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PCSK9 Inhibition-Reaching Physiologic LDL-C Levels "Endo, Goldstein and Brown's Dream is Coming True"

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Abstract

Physiologically, in the presence of an intracellular deficit of cholesterol, the LDLR synthesis, expression and function increases, thus uptaking, introducing and providing cholesterol to the cell. This process is counter-regulated by PCSK9 expression, the protease inducing LDLR proteolysis, thereby limiting its function maintaining a constant cholesterol intracellular concentration. Accordingly, the balance or Yin-Yang between PCSK9 and LDLR directly regulates the intracellular concentration of cholesterol and indirectly has a high impact on circulating LDL-cholesterol.

This article reviews the brief and amazing recent history with PCSK9 inhibition from basic science to current clinical recommendations for MAbs-PCSK9. In 2003 and 2005, respectively, the pcsk9 gene mutations, determinants of the "gain of function" of PCSK9 and severe hypercholesterolemia, and the pcsk9 gene mutations with "loss of function" of PCSK9, determinants of hypocholesterolemia were described; subsequently, in 2006, the association between the PCSK9 gene mutations and the "loss of function" of PCSK9 with hypocholesterolemia and reduction of up to 88% for the risk of a coronary event in the "mutant" population versus the control population was published.

In 2009, the effect of a "full human" monoclonal antibody *vs* PCSK9 in mice and non-human primates was first reported; MAbs-PCSK9, AMG-145 and REGN-727, produced in cynomolgus monkeys a doubling in the number of LDLR and an average 75% reduction in circulating LDL-cholesterol. In 2012, the first phase I studies with Evolocumab and Alirocumab versus placebo were reported; these programs informed very significant reductions in LDL-cholesterol in healthy subjects and patients with familial and non-familial hypercholesterolemia treated without/with statins; in both programs, tolerability and safety of MAbs were similar to placebo. With this evidence, the Phase II and III investigations with MAbs-PCSK9 initiated; four years after, pending the results of ongoing studies of cardiovascular outcomes and long-term safety (ODYSSEY-Outcomes with Alirocumab and FOURIER with Evolocumab), the OSLER trial with Evolocumab and the ODYSSEY-Long Term trial with Alirocumab, together with some recent meta-analysis of MAbs-PCSK9 allows us to establish the following scenario: MAbs-PCSK9 Evolocumab and Alirocumab have a positive and significant impact on LDL-cholesterol, other apo-B100 lipoproteins -including Lp(a)-, overall mortality and myocardial infarction; all the aforementioned with a safety profile and tolerability very favorable. In the same scenario, the recently published GLAGOV trial demonstrates for the first time that the addition of a non-statin therapy -Evolocumab-, to the optimal treatment with statins is associated with atheroregression.

Beyond currently approval indications by regulatory agencies, taking account of high cost of PCSK9 inhibitors and financial restraints within healthcare budgets, by now and before definitive evidence publication, the MAbs-PCSK9 are recommended in six clinical scenarios analyzed in this paper. However, even though it is irrefutable that the MAbs-PCSK9 showed great efficiency in reducing atherogenic lipoproteins and insinuated a promising reduction in overall mortality and myocardial infarction, the certainty of the net benefit that this therapeutic strategy adds to the optimal current treatment with statins and other non-statin lipid-lowering drugs will be conclusive when results of the two currently ongoing "ad hoc" programs with Evolocumab and Alirocumab are available. Finally, preliminary results with PCSK9-mRNA interference with Inclisiran are presented.

Keywords: PCSK9; LDL-C Levels; MAbs-PCSK9

Abbreviations: NARC-1: Neural Apoptosis-Regulated Convertase 1; SKI-1/S1P: Subtilisin Kexin Isozyme 1/Site-1 Protease; SREBP: Sterol Regulatory Element-Binding Proteins; BDNF: Brain-Derived Neurotrophic Factor; PCSK9: Proprotein Convertase Subtilisin Kexin type 9; LDLR: LDL receptor; ApoB100: Apolipoprotein B100 (LDLR ligand); HMGCoAR: Hydroxy-methyl-glutaryl-coenzyme A Reductase ; FHC3: Familial Hypercholesterolemia type 3; ASCVD: Atherosclerotic Cardiovascular Disease; EMA: European Medicines Agency; FDA: U.S. Food and Drug Administration; MAbs-PCSK9: Monoclonal antibodies *vs* PCSK9; ARIC: Atherosclerosis Risk in Communities Study; TEAE: Treatment-Emergent Adverse Events; SIRIO: Systematic Investigation and Research on Interventions and Outcomes; CPK: Creatine phosphokinase; COFEPRIS: Federal Commission for the Protection against Sanitary Risk; ACS: Acute Coronary Syndrome; RNAi: RNA interference

Basic Evidence

PCSK9-Proprotein Convertase Subtilisin Kexin type 9

Characterized in 2003 as NARC-1 by Nabil Seidah et al. [1], this protein currently called PCSK9 is the ninth member of the proprotein convertase family or subtilases. These proteins are serine proteases that regulate the activation, inactivation and/or intracellular translation of secretory proteins such as transcription or growth factors, prohormones and membrane receptors, some of which are related with cardiovascular health and disease [2]. Nine subtilases have been identified; seven belonging to the kexin subfamily in bacteria and fungi and two belonging to the kexin-like subfamily in mammals. In humans, the SKI-1/S1P subtilase regulates the activity of the SREBP and BDNF transcription factors, and the PCSK9 subtilase regulates LDLR activity [3-5].

PCSK9 is encoded by the pcsk9 gene located on the small arm of the chromosome 1, position 32. The PCSK9 primordium or prepro-PCSK9 is synthesized in the liver, small intestine and kidney as an inactive glycoprotein of 692 amino acids with 4 domains [6]. Amino acids 1-30 constitute the signal peptide domain; amino acids 31-152 constitute the inhibition propeptide domain; amino acids 153-452 constitute the subtilisin-like or catalytic peptide domain; and amino acids 453-692 constitute the cysteine-rich or C-terminal peptide domain. After the synthesis, in the endoplasmic reticulum, through two autocatalytic steps, the prepro-PCSK9 of 692 amino acids is transformed into PCSK9 of 540 amino acids. During the first step, the prepro-PCSK9 loses the 30 amino acids of the signal peptide, and during the second step, the pro-PCSK9 loses the 121 amino acids of the inhibition propeptide. The resulting molecule with 540 amino acids constitutes the secretory PCSK9 with two domains, the subtilisin-like or catalytic domain and the C-terminal domain [1,6]. The secretory PCSK9 freely circulates in plasma and probably also bound to the LDL, and its mean plasma concentration are 608 ng/ml on average for males and 646 ng/ml on average for females [3,7]. The first secretory PCSK9 target to be identified was the LDLR at the surface of liver hepatocytes [8].

