PCSK9i: Proprotein Convertase Subtilisin/kexin Type 9 Inhibitor
A New Phase of Lipid-Lowering Treatment

By
Anikah Lubaba
19146073

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements

for the degree of

Bachelor of Pharmacy (Hons).

School of Pharmacy
Brac University
February 2023

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Anikah Lubaba
19146073

Approval

The thesis titled “PCSK9i: Proprotein convertase subtilisin/kexin type 9 inhibitor. A new phase of lipid-lowering treatment” submitted by Anikah Lubaba,(19146073) of Summer, 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

Supervised By:

Luluel Maknun Fariha
Lecturer
School of Pharmacy
BRAC University

Approved By:

Program Director:

Professor Dr. Hasina Yasmin
Program Director and Assistant Dean
School of Pharmacy
BRAC University

Dean:

Professor Dr. Eva Rahman Kabir
Dean
School of Pharmacy
BRAC University

Ethics Statement

There were no trials involving humans or animals in this study. The original sources of the information included here are properly credited.

Abstract

One of the largest global causes of morbidity and mortality is cardiovascular disease (CVD). Elevated low-density lipoprotein (LDL) levels and adverse cardiovascular outcomes are linked to increased levels of the proprotein convertase subtilisin/kexin type 9 (PCSK9) in the blood. Inhibiting PCSK9 lowers the levels of circulating LDL-C by increasing the extracellular membrane density of LDL receptors. Regulators have approved the use of PCSK9 antibodies to treat patients with high LDL-cholesterol (LDL-C) levels. It can reduce LDL-C in patients receiving statin therapy by as much as 60% with clinical advantages, such as lower incidence of myocardial infarction or stroke. The outcomes of the major clinical trials, ODYSSEY and the FOURIER indicated a statistically significant decrease in mortality with a relative risk reduction of 15% in both trials. Two monoclonal antibodies (evolocumab and alirocumab) are PCSK9 inhibitors approved by the FDA for the treatment of familial hypercholesterolaemia, and patients who require additional therapies along with healthy diet and statin medication.

Keywords: PCSK9, PCSK9i, Alirocumab, Evolocumab, Cholesterol-lowering therapies, cardiovascular diseases, hyperlipidemia, Monoclonal antibody.

Dedication

I would like to express my sincere gratitude to my family for their continuous encouragement during this project and also my supervisor.

Acknowledgement

First and foremost, I would like to thank my respected supervisor for her continuous encouragement throughout the project as well as for her inspiration, patience, and enormous knowledge, all of which were crucial during the review task. In addition, I would like to express my sincere gratitude to Professor Dr. Eva Rahman Kabir, Professor and Dean of the School of Pharmacy at BRAC University for her assistance in guiding the department and the student as well as Dr. Hasina Yasmin, Program Director and Assistant Dean of the School of Pharmacy for her immense support. Second, I would like to thank my parents for constantly supporting me. Finally, I want to convey my gratitude to all the professors and personnel who have inspired me over the past four years.

Table of Contents

Declaration.....ii

Approval iii

Ethics Statement.....iv

Abstract.....v

Dedication vi

Acknowledgement.....vii

Table of Contents viii

List of Tables x

List of Figures.....xi

List of Acronyms.....xii

Chapter 1 Introduction.....1

1.1 Bcakground.....1

1.2 Objective of the study……1

1.3 Rationale of the study ……...2

Chapter 2 Methodology……3

Chapter 3 Proprotein convertase subtilisin/kexin type 9 inhibitor.....4

3.1 PCSK9……4

3.2 PCSK9i……4

3.3 Mechanism of action ……6

3.4 Non LDL-C-Lowering effects of PCSK9 inhibitor……..8

Chapter 4 Monoclonal antibodies PCSK9 Inhibitor……**10**

4.1 Evolocumab……10

4.2 Alirocumab ……11

Chapter 5 Summary of Clinical Trials……..…….……...12

Chapter 6 Discussion ……..……...16

Chapter 7 Future Directions……..17

Chapter 8 Conclusion…….18

References……...19

List of Tables

Table 1: Different CVD medicines with actions.....8

Table 2: Summary table of trials in different regions……..……..13

List of Figures

Figure 1: Interaction between PCSK9i and LDL-R.....7

Figure 2: Working mechanism of PCSK9i.....7

List of Acronyms

PCSK9i	Proprotein convertase subtilisin/kexin type 9 inhibitor
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
CVD	Cardiovascular disease
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
Lp(a)	Lipoprotein a
MACE	Major adverse cardiovascular events
ACS	Acute coronary syndrome
CHD	Congenital heart defects
ASCVD	Atherosclerotic cardiovascular disease

