the back-ground maximal dose of statin therapy could have reduced this marker of low-grade systemic inflammation [66]. Further investigations are needed to clarify if PCSK9 inhibitors could have anti-inflammatory effects.

PCSK9 is also expressed in brain, small intestine, adrenals, pancreas and kidneys, and its physiological role is still not clear [14]. Interestingly, the absence of PCSK9 in mouse increases the expression of the very low density lipoprotein receptor on the adipocytes surface with an improvement of triglycerides hydrolysis, free fatty acids uptake and visceral fat accumulation [67]. Furthermore, in rodents, it has been observed that PCSK9 is involved in the degradation of epithelial sodium channels expressed in the collecting ducts of the kidney, and this effect can reduce blood pressure [68]. In mice PCSK9 play also a critical role in liver regeneration [69] and seems to promote the lysosomal degradation of a wide number of LDL-R family members, including the hepatitis C virus (HCV) co-receptor [70]; therefore, it is possible that long term PCSK9-inhibition may reduce the physiological liver capacity to regenerate after injury or could increase HCV infectivity in humans.

Another important open question is the relation between LDL-C lowering drugs and glucose metabolism. A dose-dependently association between statin therapy and increased risk of developing diabetes has been observed [71] and mouse with loss of function in PCSK9 gene present impaired glucose tolerance [72]. Furthermore, in humans, polymorphisms in PCSK9 gene that associate with lower levels of LDL-C are also associated with a higher risk of type 2 diabetes [73, 74]; a recent pooled analysis of phase III trials failed to find any evidence that alirocumab increased

the risk of developing diabetes in 3448 hypercholesterolaemic patients without diabetes during a follow-up period of 6–18 months [53]; nevertheless, the gold standard for evaluating glucose intolerance, i.e. the oral glucose tolerance test, was not performed; furthermore, the increased risk of developing diabetes with statins has been observed after nearly 2 decades of prescriptions and millions of patients treated.

Further data are needed also for long-term concerns regarding development of neutralizing antibodies against alirocumab that could reduce the efficacy of the drug.

In addition to the long-term safety concerns, the last consideration is the cost of this therapy, especially for the present cost-conscious period of health care: the cost of $14,600 per year is still very elevated, especially compared to classic LLT, and will be a prominent consideration in establishing which high-risk patient subjects will be treated [75].

Based on all these considerations and the data available, statins still remain the standard of care in primary and secondary prevention; the prescription of PCSK9 inhibitors must be limited, at least for now, to some very-high risk patients unable to reach LDL-C targets, despite maximal tolerated statin therapy, such as patients with HeFH, or those in secondary prevention with very-high residual risk. Statin intolerance represents another condition where PCSK9 inhibitors could be used. However, presently, the proportion of patients with real intolerance to statins is very difficult to evaluate [76, 77] since most of these cases are simply bothersome rather than dangerous adverse events [78].

7. Five year view

Most of the above uncertainties will likely be clarified in the upcoming years by the results of the ongoing studies. In fact, there is growing interest relatively to the results of ODYSSEY OUTCOMES, available in 2018; this trial, which was performed to evaluate the effect of PCSK9 inhibition on MACE incidence, will provide definitive evidence of the clinical efficacy of alirocumab on CV events reduction [42]. In the upcoming years, possible concerns regarding long-

term safety, efficacy, and immunogenicity of alirocumab will be available from ODYSSEY OLE, which is expected to be complete in by mid-2017 [43]. Finally, in the next few months, data from ODYSSEY DM-DYSLIPIDEMIA [44] and ODYSSEY DM-INSULIN [45] will be presented; these two trials have been performed to evaluate the efficacy of alirocumab in diabetic patients, a particular population with high CV risk and frequently mixed dyslipidemia. The results of the four ongoing studies will determine the fate of this innovative biological drug, and the possible opening of a modern era in the pharmacological management of hypercholesterolaemia and CVD.

8. Key issues

- Over the three last decades, the treatment of hypercholesterolaemia with statins has shown a significant effect in lowering LDL-C levels and prevent cardiovascular events.
- Despite the validated efficacy of statins, a significant number of patients is unable to reach the recommended LDL-C treatment targets.
- Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a recently discovered protein that plays a crucial role in LDL-C homeostasis favoring the intracellular lysosomal degradation of LDL-C receptor.
- Alirocumab is a monoclonal antibody that targets selectively PCSK9. By binding to PCSK9 and by inhibiting the interaction between PCSK9 and LDL-R, alirocumab increases the number of LDL receptors on hepatocytes with a consequently reduction in circulating levels of LDL-C.
- Data of Phase III clinical trials show that alirocumab reduced LDL-C by 31.6–64.1% compared with control, and it allows to reach LDL-C targets in several patient categories.
- Alirocumab is generally well tolerated and presents a favorable safety profile.