LDLR-Low Density Lipoprotein Receptor

The exact dimension of the importance of the secretory PCSK9 in cellular cholesterol metabolism is understood in terms of its interaction with the LDLR. The LDLR was identified by Goldstein and Brown in the 70s [9]. It is encoded on chromosome 19 and is constituted as a glycoprotein structure with five domains; the extracellular ligand-binding domain 1 (apo B100 binding site), the extracellular epidermal growth factor homology domain 2 (PCSK9 binding site), the extracellular sugar-rich domain 3, the transmembrane domain 4 or LDLR adaptor protein 1 and the intracytoplasmic domain 5 [9,10].

Under physiological conditions, circulating LDLs, each with an average content of 1,500 esterified cholesterol molecules, are identified in the single molecule of apo-B100 by the extracellular ligand-binding domain 1 of the LDLR. LDL-LDLR binomial is introduced into the cell by receptor-mediated endocytosis. In the endosome, by V type ATPase-induced acidification, the LDLR is allosterically dissociated from the LDL and is recycled to the cell membrane; by membrane fusion, the LDL is transferred from endosomes to lysosomes where the lipid and protein components are hydrolyzed and incorporated into the cellular metabolism; the half-life of a LDLR is 20 hours and the time of a membrane-endosome-membrane cycle is 10 minutes [9].

In other words, each LDLR is able to uptake 120 LDL molecules, each with 1,500 esterified cholesterol molecules equivalent to 180,000 esterified cholesterol molecules up taken by each LDLR. The aforementioned reflects the high efficiency of the LDLR to fulfill the function of up taking LDL, integrating its esterified cholesterol content to the cellular metabolism and facilitating the hepato-biliary-enteral elimination of the excess of this pro-atherogenic lipid.

PCSK9 and LDLR. The Yin-Yang

The incorporation of esterified cholesterol into the cell *via* LDLR is a process finely counter-regulated by the PCSK9. The concentration of intracellular cholesterol, especially in the Golgi apparatus and the endoplasmic reticulum membranes, is the biological constant that regulates the expression of the transcription factor SREBP1-2 [11,12]. In the presence of an intracellular cholesterol reduction, the SREBP1-2 transcription factor is activated. The activation of this transcription factor determines the following processes: activation of intracellular cholesterol synthesis by activation of the synthesis and expression of HMGCoAR, activation of the uptake and incorporation of LDL-cholesterol by activation of the synthesis and expression of LDLR, and activation of cell autophagy [9]. All the aforementioned with the purpose of correcting intracellular cholesterol depletion.

However, in parallel and paradoxically at first sight, in the presence of intracellular cholesterol reduction, the SREBP1-2 transcription factor also determines the activation of the synthesis and expression of PCSK9 [12]. This counter-regulation aims to maintain the intracellular cholesterol concentration stable, since the absence of the counter-regulation of cholesterol synthesis by HMGCoAR, the uptake of LDL-cholesterol by the LDLR and autophagy, would cause an intracellular cytotoxic cholesterol increase. This phenomenon is prevented by the PCSK9 action that keeps the number of functional LDLR constant (Yin-Yang phenomenon) [13,14]. The circulating secretory PCSK9 (extracellular pathway) has as a ligand the domain 2 with homology to the endothelial growth factor of the LDLR [10]. Once PCSK9 is bound to LDLR, in the presence or absence of LDL, the PCSK9-LDLR binomial is endocytosed and is unable to dissociate at acidic pHs, being transferred from the endosomes to the lysosomes for proteolysis of both molecules [8,15].

Genetic Evidence

Gene pcsk9 mutations with "gain of function"

Marianne Abifadel, Catherine Boileau et al. [16] reported in a brief communication, the characterization of a third form of autosomal dominant hypercholesterolemia. In a French population with familial hypercholesterolemia phenotype, the authors described the association with two mutations in the pcsk9 gene, the S127R (625T → A) mutation in exon 2 and F216L (890T → C) mutation in exon 4. This third form of autosomal dominant hypercholesterolemia was called HCHOLA3 or HF3.

Prior to the report of Abifadel/Boileau, the familial hypercholesterolemia phenotype was associated with mutations in the LDLR gene and mutations in the APOB gene. Hypercholesterolemia associated with mutations in the LDLR gene or Familial Hypercholesterolemia, the one associated with mutations in APOB gene or Familial Hypercholesterolemia by defective apo-B100 and the one associated with mutations described by Abifadel/Boileau et al. [16] determine a phenotype characterized by severe hypercholesterolemia (LDL cholesterol>500 mg/dL), cholesterol deposits in tissues, tendon and skin xanthomas, corneal arcus and early atherosclerotic cardiovascular disease.

During discussion of the results, Abifadel postulated that given the association between hypercholesterolemia with an autosomal dominant pattern and mutations in the pcsk9 gene, such mutations in the gene encoding the PCSK9, may constitute a "gain of function" of the protease as the determining mechanism of hypercholesterolemia. Based on this observation, even in a lack of awareness of the intrinsic mechanism of action of PCSK9, the authors postulated that research on this topic would be the key to discovering new ways in cholesterol metabolism with therapeutic potential. Clearly, this discovery, in addition to describing a third etiology of autosomal dominant hypercholesterolemia, was the key to start researching a new therapeutic strategy against hypercholesterolemia.

Gene pcsk9 mutations with "loss of function"

Jonathan Cohen, Helen Hobbs et al. [17], in a letter, published the characterization of a new form of hypocholesterolemia. In the cohort of the Dallas Heart Study (52% African American, 29% European-American, 17% Hispanic, and 2% other ethnicities), subjects with hypocholesterolemia (LDL cholesterol<58 mg/dL) were selected. In this subpopulation of 32 subjects, the authors described two "nonsense" mutations in the pcsk9 gene; the Y142X (426C → G) mutation in exon 3, which introduces a stop codon at residue 142 and the C679X (20137C → A) mutation in exon 12, which introduces an early termination signal at

codon 679. These mutations had a prevalence of 0.4% (Y142X) and 1.4% (C679X) in African Americans and a negligible prevalence in European Americans (<0.1%) and Hispanics (<0.2%) [7].

Expanding the research to a Cook County population in Illinois and a Nigeria native population, Cohen found that the prevalence of Y142X and C679X mutations was 0.6% and 1.6% in the Cook population and 0% and 1.4% in the Nigeria population (average prevalence of both mutations 2% in African American subjects). The phenotype of these subjects was characterized by LDL-cholesterol levels between the 1st percentile and the 50th percentile, with an average LDL-cholesterol value 40% lower than the control population (p<0.001). By measuring latosterol (cholesterol biosynthesis) and camposterol (cholesterol absorption), the authors discarded the inhibition of the synthesis and/or the inhibition of the absorption of cholesterol as determinant factors of hypocholesterolemia [17].

With these findings, Cohen et al. [17] postulated a "loss of function" of the PCSK9 as the determining mechanism of hypocholesterolemia, anticipating the interaction between PCSK9 and LDLR as the mechanism potentially involved.