Chapter 1

Introduction

1.1 Background

Cardiovascular disease continues to be the main cause of mortality and morbidity, despite the development of therapies which reduce low-density lipoprotein cholesterol (LDL-C) (Wadhera et al., 2016). According to WHO, the biggest cause of death worldwide CVDs claim 17.9 million lives annually (WHO, 2021). Globally, CVD was estimated to be responsible for 19.1 million deaths in 2020 (Tsao et al., 2022). Age-adjusted mortality rate was 239.8 per 100,000 people according to American heart association (Tsao et al., 2022). Lipids are vital for cell construction, division, signal, and supply of energy and also make up the majority of the membrane's structural elements. Cholesterol is a component of cell membrane that affects the fluidity of the membrane, the ability of cells to bind to extracellular matrix, and the beginning of signaling (Mahboobnia et al., 2021). Due to the exposure to high levels of LDL-C since birth, familial hypercholesterolemia (FH), a hereditary illness caused by gene mutations encoding proteins that play a role of (LDL), is known by premature CVD (Raal, Hovingh and Catapano, 2018). Hypercholesteremia is managed and treated with statin medicines as they reduce liver cholesterol synthesis (Coppinger et al., 2022). They are recognized as hydroxymethylglutaryl-CoA reductase inhibitors. Patients who cannot take statins or who do not meet their treatment objectives while receiving high-intensity statin medication, like people with familial hypercholesterolemia, continue to be at concern (Castilla-Guerra et al., 2019). Leukemia, lung, pancreatic cancers and glioblastoma, CVD have all been linked to an increase in LDLR and high plasma LDL-C (Huang et al., 2016). This review aims to provide a comprehensive overview of how PCSK9i can provide the world a glimmer of hope for the treatment of hyperlipidemia and incorporation of statins with monoclonal antibody for more of LDL reduction. Additionally, limitations behind reduced monoclonal antibody entrance into the bloodstream and physiological dysfunction as once monoclonal antibody is received were discussed in this article.

1.2 Aim & Objectives of the Study

Aim: The aim of this review is to show the ray of hope for the management of hyperlipidemia with the combination of monoclonal antibody and statins to further LDL reduction.

Objectives: The objective of the project is to compile information on recent clinical studies and acquire more knowledge about PCSK9i and give a concise assessment of the clinical trials done previously in reduction of LDL using monoclonal antibody along with statins. And also to inspire doing more research into monoclonal antibodies, not just as a LDL reduction treatment, but for a wide range of other pharmacological purposes and make it cost efficient.

1.3 Rationale of the Study

Cardiovascular disease has been major cause of death now-a-days worldwide and statin therapy alone is unable to function as effectively as addition of PCSK9i therapy. Inhibition of PCSK9 is safe for statin intolerant patients and reduction of CVD risk is higher than statins. Kidney disease, Lipodystrophy, Hypothyroidism, Hyperinsulinemia, Nonalcoholic fatty liver disease are reasons behind PCSK9 increasing and inhibition of PCSK9 is done by two FDA approved monoclonal antibodies that were discussed to know more about their pharmacodynamics. This article doesn't include any studies using human or animal subjects; instead, it is based on prior completed research. The study is important because in a record amount of time, the significant scientific discoveries have been transformed into a successful medical treatment. This discovery's transformation made up as a breakthrough therapy is one of the best instances of how genetic knowledge may be used to find targets for sophisticated drugs. Although the PCSK9 controversy is developing swiftly, it is still far from end. So, this article will help in further research on PCSK9i and their effect on human.

Chapter 2

Methodology

This review was supported by current, significant research publications and articles from reputable journals. Peer-reviewed journals, clinical study papers, and articles were all thoroughly searched. To support the review study, basic and supplemental information was acquired from books. For this paper's data collection, electronic databases such as ResearchGate, PubMed, Google scholar, and Embase were used. Additionally, this article's research was done using both primary and secondary sources. The keywords that have been used to avail the data are “PCSK9”, “PCSK9i”, “Alirocumab”, “Evolocumab”, “Cholesterol-lowering therapies”, “cardiovascular diseases”, “hyperlipidemia”, “Monoclonal antibody”. PCSK9 and PCSK9 inhibitors, Clinical trials determining the safety and efficacy of PCSK9 inhibitors, comparison of PCSK9i with statin in LDL reduction articles were included and unrelated, duplicates and abstract only journals were excluded. Proprotein convertase subtilisin/kexin type 9 inhibitor, a comprehensive search of journals was carried out, followed by a filtering down to the most recent and pertinent ones. Websites like WHO, FDA, American heart association were searched for relevant information.

Chapter 3

3.1 What is PCSK9?

It is an enzyme that is produced in humans by the PCSK9 gene on chromosome 1 (Seidah et al., 2003). It is the ninth member of the family of proteins called proprotein convertases that trigger additional proteins (Seidah et al., 2003).