- The results described above are promising and suggest that monoclonal antibodies against PCSK9 will influence the future of hypercholesterolaemia management, particularly for patients at high CV risk with inadequate LDL-C targets and maximal statin dose or unable to tolerate statins. The first results on CV events provided by the FOURIER trial reinforce this suggestion, although, longer term safety data are still needed.

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Declaration of Interest

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Table 1. Efficacy of alirocumab in healthy volunteers and in subjects with familial or nonfamilial hypercholesterolemia (Phase I and Phase II trials).

Author, Phase of clinical trial [reference]	**Study Population** participants, use of statin/LLT[a], LDL-C values, CV risk	**Mean baseline LDL-C** (mg/dL)	**Treatment**[b] (mg)	**Duration** (weeks)	**% LDL-C change from baseline to study end**[e]	**Difference between % LDL-C change** (ALI *vs* EZE/PL/STATIN)
Stein et al. 2012 Phase I trial [**25**]	40 M/F no statin/LLT LDL-C ≥100 mg/dL healthy subjects in absence of CV risk factors	135.3	ALI 0.3 mg/kg ALI 1 mg/kg ALI 3 mg/kg ALI 6 mg/kg ALI 12 mg/kg PL	single i. v. dose	– 34.2 – 48.3 – 63.5 – 62.8 – 71.5 – 6.1	**– 28.1** **– 42.2** **– 57.4** **– 56.5** **– 65.4**
Stein et al. 2012 Phase I trial [**25**]	32 M/F no statin/LLT LDL-C ≥100 mg/dL healthy subjects in absence of CV risk factors	129.7	ALI 0.3 mg/kg ALI 1 mg/kg ALI 3 mg/kg ALI 6 mg/kg PL	single s. c. dose	– 45.8 – 53.2 – 51.8 – 59.0 – 13.3	**– 32.5** **– 39.9** **– 38.5** **– 45.7**
Stein et al. 2012 Phase I trial [**25**]	21 HeFH M/F 41 no HeFH M/F statin (A 10, 20, 40)/diet alone LDL-C ≥100 mg/dL moderate/high CV risk	134.1[e] 165.0[f]	ALI 50 ALI 100 ALI 150 PL	s. c. dose on day 1, 29, 43	– 35.4 – 49.9 – 57.2[e] – 53.9[f] 3.8[e] 3.1[f]	**– 39.2** **– 53.7** **– 61.0**[e] **– 57.0**[f]
Stein et al. 2012 Phase II trial [**26**]	77 HeFH M/F statin/LLT LDL-C ≥100 mg/dL high/very high CV risk	149.2	ALI 150 q4w ALI 200 q4w ALI 300 q4w ALI 150 q2w PL q2w	12	– 28.8 – 31.5 – 42.5 – 67.9 – 10.6	**– 18.2** **– 20.9** **– 31.9** **– 57.3**
McKenney et al. 2012 Phase II trial [**27**]	183 M/F statin (A 10, A 20, A 40) LDL-C **≥100 mg/dL** moderate to very high CV risk	127.3	ALI 50 q2w ALI 100 q2w ALI 150 q2w ALI 200 q4w ALI 300 q4w PL q2w	12	– 39.6 – 64.2 – 72.4 – 43.2 – 47.7 – 5.1	**– 34.5** **– 59.1** **– 67.3** **– 38.1** **– 42.6**

Roth et al. 2012 Phase II trial [**28**]	92 F/M statin (A 10) LDL-C ≥ 100 mg/dL moderate to very high CV risk	122.6	A 10 + ALI 150 q2w A 80 + ALI 150 q2w A 80 + PL	8	– 66.2 – 73.2 – 17.3	**– 48.9** **– 55.9**

LLT lipid lowering therapy, *LDL-C* low-density lipoprotein cholesterol, *CV* cardiovascular, *vs* versus, *i.v.* intravenous, *s.c.* subcutaneous, *q2w* every 2 weeks, *q4w* every 4 weeks, *HeFH* Heterozygous familial hypercholesterolaemia, *M* male, *F* female, *ALI* alirocumab, *PL* placebo, *EZE* ezetimibe, *A* atorvastatin, *od* once daily.