Genetic-Epidemiological Evidence

Gene pcsk9 mutations with "loss of function" and ASCVD

Jonathan Cohen, Helen Hobbs et al. [18], published as an original article, the association between the pcsk9 gene mutations with PCSK9 "loss of function", low LDL-cholesterol levels and low prevalence of coronary events [18]. In Study Cohort ARIC, of 3,363 African American subjects, 2.6% had a pcsk9 gene mutation with PCSK9 "loss of function" with a 28% reduction in the average value of LDL-cholesterol (P=0.008) and 88% risk reduction of a coronary event (H.R 0.11-0.02 to 0.81- with P=0.03); of 9,524 Euro-American subjects, 3.2% had a pcsk9 gene mutation with PCSK9 "loss of function", with 15% reduction in the average value of LDL-cholesterol and 47% risk reduction of a coronary event (HR 0.53 -0.32 to 0.79 - with P=0.003). Considering the high frequency of mutations Y142X and C679X in African Americans and mutation R46L in White subjects [19], Cohen analyzed the prevalence of such mutations and their correlation with LDL-cholesterol level and the incidence of coronary events within a period of 15 years; to that end, Cohen assessed 3,363 African Americans subjects and 9,524 Euro-American subjects aged between 45 and 64 years of the study ARIC.

Mutations Y142X and C679X had a prevalence of 0.8 and 1.8% in African-American subjects and <0.01 in White subjects; the average level of LDL-cholesterol was 100 mg/dL in the carrier population and 138 mg/dL in the control population (P<0.001). During a 15-year follow-up period, the incidence of coronary events was 1.2% in the carrier population and 9.7% in the control population, representing a H.R of 0.11 in the carrier population *vs* the control population (P=0.003), or a reduction of 88% of the relative risk of coronary events. Mutation R46L had a prevalence of 3.2% in White subjects and 0.7 in African-American subjects; the average level of LDL-cholesterol was 116 mg/dL in the carrier population and 137 mg/dL in the control population (P<0.001). During a 15-year follow-up period, the incidence of coronary events was 6.3% in the carrier population and 11.8% in the control population, representing a H.R of 0.53 in the carrier population *vs* the control population (P=0.003) or a relative risk reduction of 47% of coronary events. In all three types of mutations, the numbers remained statistically significant after multivariable adjustment for age, sex and non-lipid cardiovascular risk factors (P<0.05).

Based on these findings the following statement was postulated: "*The reduction of 1 mmol equivalent to 40 mg/dL or 0.5 mmol equivalent to 20 mg/dL of LDL-cholesterol throughout the entire life of an individual represents a reduction of up to 88% in the relative risk of coronary events*".

Such "genetic benefit" is much higher than the reported with an equivalent drug reduction of LDL-cholesterol initiated in adulthood and opened the door to the concept of "starting early and maintaining for a long-term the reduction in LDL-cholesterol". During discussion, while recognizing that the intrinsic mechanism explaining the association between pcsk9 gene mutations and LDL-cholesterol reduction was not completely defined, Cohen postulated the inhibition of PCSK9 as a highly attractive therapeutic strategy focused on LDL-cholesterol reduction and the incidence of cardiovascular events associated with atherosclerosis. Additionally, he emphasized that the protection conferred by the low "for life" level of LDL-cholesterol was observed in a population such as the African American ARIC with 50% prevalence of hypertension, 30% prevalence of smoking and 20% prevalence of diabetes mellitus [18].

Animal evidence with MAbs-PCSK9

Animal experimental evidence in non-human primates

The Joyce Chan et al. (AMGEN) [20] and Victoria Gusarova et al. (REGENERON-SANOFI) [21] groups pioneered the development of "full human" MAbs-PCSK9. Briefly, these antibodies are produced in the mouse by removing the genomic sequences encoding immunoglobulin synthesis, followed by replacing the corresponding human genomic sequences; thus, the "humanized" mouse produces 100% human immunoglobulins and by being immunized with human PCSK9, "full human" MAbs- PCSK9 are synthesized. "High quality" secretory B lymphocytes of MAbs-PCSK9 are selected and used for creating producer hybridomas of MAbs-PCSK9- [22].

Both groups of Investigators showed that "full human" MAbs-PCSK9, AMG 145 and REGN 727, have a high affinity for the catalytic site of secretory PCSK9, inhibiting its binding to the LDLR and increasing recycling and survival of the receptor. In mice analyses and especially in non-human primates "cynomolgus monkeys", both groups reported that the administration of MAbs-PCSK9 produced an almost total reduction in the level of circulating PCSK9, a doubling of the number of LDLR and a reduction up to 75% in circulating LDL-cholesterol [20,21].

This proof of concept expanded the knowledge of the PCSK9 mechanism of action and opened the door for the efficacy, safety and tolerability study of MAbs-PCSK9 in human phase I studies.

Early Clinical Evidence

Phase I evidence in humans

Clampton Dias et al. reported in November 2012 [23], the results of two studies of their phase I program with Evolocumab. The first study included seven cohorts of healthy subjects and the second study included 7 cohorts of subjects with hypercholesterolemia treated with statins (6 non-FHC and 1 HeFHC). In the first study, five single S.C doses (7, 21, 70, 210 and 420 mg) and two single I.V. doses (21 and 420 mg) of Evolocumab versus placebo were tested; in the second group, five multiple S.C doses (14 mg/week, 35 mg/week, 140 mg/2 weeks, 280 mg/2 weeks and 420 mg/4 weeks) of Evolocumab versus placebo were tested [14].

The TEAE incidence was similar between the active treatment groups and placebo. In the first single dose study, LDL-cholesterol reduction versus placebo was dose-dependent; of up to -64.0% with a maximum S.C dose of 420 mg and of -61% with a maximum I.V. dose of 420 mg. In the second multiple dose study, LDL-cholesterol reduction was also dose dependent; up to -73%, -75% and -66% with doses of 140 and 280 mg/2 weeks and 420 mg/4 weeks, respectively. The type of hypercholesterolemia and type of the baseline treatment did not determine a difference in the therapeutic response. The levels of non-HDL cholesterol, apoB and lipoprotein (a) had significant reductions, and the levels of HDL-cholesterol and apoA1 had also significant increases.

During discussion, the authors highlighted several important aspects, including: the significant and equivalent reduction in LDL-cholesterol and other atherogenic lipoproteins with doses of 140 mg S.C/2 weeks and 420 mg S.C/4 weeks; the direct relationship between the reduction of the level of circulating PCSK9 and LDL-cholesterol; and ultimately the safety and tolerability of Evolocumab.