[a]Fenofibrate, omega-3 fatty acid, nicotinic acid or bile acid sequestrants.

[b]Alirocumab was administered intravenous in the first trial descripted and subcutaneous in the other trials.

[c]In the first two trials was reported LDL-C change from baseline to study day with lowest value.

[e]Patients in statin therapy.

[f]Patients in only diet therapy.

Table 2. Efficacy of subcutaneous alirocumab in hypercholesterolaemic patients not receiving statin therapy (Phase III trials).

Author, trial name [reference]	**Study Population** participants, use of statin/LLT[a], LDL-C values CV risk	**Mean baseline LDL-C** (mg/dL)	**Treatment** (mg)	**Duration** (weeks)	**% LDL-C change from baseline to week 24**	**Difference between % LDL-C change** (ALI *vs* EZE/PL)
Roth et al. 2014 **ODYSSEY MONO** [**29**]	103 M/F no statin/LLT LDL-C between 100-190 mg/dL and moderate CV risk	139.7	ALI 75/150 q2w[b] EZE 10 od	24	– 47.2 – 15.6	**– 31.6**
Moriarty et al. 2015 **ODYSSEY ALTERNATIVE** [**30**]	314 M/F LLT LDL-C ≥70 mg/dL and very high CV risk or LDL-C ≥100 mg/dL and high or moderate CV risk	191.3	ALI 75/150 q2w EZE 10 od	24	– 45.0 – 14.6	**– 30.4**
Stroes et al. 2016 **ODYSSEY CHOICE II** [**31**]	233 M/F LLT LDL-C ≥70 mg/dL and very high CV risk or LDL-C ≥100 mg/dL and high or moderate CV risk	158.9	ALI 150 q4w ALI 75/150 q2w PL q2w	24	– 51.7 – 53.5 + 4.7	**– 56.4** **– 58.2**

LDL-C low-density lipoprotein cholesterol, *M* male, *F* female, *LLT* lipid lowering therapy, *CV* cardiovascular, *q2w* every 2 weeks, *q4w* every 4 weeks, *ALI* alirocumab, *PL* placebo, *EZE* ezetimibe, *vs* versus, *od* once daily.

[a]Fenofibrate, omega-3 fatty acid, nicotinic acid or bile acid sequestrants.

[b]The alirocumab dosing regimen was 75 mg Q2W starting dose titrated to 150 mg q2w at week 12 if LDL-C targets were not achieved.

Table 3. Efficacy of subcutaneous alirocumab in hypercholesterolaemic patients receiving maximal tolerated statin therapy with or without other LLT (Phase III trials).

Author, trial name [reference]	**Study Population** participants, use of statin[a]/LLT[b], LDL-C values CV risk	**Mean baseline LDL-C** (mg/dL)	**Treatment** (mg)	**Duration** (weeks)	**% LDL-C change from baseline to week 24**	**Difference between % LDL-C change** (ALI *vs* EZE/PL/STATIN)
Kereiakes et al. 2015 **ODYSSEY COMBO I** [**32**]	316 M/F statin±LLT LDL-C ≥70 mg/dL and very high CV risk or LDL-C ≥100 mg/dL and moderate/high CV risk	102.4	ALI 75/150 q2w[c] PL q2w	52	– 48.2 – 2.3	**– 45.9**
Cannon et al. 2015 **ODYSSEY COMBO II** [**33**]	720 M/F statin LDL-C ≥70 mg/dL and very high CV risk or LDL-C ≥100 mg/dL and moderate/high CV risk	107.3	ALI 75/150 q2w EZE 10 od	104	– 50.6 – 20.7	**– 29.9**
Basing et al. 2015 **ODYSSEY OPTIONS I** [**34**]	355 F/M 720 M/F statin (A 20/A 40) LDL-C ≥70 mg/dL and very high CV risk or LDL-C ≥100 mg/dL and moderate/high CV risk	105.1	ALI 75/150 q2w (A 20/A 40) EZE 10 od (A 20/A 40) A 40 od (double A 20) A 80 od (double A 40) R 40 od (switch A 40)	24	– 49.0 – 21.5 – 5.0 – 4.8 – 21.4	**– 27.5** **– 44.0** **– 44.2** **– 27.6**
Farnier et al. 2015 **ODYSSEY OPTIONS II** [**35**]	305 F/M statin (R 10/R 20) LDL-C ≥70 mg/dL and very high CV risk or LDL-C ≥100 mg/dL and moderate/high CV risk	111.3	ALI 75/150 q2w (R 10/R 20) EZE 10 od (R 10/R 20) R 20 od R 40 od	24	– 43.5 – 12.7 – 16.3 – 15.9	**– 30.8** **– 27.2** **– 27.6**
Teramoto et al 2015 **ODYSSEY JAPAN** [**36**]	216 F/M, 19% HeFH statin±LLT LDL-C ≥100 mg/dL and very high CV risk or LDL-C ≥120 mg/dL and moderate/highCV risk	143.0	ALI 75/150 q2w PL q2w	52	– 62.5 + 1.6	**– 64.1**
Robinson et al. 2015 **ODYSSEY LONG TERM** [**37**]	2341 F/M, 17% HeFH statin±LLT LDL-C ≥70 and high/very high CV risk	122.4	ALI 75/150 q2w PL q2w	78	– 61.0 + 0.8	**– 61.8**

Roth et al. 2016 **ODYSSEY CHOICE I** [**38**]	803 F/M 68% statin±LLT 32% ±LLT LDL-C ≥70 mg/dL and very high CV risk or LDL-C ≥100 mg/dL and moderate/high CV risk	141.8[d] 114.1[e]	ALI 300 q4w ALI 75/150 q2w PL q2w	48	– 58.8[d] – 52.7[e] – 51.6[d] – 50.2[e] – 0.3[d] – 0.1[e]	**– 58.5[d]** **– 52.6[e]** **– 51.3[d]** **– 50.1[e]**

LLT lipid lowering therapy, *LDL-C* low-density lipoprotein cholesterol, *CV* cardiovascular, *vs* versus, *q2w* every 2 weeks, *q4w* every 4 weeks, *HeFH* Heterozygous familial hypercholesterolaemia, *M* male, *F* female, *ALI* alirocumab, *PL* placebo, *EZE* ezetimibe, *A* atorvastatin, *R* rosuvastatin, *od* once daily.

[a]Maximal tolerated daily dose of statin (i.e. Atorvastatin 40–80 mg, Rosuvastatin 20–40 mg, Simvastatin 80 mg).

[b]Fenofibrate, omega-3 fatty acid, nicotinic acid or bile acid sequestrants.

[c]The alirocumab dosing regimen was 75 mg Q2W starting dose titrated to 150 mg q2w at week 12 if LDL-C targets were not achieved.

[d]Statin±LLT

[e]±LLT

Table 4. Efficacy of subcutaneous alirocumab in hypercholesterolaemic patients with HeFH receiving maximal tolerated statins therapy (Phase III trials).

Author, trial name [reference]	**Study Population** participants, statin[a]/LLT[b] therapy, LDL-C values, CV risk	**Mean baseline LDL-C** (mg/dL)	**Treatment** (mg)	**Duration** (weeks)	**% LDL-C change from baseline to week 24[c]**	**Difference between % LDL-C change** (ALI *vs* PL)
Kastelein et al. 2015 **ODYSSEY FH I** [**39**]	HeFH, 486 M/F statin±LLT LDL-C ≥70 mg/dL and very high CV risk or LDL-C ≥100 mg/dL and high CV risk	144.6	ALI 75/150 q2w[d] PL q2w	78	– 48.8 + 9.1	**– 57.9**
Kastelein et al. 2015 **ODYSSEY FH II** [**39**]	HeFH, 249 M/F statin±LLT LDL-C ≥70 mg/dL and very high CV risk or LDL-C ≥100 mg/dL and high CV risk	134.4	ALI 75/150 q2w PL q2w	78	– 48.7 + 2.8	**– 51.5**
Ginsberg et al. 2016 **ODYSSEY HIGH FH** [**40**]	HeFH, 105 F/M statin±LLT LDL-C ≥160 mg/dL high/very high CV risk	197.8	ALI 150 q2w PL q2w	78	– 47.5 – 6.6	**– 39.1**
Moriarty et al. 2016 **ODYSSEY ESCAPE** [**41**]	HeFH, 62 F/M statin±LLT weekly lipoprotein apheresis high/very high CV risk	184.5	ALI 150 q2w PL q2w	18	– 53.7 + 1.6	**– 55.3**

HeFH Heterozygous familial hypercholesterolaemia, *LDL-C* low-density lipoprotein cholesterol, *M* male, *F* female, *LLT* lipid lowering therapy, *CV* cardiovascular, *q2w* every 2 weeks, *ALI* alirocumab, *PL* placebo, *vs* versus.