Evan Stein et al. [24] reported in April 2012 the results of the three studies of their phase I program with Alirocumab. The first single IV dose study included 40 healthy subjects with LDL-cholesterol>100 mg/dL; the second single S.C dose study included 32 healthy subjects with LDL-cholesterol>100 mg/dL; and the third multiple S.C dose study included three subgroups: a) 21 subjects with HeFHC treated with atorvastatin and LDL-cholesterol>100 mg/dL; b) 30 subjects with non-FHC treated with atorvastatin and LDL-cholesterol>100 mg/dL; c) 10 subjects with non-FHC treated only with diet and LDL-cholesterol>130 mg/dL.

The TEAE incidence was similar between the active treatment groups and placebo. The LDL-cholesterol reduction in single I.V and S.C dose groups was dose-dependent; up to -65.4% with the maximum I.V dose of 12 mg/kg and of -45.7% with the maximum S.C dose of 6.0 mg/kg. The LDL-cholesterol reduction in the multiple S.C dose groups treated with atorvastatin was also dose-dependent, -39.2% with 50 mg SC/2 weeks, -53.7% with 100 mg S.C/2 weeks and -61.0% with 150 mg S.C/2 weeks. The type of hypercholesterolemia and type of the baseline treatment did not determine a difference in the therapeutic response. The levels of non-HDL cholesterol, apoB and lipoprotein (a) had significant reductions, and the levels of HDL-cholesterol and apoA1 had also significant increases.

During discussion, the authors highlighted several important aspects, including: the significant and consistent reduction in LDL-cholesterol and other atherogenic lipoproteins, regardless of the type of hypercholesterolemia and/or background treatment; the direct relationship between reduction of the level of circulating PCSK9 and LDL-cholesterol, a fact confirmed by the therapeutic hypothesis; the additive but not synergistic effect of Alirocumab with atorvastatin; and finally the excellent safety and tolerability of this MAb-PCSK9.

Clinical Evidence

OSLER study

Since short-term studies (Phase II and Phase III) with Evolocumab showed an efficient LDL-cholesterol reduction (60% average) with an excellent safety and tolerance profile, the Open-Label Study of Long-Term Evaluation against LDL-Cholesterol -OSLER- [25] was planned with the aim of evaluating the medium-term safety, tolerance and efficacy in reducing LDL-cholesterol of Evolocumab; this analysis also included the analysis of the blindly adjudicated incidence of cardiovascular events: death, myocardial infarction, angina pectoris requiring hospitalization, coronary revascularization, stroke, transient ischemic attack, and heart failure requiring hospitalization.

The OSLER-1 study included subjects who had completed any of the 5 phase II studies, and the OSLER-2 study included subjects who had completed any of the 7 phase III studies. Of the 12 "parent" studies, those subjects who had no adverse events leading to treatment discontinuation during the study, who did not require an "urgent" modification of their baseline therapy and who achieved clinical stability, were randomly assigned in a 2:1 ratio to receive open-label Evolocumab 420 mg S.C/4 weeks (OSLER 1) *vs* baseline therapy without Evolocumab for 56 weeks, or Evolocumab 140 mg S.C/2 weeks or 420 mg S.C/4 weeks (patient's choice in OSLER 2) versus baseline therapy without Evolocumab for 48 weeks.

The study included 74.1% of the parent study cohort, totaling 4,465 subjects (1,324 OSLER 1 and 3,141 OSLER 2); 2,976 were assigned to Evolocumab and 1,489 continued their baseline therapy without Evolocumab. The mean follow-up was 11.1 months. With a baseline, LDL-cholesterol level of 120 mg/dL at week 12, the LDL-cholesterol reduction was -61% in the Evolocumab group (-59% to -63%; P<0.001); the mean absolute reduction of LDL-cholesterol was 73 mg/dL with a mean circulating LDL-cholesterol value of 48 mg/dL; 90.2% and 73.6% of subjects in the Evolocumab group had an LDL-cholesterol level of <100 mg/dL and <70 mg/dL, respectively, against 26% and 3.8% in the control group. These figures remained stable throughout the follow-up. The figures for non-HDL cholesterol, apoB, total cholesterol and triglycerides had a reduction of -52%, -47.3%, -36.1% and -12.6%, respectively. The HDL-cholesterol and apoA figures increased 7% and 4.2%, respectively.

The incidence of adverse events, serious adverse events and hepatic and muscle enzyme elevations were similar between the Evolocumab group and the control group, with no difference related with LDL cholesterol levels during treatment (<40 mg/dL or <25 mg/dL); in the Evolocumab group there was a low (<1%) although a higher incidence of neurocognitive adverse events, with no association to LDL cholesterol level. Injection site reactions with Evolocumab were reported in 4.3%, leading to discontinuation in 0.2%; anti-evolocumab antibodies were reported in 0.3% in both groups and anti-evolocumab neutralizing antibodies were reported in 0%. The estimated incidence of cardiovascular events at 12 months was 0.95% in the Evolocumab group versus 2.18% in the control group, representing a H.R of 0.47 (0.28 to 0.78; P=0.003); this trend was progressive throughout the follow-up.

In their discussion, the authors note that this study confirms the medium-term efficacy, safety and tolerability of Evolocumab, providing a positive signal regarding the reduction of cardiovascular events with Evolocumab compared to that achieved with statins and other non-statin therapies. Finally, they recognize the methodological limitations of the study and are optimistic concerning the future results of the FOURIER study.

ODYSSEY long-term study

As with Evolocumab, since short-term studies (Phase II and Phase III) with Alirocumab showed an efficient LDL-cholesterol reduction (55% average) with an excellent safety and tolerance profile, the study Long-Term Safety and Tolerability of Alirocumab in High Cardiovascular Risk Patients with Hypercholesterolemia Not Adequately Controlled with Their Lipid Modifying Therapy -ODYSSEY Long-Term- [26] was planned with the aim of evaluating the medium-term safety, tolerance and efficacy in reducing LDL-cholesterol of Alirocumab; this analysis also included the analysis of the blindly adjudicated incidence of cardiovascular events: death from coronary heart disease, nonfatal myocardial infarction, fatal and nonfatal ischemic stroke, and unstable angina requiring hospitalization.

The ODYSSEY Long-Term study included subjects aged ≥18 years with HeFH, with established coronary heart disease or a coronary heart disease risk equivalent, on a stable statin treatment with the maximum tolerated dose and/or on some other non-statin drug and an LDL cholesterol level ≥70 mg/dL. Subjects included were randomized in a 2:1 ratio to receive Alirocumab 150 mg S.C/2 weeks *vs* placebo S.C/2 weeks for 78 weeks.

In this study, 2,341 subjects were randomized, 1,553 to receive Alirocumab and 788 to receive placebo. With a baseline, LDL-cholesterol level of 122 mg/dL in both groups at week 24, the LDL-cholesterol reduction was -61% in the Alirocumab group *vs* 0.8% in the placebo group (absolute difference -61.9%; P<0.001); the absolute value of LDL cholesterol at week 24 was 48 mg/dL in the Alirocumab group versus 119 mg/dL in the placebo group (absolute differences of -74 mg/dL and -4 mg/dL respectively). At week 24, 70.3% *vs* 8% of subjects on Alirocumab and placebo, respectively achieved LDL cholesterol levels of <70 mg/dL (P<0.001). These results were maintained without significant variation

throughout the study and showed no significant differences according to the baseline characteristics. The absolute differences (Alirocumab-placebo) at week 24 in the non-HDL cholesterol, apoB, total cholesterol, triglycerides and lipoprotein (a) figures were -52.3%, -54%, -37.5%, -17.3 and -25.6%, respectively (P<0.001). In addition, the HDL-cholesterol and apoA levels had an absolute difference of +4.6% and +2.9%, respectively (P<0.001).

The incidence of adverse events, leading to study treatment discontinuation and elevations of liver or muscle enzymes was similar between both groups. The Alirocumab group had a higher incidence of adverse events in the application site (5.9% *vs* 4.2%), myalgias (5.4% *vs* 2.9%), neurocognitive events (1.2% *vs* 0.5%) and ophthalmological events (2.9% *vs* 1.9%); the incidence of *de-novo* Diabetes Mellitus was similar in both groups (1.8% *vs* 2%). In the Alirocumab group, 37.1% (575 subjects) had LDL-cholesterol levels <25 mg/dL twice during the study; the incidence of adverse events in this subgroup was similar to the average incidence of the study. The incidence of total adjudicated cardiovascular events (including cardiac failure requiring hospitalization and/or myocardial ischemia with revascularization) during the study was 4.0% in the Alirocumab group *vs* 5.1% in the placebo group (P=NS); the incidence of pre-specified cardiovascular events during the study was 1.7% (27/1550) in the Alirocumab group *vs* 3.3% (26/778) in the placebo group, representing a HR of 0.52 (0.31 to 0.90; P=0.02); this trend was progressive throughout the follow-up.

Similarly, to the OSLER study, in their discussion, the authors commented that this study confirms the medium-term efficacy, safety and tolerability of Alirocumab, and provides a positive signal regarding the reduction of cardiovascular events with Alirocumab compared to that achieved with statins and other non-statin therapies. In conclusion, they recognize the methodological limitations of the study and are optimistic concerning the future results of the ODYSSEY OUTCOMES study.

With the purpose of evaluating the mental state neurocognitive function with the use of MAbs-PCSK9, the "ad-hoc" studies ODYSSEY-Neurocognitive with Alirocumab -NCT 02957682- (recruiting) [27] and EBBINGHAUS with Evolocumab -NCT 0220734- (completed) [28] were designed; in both trials the primary outcome is evaluating the change in neurocognitive scales scores between Alirocumab and Evolocumab versus placebo.

GLAGOV study

Based on the evidence of atheroregression with the use of high-intensity statins -SATURN study- [29], the study Global Assessment of Plaque Regression with a PCSK9 Antibody as Measured by Intravascular Ultrasound -GLAGOV- [30] was planned with the aim of exploring at what extent the progression of coronary atherosclerosis is reduced by the use of Evolocumab as assessed by intracoronary ultrasound.

The GLAGOV study included adult subjects with clinical indication of coronary angiography for suspicion of coronary atherosclerosis. Subjects included were required to have been treated with a stable statin therapy for 4 weeks and to have an LDL-cholesterol level between 60-80 mg/dL (high risk cohort) or >80 mg/dL (intermediate risk cohort). In all included subjects, a coronary ultrasound was performed at baseline and week 78; the analysis of the segments selected for analysis was centralized, standardized and "blind". The selected subjects were randomly assigned in a 1:1 ratio to receive "in a blinded manner", Evolocumab 420 mg S.C/4 weeks or placebo for 76 weeks. The primary endpoint of the study was the change in percent atheroma volume -PAV- and the secondary endpoint was the change in normalized total atheroma volume -TAV-. Other (exploratory) objectives included the analysis of the relationship between LDL-cholesterol reduction and atheroregression and adjudicated incidence of cardiovascular events.

The study included 968 individuals, 484 in the Evolocumab group and 484 in the placebo group; 87.2% of the individuals included had baseline and control studies and 423 individuals in each group were included in the analysis. The mean follow-up was 17.6 months. With a baseline, LDL-cholesterol of 92.5 mg/dL in both groups, in the Evolocumab group, the mean LDL-cholesterol level was 36.6 mg/dL *vs* 93 mg/dL in the placebo group with an absolute difference of -56.5 mg/dL (-59.7 to -53.4% with P<0.001). The numbers of apoB, triglycerides and lipoprotein (a) had an absolute difference of -40.6 mg/dL, -19.1 mg/dL and -6.7 mg/dL, respectively. HDL-cholesterol levels had an absolute difference of 2.5 mg/dL and CRP did not differ between groups (1.4 mg/L in both groups). The PAV increased +0.05% in the control group and reduced -0.95% in the Evolocumab group, with an absolute difference of -1.0% (-1.8% to -0.64%; P<0.001). Similarly, the TAV decreased -0.9 mm^3 in the placebo group and reduced -5.8 mm^3 in the Evolocumab group, with an absolute difference of -4.9 mm^3 (-7.3 mm^3 to -2.5 mm^3; P<0.001). Regression by PAV was observed in 64.3% of the Evolocumab group versus 47.3% of the placebo group (P<0.001) and by TAV in 61.5% versus 48.9%, respectively (P<0.001), with no difference or interaction between the pre-specified groups. In 144 subjects with baseline LDL cholesterol levels <70 mg/dL, the PAV reduced-1.97% in the Evolocumab group *vs* -0.35% in the placebo group (absolute difference -1.62%: -2.5% to -0.74%; P<0.001) with a regression by PAV of 81.2% *vs* 48% 1 in the Evolocumab group versus placebo, respectively. The results show a linear association between levels of 110 to 20 mg/dL of LDL-cholesterol and the PAV; the lower the LDL, the greater the regression.

The safety and tolerability profile of the Evolocumab group did not show significant differences compared to that observed in the placebo group, and finally the incidence of blinded cardiovascular events was numerically lower in the Evolocumab group (12.2%) than in the placebo group (15.3%).

In their discussion, the authors note that this study demonstrates for the first time that the addition of a non-statin therapy, in this case Evolocumab, to the optimal treatment with statins is associated with atheroregression, whereas the treatment with statins only favors stabilization or a non-progression status. The above with an appropriate safety and tolerability profile and a promising sign of reduction of cardiovascular events by atherosclerosis.

MAbs-PCSK9 Meta-Analysis

Even though the first publications of phase 1 Alirocumab and Evolocumab are very recent -year 2012-, in the three subsequent years 24 phase II and III studies of MAbs-PCSK9 had been published or reported. The results of these studies have been analyzed by many authors and support the platform that served to justify the approval of Alirocumab and Evolocumab for clinical use by the "EMA", "FDA" and "COFEPRIS" in México.