[a]Maximal tolerated daily dose of statin (i.e. Atorvastatin 40–80 mg, Rosuvastatin 20–40 mg, Simvastatin 80 mg).

[b]Fenofibrate, omega-3 fatty acid, nicotinic acid or bile acid sequestrants.

[c]In ODYSSEY ESCAPE %LDL-C change/reduction from baseline to week 6.

[d]The alirocumab dosing regimen was 75 mg Q2W starting dose titrated to 150 mg q2w at week 12 if LDL-C targets were not achieved.

Table 5. Treatment-emergent adverse events rates following alirocumab, placebo or ezetimibe treatments in Phase III trials.

Event	Placebo-controlled pool				Ezetimibe-controlled pool			
	ALIROCUMAB n = 3248		PLACEBO n = 1554		ALIROCUMAB n = 864		EZETIMIBE n = 618	
	event/total	%	event/total	%	event/total	%	event/total	%
Treatment-emergent adverse events								
Any	2615/3248	**80.0%**	1290/1554	**83.0%**	607/864	**70.2%**	421/618	**68.1%**
Serious	441/3248	**13.5%**	257/1554	**16.5%**	113/864	**13.0%**	69/618	**11.1%**
Leading to treatment discontinuation	198/3248	**6.0%**	94/1554	**6.0%**	76/864	**8.7%**	60/618	**9.7%**
Leading to death	18/3248	**0.5%**	14/1554	**0.9%**	2/864	**0.2%**	7/618	**1.1%**
Common (≥5%) treatment-emergent adverse events								
Nasopharyngitis	428/3248	**13.1%**	198/1554	**12.7%**	52/864	**6.0%**	41/618	**6.6%**
Upper respiratory tract infection	222/3105	**7.1%**	117/1482	**7.8%**	62/864	**7.1%**	40/618	**6.4%**
Influenza	170/3248	**5.2%**	92/1554	**5.9%**	38/864	**4.3%**	23/618	**3.7%**
Back pain	185/3248	**5.6%**	172/1554	**5.9%**	33/864	**3.8%**	26/618	**4.2%**
Arthralgia	175/3248	**5.3%**	104/1554	**6.6%**	19/864	**2.1%**	17/618	**2.7%**
Myalgia	158/3248	**4.8%**	67/1554	**4.3%**	65/864	**7.5%**	49/618	**7.9%**
Headache	180/3248	**5.5%**	77/1554	**4.9%**	42/864	**4.8%**	24/618	**3.8%**
Diarrhoea	163/3105	**5.2%**	99/1482	**6.6%**	30/864	**3.4%**	21/618	**3.3%**
Treatment-emergent adverse events of special interest								
General allergic events[a]	255/3105	**8.2%**	144/1482	**9.7%**	11/207	**5.3%**	7/202	**3.4%**
Neurological events[b]	108/2532	**4.2%**	59/1482	**3.9%**	5/207	**2.4%**	4/202	**1.9%**
Neurocognitive disorders[c]	32/3105	**1.0%**	13/1482	**0.8%**	10/864	**1.1%**	8/618	**1.2%**
Ophthalmologic disorders[d]	87/3041	**2.8%**	44/1447	**3.0%**	4/582	**0.6%**	2/342	**0.5%**
ALT/AST > 3 x ULN	83/3041	**2.7%**	51/1447	**3.5%**	9/864	**1.0%**	1/618	**0.1%**

CK > 3 x ULN	109/3041	**3.5%**	67/1447	**4.6%**	21/864	**2.4%**	12/618	**1.9%**
Events associated with injectable protein therapies								
Injection site reaction[e]	287/3248	**8.8%**	86/1554	**5.5%**	26/864	**3.0%**	13/618	**2.1%**

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *CK* creatine kinase, *ULN* upper limit of normal.
[a]Defined by *Medical Dictionary for Regulatory Activities* search terms that include hypersensitivity.
[b]Defined by *Medical Dictionary for Regulatory Activities* search terms that include: demyelination, peripheral neuropathy, and Guillain-Barré syndrome.
[c]Defined by *Medical Dictionary for Regulatory Activities* search terms that include: deliria, cognitive and attention disorders, dementia and amnestic conditions, disturbances in thinking and perception, and mental impairment disorders.
[d]Defined by *Medical Dictionary for Regulatory Activities* search terms that include: optic nerve disorders, retinal disorders, and corneal disorders.
[e]Defined by *Medical Dictionary for Regulatory Activities* search terms that include: erythema/redness, itching, swelling, pain/tenderness.