Meta-analysis S.I.R.I.O 2015. 10,159 subjects in 24 RCT

Eliano Pio Navarese, at the head of the S.I.R.I.O group, published in April 2015 the first "study by study" meta-analysis which included publications with Alirocumab, Evolocumab and Bococizumab [31]. In this meta-analysis of 24 studies based on the Cochrane guidelines for conducting meta-analyzes and the PRISMA system for reporting them, Navarese summarizes the results on cardiovascular outcomes (preliminary), efficacy and safety of MAbs-PCSK9. The meta-analysis included 10,159 randomized subjects allocated in eight phase II studies and sixteen phase III studies, all placebo-controlled or with another control (ezetimibe). Of the 24 studies, twelve include subjects with FHC, nine include subjects with non-FHC, two include subjects with statin intolerance and one include subjects with FHC and non-FHC.

Primary clinical outcomes: a) Overall mortality: The analysis of 24 studies and 10,159 subjects showed an overall mortality for MAbs-PCSK9 of 0.31% (19 of 6,187 subjects) *vs* 0.53% (21 of 3,971 subjects), corresponding to an O.R of 0.45 with a 0.23 to 0.86 confidence interval and a P value of 0.015; b) Cardiovascular mortality: The analysis of 24 studies and 10,159 subjects demonstrated a cardiovascular mortality for MAbs-PCSK9 of 0.19% (12 of 6,187 subjects) *vs* 0.33% (13 of 3,971 subjects), corresponding to an O.R of 0.50 with a 0.23 to 1.10 confidence interval and a P value of 0.084.

Secondary clinical outcomes: a) Myocardial infarction: The analysis of 10 studies and 5,195 subjects demonstrated an incidence of myocardial infarction for MAbs-PCSK9 of 0.58% (19 of 3,289 subjects) *vs* 1.00% (19 of 1,906 subjects), corresponding to an O.R of 0.49 with a 0.26 to 0.93 confidence interval and a P value of 0.030; b) Unstable angina: The analysis of 6 studies and 3,894 subjects revealed an incidence of unstable angina for MAbs-PCSK9 of 0.040% (1 in 2,515 subjects) *vs* 0.07% (1 in 1,379 subjects), corresponding to an O.R of 0.61 with a 0.06 to 6.14 confidence interval and a P value of 0.676.

Lipid profile: a) LDL-cholesterol level *vs* placebo: The analysis of 24 studies and 10,159 subjects indicated a mean reduction in LDL-cholesterol for MAbs-PCSK9 *vs* placebo of -47.49% (-69.64% to -25.35% with P<0.001); b) LDL-cholesterol level *vs* ezetimibe: The analysis of the MAbs-PCSK9 *vs* control (ezetimibe) studies revealed a mean reduction of LDL-cholesterol for MAbs-PCSK9 of -58.77% (-61.03% to -56.51%) *vs* -36.17% for ezetimibe (-39.28% to -33.06%) with P<0.001; c) Total cholesterol level: The analysis of 10 studies and 5,357 subjects demonstrated a mean reduction in total cholesterol for MAbs-PCSK9 *vs* no antibody of -31.49% (-46.35% to -16.64% with P<0.001).

In studies of MAbs-PCSK9 *vs* placebo, the mean reduction for MAbs-PCSK9 was -38.99% (-40.72% to -37.26% with P<0.001) and *vs* control (ezetimibe) of -23.83% (-27.35% to -20.32% with P<0.001); d) Lipoprotein level (a): The analysis of 12 studies and 6,566 subjects revealed a mean reduction of lipoprotein (a) for MAbs-PCSK9 *vs* no antibody of -26.45% (-30.19% to -22.71% with P<0.001). Similar data were found when MAbs-PCSK9 *vs* placebo were compared -27.96% (-31.21% to -24.71% with P<0.001) or *vs* control (ezetimibe) -24.05% (-28.94% to -19.16% with P<0.001); e) HDL-cholesterol level: The analysis of 14 studies and 4,378 subjects showed a mean increase in HDL-cholesterol for MAbs-PCSK9 *vs* no antibody of +6.30% (+5.58% to +6.97% with P<0.001). Similar data were found when MAbs-PCSK9 *vs* placebo were compared +6.14 (+5.31% to +6.97% with P<0.001) or *vs* control (ezetimibe) +6.8% (+5.33% to +8.26% with P<0.001).

Safety and tolerability: a) CPK level: The analysis of 24 studies and 10,159 subjects demonstrated an incidence of elevated CPK for MAbs-PCSK9 of 1.96% (121 of 6,187 subjects) *vs* 2.31% (92 of 3,972 subjects), corresponding to an O.R of 0.72 with 0.54 to 0.96 confidence intervals and a P value of 0.026; b) Serious adverse events: The analysis of 24 studies and 10,159 subjects demonstrated an incidence of serious adverse events for MAbs-PCSK9 of 9.26% (573 of 6,187 subjects) *vs* 7.73% (307 of 3,972 subjects), corresponding to an O.R of 1.01 with 0.87 to 1.18 confidence intervals and a P value of 0.879. There was no difference in the percentage of discontinuation between MAbs-PCSK9 and placebo or control (ezetimibe).

Recent meta-analysis and systematic reviews

After Navarese´s meta-analysis publication, other important meta-analysis [32-34] and one systematic review [35] have been published. The Li meta-analysis [32] included 20 trials and 6,464 individuals; the Zhang meta-analysis [33] included 25 trials and 12,200 individuals and the Lipinski network meta-analysis [34] included 17 trials and 13,083 individuals. The McDonagh systematic review [35] analyzed the quality and results of 17 trials in 19 publications. These meta-analysis reported the efficacy and safety of MAbs-PCSK9 with concordant results (LDL-cholesterol reduction against placebo from 53.8% to 60.4% and against ezetimibe from 29.9% to 38.2%); the Lipinski network meta-analysis also reported the impact of MAbs-PCSK9 on cardiovascular events with favorable impact on overall mortality (O.R 0.43: 95% CI 0.22 to 0.82 with P value 0.001), non-significant impact on cardiovascular mortality (O.R 0.50: 95% CI 0.22 to 1.13 with P value 0.10) and borderline significant impact on cardiovascular events (O.R 0.67: 95% CI 0.43 to 1.04 with P value 0.7); in this analysis an increase in neurocognitive adverse events was reported (O.R 2.34: 95% CI 1.11 to 4.93 with P value 0.02).

As resume from all these analysis we can conclude that until now, the MAbs-PCSK9 compared to standard treatment with no antibodies showed: a) a very significant reduction of all atherogenic lipoproteins [31-35]; b) a significant reduction in overall mortality [31,34]; c) a significant reduction of myocardial infarction [31]; d) a trend to reduced cardiovascular mortality not yet significant [31,34]; e) minor increase in CPK [31]; f) no increase in serious adverse events or therapeutic discontinuation [31-35]; a trend to increased neurocognitive adverse events [34]. All authors consider results to be very promising, which could show an amplification of the benefit in very high-risk populations, especially in acute coronary syndromes with longer follow-up periods.

Finally, they recognized as a limitation that these meta-analyses were "study by study" analysis instead of a "patient by patient" analysis, denoting the importance of "ongoing" studies specific for the MAbs-PCSK9 impact analysis on "hard" cardiovascular outcomes and long term security, especially neurocognitive security. Finally, authors suggest that in order for the MAbs-PCSK9 to be cost-effective, the price should be lower, around $ 3,500 per year instead the current price [35].

Predicting MAbs-PCSK9 benefit on top of statins

Recently, a pooled analysis of 10 double-blind Alirocumab trials was published by Ray et al. [36]. This "post-hoc" pooled analysis included 4,974 patients: 3,184 Alirocumab-treated patients, 1,174 placebo-treated patients and 618 ezetimibe-treated patients; the authors reported that in trials comparing Alirocumab *vs* placebo, with a baseline LDL-cholesterol of 126.8 mg/dL in both arms, the LDL-cholesterol level on-treatment was 56.9 mg/dL for Alirocumab and 126.5 mg/dL for placebo; in trials comparing Alirocumab *vs* ezetimibe, with a baseline LDL-cholesterol level of 123.2 mg/dL and 125.5 mg/dL respectively, the LDL-cholesterol on-treatment was 64.0 mg/dL for Alirocumab and 100.9 mg/dL for ezetimibe. In trials comparing Alirocumab *vs* placebo, a LDL-cholesterol level <50 mg/dL was reported in 52.6% of Alirocumab-treated patients and in 0% of placebo-treated patients and in 44.7% of Alirocumab-treated patients and 6.5% in ezetimibe-treated patients for trials comparing Alirocumab *vs* ezetimibe. The average percentage change from baseline in LDL-cholesterol level was -55.4% for Alirocumab, and 2.7% for placebo in trials comparing Alirocumab *vs* placebo and -48.1% for Alirocumab and -18.0% for ezetimibe in trials comparing Alirocumab *vs* ezetimibe. A total of 104 first MACEs were reported among 4,974 patients treated during 6,699 patient-years of follow-up; the H.R for MACE was 0.76 (0.63-0.91 with a P value of 0.0025) per 39 mg/dL lower achieved LDL-cholesterol and 0.71 (0.57-0.89 with a P value of 0.003) per additional 50% LDL-cholesterol reduction achieved, with a strong and significant correlation between LDL-cholesterol, non-HDL-cholesterol and apoB levels. Treatment-emergent adverse events were similar between the Alirocumab and placebo-control groups, regardless of the LDL-cholesterol level on treatment.

In their discussion, the authors underlie that on top of statins, every 39 mg/dL LDL-cholesterol level reduction or every 50% LDL-cholesterol level reduction is associated with a 24% or 29% risk reduction, respectively, with

no attenuation even at LDL-cholesterol levels of <50 mg/dL. Finally, they recognize the limitations of their post-hoc analysis and the importance of ongoing ad-hoc trials.

Indications and Recommendations 2017

Already approved indications for Evolocumab and Alirocumab

As of the printing of this paper, both "full human" MAbs-PCSK9, Alirocumab (Praluent®) and Evolocumab (Repatha®), had been approved in the United States and the European Union, and are in process of being approved in many other countries; in Mexico, both MAbs-PCSK9 have already been approved for marketing under the equivalency agreement between COFEPRIS and EMA [37-39].

Evolocumab (Repatha®) is indicated in patients with HeFH and non-FH as a complement to diet, in combination with statins or other non-statin lipid lowering therapies in subjects who do not achieve LDL-cholesterol target levels with maximum therapeutic statin doses or either alone or in combination with other non-statin lipid lowering therapies when there is intolerance and/or contraindication for statins. The Evolocumab recommended doses are 140 mg S.C every 2 weeks or 420 mg S.C every 4 weeks; the scheme selection (both having the same effectiveness) is made by the patients [37].

Based in the TESLA pilot and TESLA-B trial results [40,41], Evolocumab 420 mg S.C every 4 weeks is indicated in individuals ≥12 years of age with HoFH. In the TESLA-B trial, individuals with HoFH receiving lipid-lowering treatment and not on apheresis, Evolocumab compared to placebo reduced ultracentrifugation LDL-cholesterol by 30.9% (95% CI 43.9% to 18.0%: p<0.0001) and apolipoprotein B by 23.1% (95% CI 34.8 to 11.5%: p 0.002). It is very relevant to note that LDL-cholesterol responses were according to LDLR function; patients with LDLR negative mutations in both alleles and one patient with autosomal recessive HoFH showed no response to Evolocumab, whereas in patients with LDLR mutations in both alleles of which at least one was defective the LDL-cholesterol reduction was 40.8%. In this cohort of patients with very high levels of Lp (a), the overall reduction in this lipoprotein did not reach statistical significance (11.8% with 95% CI 25.5% to 1.8: P=0.09). The safety and tolerability profile of Evolocumab was similar to placebo.

Alirocumab (Praluent®) is indicated in patients with HeFH and non-FH as complement to diet, in combination with statins or other non-statin lipid lowering therapies in subjects who do not achieve LDL-cholesterol target levels with maximum therapeutic statin doses or either alone or in combination with other non-statin lipid lowering therapies when there is intolerance and/or contraindication for statins. The Alirocumab recommended doses are 75 mg or 150 mg S.C every 2 weeks; the scheme selection depends on the treatment gap (actual LDL cholesterol - target LDL cholesterol) [39].

In the ODYSSEY ESCAPE trial [42], individuals with HeFH on stable weekly or Q2W apheresis, Alirocumab compared to placebo reduced in 75% (95% CI 67% to 0.83%: p<0.0001) the standardized rate of apheresis treatment (apheresis treatments done on-treatment/apheresis treatments planed). In this trial apheresis was not performed when the LDL-cholesterol at the on-treatment visit was ≥30 lower than the baseline pre-apheresis value. Alirocumab reduced calculated LDL-cholesterol by 53.7% (95% CI 58.2% to 49.2%: p<0.0001) compared with 1.6% (95% CI 4.7% to +7.9%) with placebo. It is relevant to underlie that in this trial apheresis was not performed based in a percentage criteria (≥30% LDL-cholesterol lowering) not based in a target approach. The safety and tolerability profile of Alirocumab was similar to placebo.

For both MAbs-PCSK9, all Regulatory Agencies stressed that at the time of its initial approval there was no definitive evidence on the impact of treatment with MAbs-PCSK9 in the incidence of ASCVD [37-39].