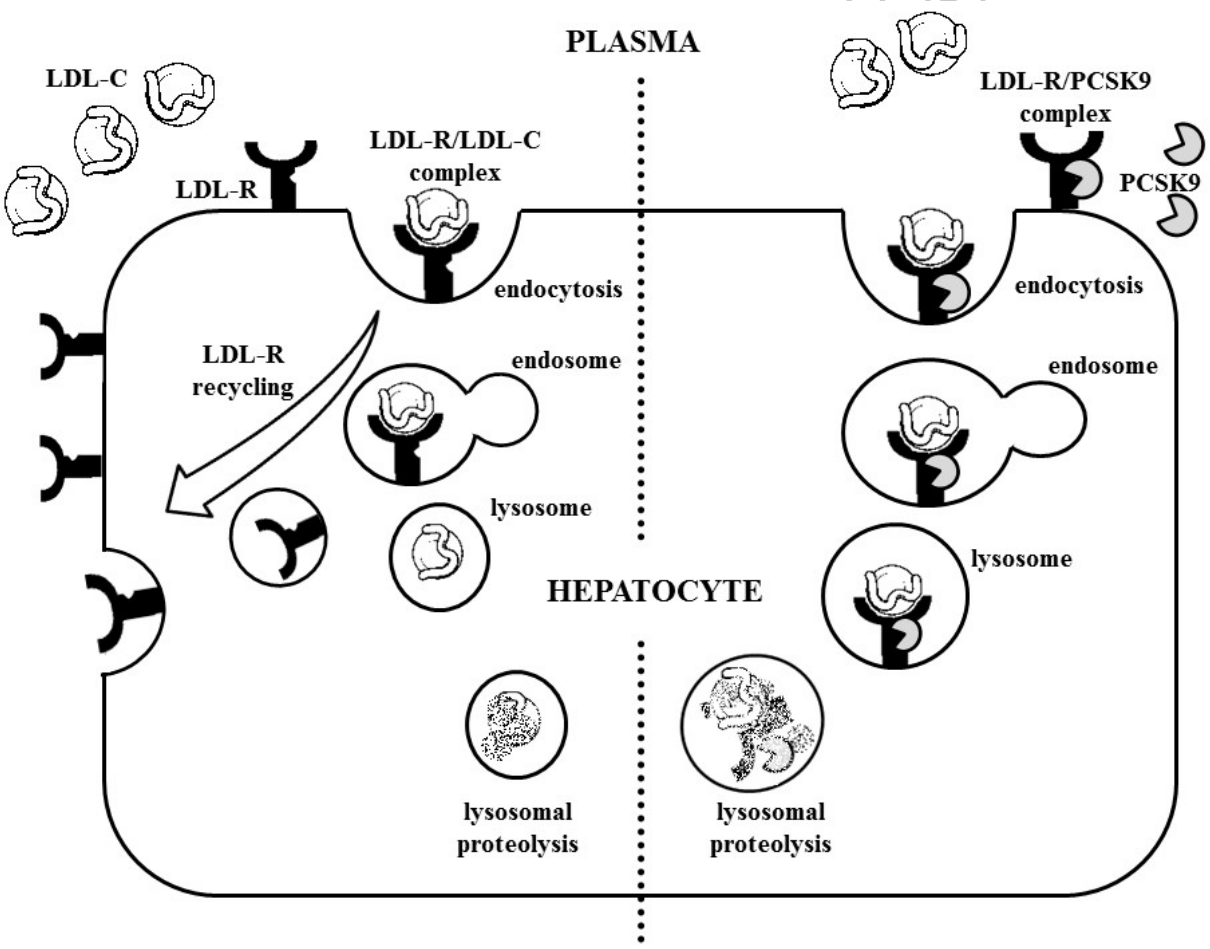

Figure 1