ESC/EAS 2016 recommendations for MAbs-PCSK9

In 2016, a ESC/EAS Task Force Consensus on PCSK9: practical guidance for use in patients at very high cardiovascular risk was published by Landmesser et al. [43]. This paper aims to provide support in appropriately allocating a highly effective LDL-cholesterol lowering therapy taking account of high cost of PCSK9 inhibitors and financial restraints within healthcare budgets. Specifically very high risk patients for whom PCSK9 inhibitors may be considered are the following: a) Individuals with ASCVD or Diabetes Mellitus with target organ damage or other major risk factor on maximally tolerated efficacious statins (atorvastatin or rosuvastatin) plus ezetimibe and LDL-cholesterol >140 mg/dL; b) Individuals with rapid progression of ASCVD on maximally tolerated efficacious statins (atorvastatin or rosuvastatin) plus ezetimibe and LDL-cholesterol >100 mg/dL; c) Severe HeFH without ASCVD on maximally tolerated efficacious statins (atorvastatin or rosuvastatin) plus ezetimibe and LDL-cholesterol >200 mg/dL; d) Severe HeFH without ASCVD with ≥1 other major risk factor or very high risk on maximally tolerated efficacious statins (atorvastatin or rosuvastatin) plus ezetimibe and LDL-cholesterol >175 mg/dL; e) HoFH without ASCVD on maximally tolerated lipid lowering therapy with or without apheresis, except individuals with negative/negative LDLR mutations; f) Statin intolerant individuals on ezetimibe and any of the above conditions.

Justified by the lack of definitive cardiovascular outcomes results with PCSK9 inhibitors and the intrinsic differences among health care systems, the European recommendations appear to be more conservative than other recent American recommendations [44,45]. Both recommendations should be re-evaluated with the availability of data from large, randomized cardiovascular outcomes studies evaluating the impact of these novel agents on ASCVD and related thromboembolic events.

Cardiovascular End-Points and Long-Term Safety Studies

Study **FOURIER** "Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk" (NCT01764633) [46] is comparing Evolocumab *vs* placebo in 27,500 subjects with evident ASCVD (secondary prevention). The primary objective of this study is to demonstrate the superiority of Evolocumab 140 mg S.C/2 weeks or 420 mg S.C/4 weeks *vs* placebo S.C/2 or 4 weeks in the incidence of the composite endpoint of: cardiovascular death, non-fatal myocardial infarction, non-fatal cerebral infarction, hospitalization for unstable angina and/or bypass graft surgery. Population included in this phase III, randomized, double-blind, placebo-controlled, parallel-group study, are subjects aged between 40 to 85 years with evident ASCVD under optimal treatment for risk factors and ASCVD and LDL-cholesterol ≥70 mg/dL. The study initiated in January 2013, the enrollment is currently completed and results are originally expected by February 2018. However, by AMGEN extra official communication, FOURIER results could be presented the first quarter 2017.

Study **ODYSSEY** Outcomes "Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab" (NCT01663402) [47], is comparing Alirocumab *vs* placebo in 18,000 subjects with a history of ACS (secondary prevention). The primary objective of this study is to demonstrate the superiority of Alirocumab 150 mg S.C/2 weeks *vs* placebo S.C/2 weeks in the incidence of the composite endpoint of: cardiovascular death, non-fatal myocardial infarction, non-fatal cerebral infarction and/or hospitalization for unstable angina. Population included in this phase III, randomized, double-blind, placebo-controlled, parallel-group study are subjects aged ≥40 years with a history of ACS (4 to 52 weeks prior to enrollment), under optimal treatment for risk factors and ASCVD and LDL-cholesterol ≥70 mg/dL. The study initiated in October 2012, enrollment is currently completed and results are expected by December 2017.

Studies **SPIRE I y SPIRE II** "The Evaluation of Bococizumab in Reducing the Occurrence of Mayor Cardiovascular Events in High Risk Subjects" (NCT01975376 and NCT01975389) [48,49], were comparing Bococizumab *vs* placebo in 17,000 and 11,000 subjects at risk of or with ASCVD (primary and secondary prevention). The primary endpoint of both studies was to demonstrate the superiority of Bococizumab 150 mg S.C/2 weeks *vs* placebo S.C/2 weeks in the incidence of the composite endpoint of: cardiovascular death, non-fatal myocardial infarction, non-fatal cerebral infarction and/or hospitalization for unstable angina requiring revascularization. Population included in both phase III, randomized, double-blind, placebo-controlled, parallel group studies were subjects aged ≥18 years at high risk of ASCVD or with ASCVD, under optimal treatment for risk factors and ASCVD and LDL-cholesterol ≥70 mg/dL (SPIRE I) or ≥100 mg/dL (SPIRE II). Both studies initiated in October 2013, enrollment was completed in 2016.

Regarding this program, on November 01, 2016 the Global Clinical Lead (James H. Revkin) issued a letter to Investigators announcing the termination of the Bococizumab clinical development program, concluding that based in phase II and III clinical trial results, Bococizumab was unlikely to provide additional therapeutic value compared to other agents in the same class [50].

PCSK9 Inhibition by mRNA Interference

The knowledge of the natural interference pathway of RNA or RNAi, and the development of small molecules activating this pathway or siRNA, have allowed the development of the messenger RNA interference strategy that dictates the translation for the PCSK9 synthesis [51]. The lipid nanoparticle ALN-PCS conjugated with N-acetyl-galactosamine or Inclisiran is a small molecule avidly captured by hepatic asialo-glycoprotein receptors. This molecule inhibits by interference the mRNA dictating the translation for the PCSK9 synthesis and thus reducing its circulating levels significantly [52].

The simple blind-design phase I study published by Fitzgerald et al. [53] showed that Inclisiran *vs* placebo (3:1) in subjects aged ≥18 years, naive or on stable statin treatment, and LDL cholesterol levels of >100 mg/dL, reduced the circulating level of PCSK9 from 69.9% to 74.5% (single SC dose cohort: 25 mg, 100 mg, 300 mg, 500 mg and 800 mg) and from 71.8% to 83.8% (multiple SC dose cohort: 125 mg/wk, 250 mg/2 wk, 300 mg/4 wk, and 500 mg/4 wk). The reduction in circulating PCSK9 was accompanied by a reduction in LDL-cholesterol from 36.7% to 50.6% (single-dose cohort) and from 45.1% to 59.7% (multiple-dose cohort). Both the reduction in circulating PCSK9 and LDL-cholesterol measured at day 84 after the first dose were dose-dependent and their duration was also prolonged in a dose-dependent manner up to ≥180 days; the SC dose of 300 mg/wk showed the best pharmacodynamic profile.

No serious adverse events or events leading to treatment discontinuation were reported in this study. The most frequent adverse events included cough, musculoskeletal pain and nasopharyngitis (single-dose cohort) and headache, back pain and diarrhea (multiple-dose cohort); only 1 patient had a significant elevation of liver enzymes related to high-intensity statins. The results of this study favored the development of the ORION phase II program, which recently reported very favorable preliminary results with Inclisiran 300 mg each 3 and inclusive each 6 month [54].

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