3.2 What is PCSK9i?

PCSK9i is Proprotein convertase subtilisin/kexin type 9 inhibitor. It inhibits PCSK9 gene. Alirocumab, evolocumab, and inclisiran are three pharmaceuticals that are approved in the FDA-US to suppress PCSK9 activity (Pokhrel, Yuet and Levine, 2022). Entirely humanized mAb alirocumab and evolocumab, which are administered for two to four weeks, are quite effective at reducing both total and LDL cholesterol (Pokhrel, Yuet and Levine, 2022). A tiny interfering mRNA called inclisiran prevents the creation of PCSK9 inside cells. Inclisiran decreases LDL cholesterol by 50% when given to patients taking the highest tolerable dose of a statin (Pirillo and Catapano, 2022). In 2016, bococizumab, an upgraded phase III clinical trial for a third PCSK9 antibody, was shelved (Mahajan, 2018). Even after implementation of six bococizumab studies, substantial rates of injection-site sensitivity because of strong immunogenicity, as well as an unanticipated reduction of effect on LDL-C over time, were identified (Kaddoura et al., 2020). Antisense oligonucleotides and tiny interfering RNAs are examples of molecules that stop PCSK9 from forming (Katzmann et al., 2020). Monoclonal antibodies and tiny adnectin polypeptides are among the molecules that bind to mature PCSK9 and inhibit it from interacting with LDL receptors (Page & Watts, 2016). Various cell types have low-density lipoprotein receptors on their outer surfaces. These receptors allow LDLs to be taken up and transported into cells from the circulation. Once inside the tissue, the LDL is degraded to liberate cholesterol. The cholesterol is subsequently utilized by the cell, kept, or the body expels it (Cox and García-Palmieri, 1990). Alirocumab, evolocumab treatment was linked with a larger decline in LDL-C, according to a network meta-analysis of clinical trials (Rallidis et al., 2019). Alirocumab effectively decreased atherogenic cholesterol and LDL compared to control among patients with T2DM and ASCVD who had high non-HDL-C/LDL-C levels even after using a maximum tolerant statin (Ray et al., 2019).

- Ezetimibe + statin decrease LDL-C upto 15-20%.
- PCSK9I + statin decrease LDL-C upto 54-74%
- Evolocumab decrease 14-20% more LDL-C compared with Alirocumab (Coppinger et al., 2022).

They are altering the treatment options for dyslipidemia because it has been demonstrated that they significantly lower LDL-C levels when used single or in conjunction along with statin therapy (Ding et al., 2022). Statins may cause MAEs but PCSK9i is a safer option. Anyway, Nasopharyngitis and upper respiratory tract infections were the quite frequent adverse events (AEs) in PCSK9i, but MAEs were reported less frequently (Ding et al., 2022). According to the results of the main clinical studies FOURIER and ODYSSEY OUTCOMES Evolocumab or alirocumab coupled to statin therapy in patients with CVD confers additional CV benefit above that achieved by statin alone. Contrary to predictions, PCSK9i are not as widely utilized as may be expected mostly because of their high price and obstacles posed by regulatory health authorities (Rallidis et al., 2019). In the FOURIER trial, participants were monitored for 2.2 years while receiving either evolocumab or a matching placebo. 27 564 patients with steady atherosclerotic disease on statin therapy were included in the study (Sabina A. Murphy, 2019). The average follow-up period was 2.8 years in ODYSSEY trial. At 1315 places in 57 countries, a sum of 18,924 patients were randomly assigned; 9462 were given alirocumab, while 9462 received a placebo (Schwartz et al., 2018).

3.3 Mechanism of Action

The apoprotein B100 on the exterior of LDL interacts to LDLR on hepatic and extrahepatic organs and LDLC is eliminated from the bloodstream (figure1). Endocytosis occurs as a result of LDL binding to its receptors (Pokhrel, Yuet and Levine, 2022). As lysosomes and endocytic vesicles combine, the amount of unbound cholesterol inside the cell rises. Three things happen as cholesterol concentrations inside cells rise (Pokhrel, Yuet and Levine, 2022).

- Reduce HMG-CoA reductase activity (the rate of cholesterol synthesis control enzyme).
- Activing of the enzyme ACAT (rise in the deposition of cholesterol in the form of cholesterol ester)
- Decreased cell surface activation of LDL receptors.

In order to bind and excrete more LDL cholesterol, LDLR are constantly recycled back to the cell surface (Pokhrel, Yuet and Levine, 2022) (figure2). Hepatocyte-produced PCSK9, which binds to LDLR to promote their lysosomal breakdown, is discharged into the bloodstream. In this way, PCSK9 lessens the expression of LDL receptors on cell membranes, hence reducing LDL cholesterol clearance (Pokhrel, Yuet and Levine, 2022). PCSK9i inhibit the PCSK9 enzyme and increase cholesterol clearance. The LDLR on the exterior of the hepatic cells binds to LDL, the LDL-LDL-R complex is internalize and the LDLR is then typically reconverted to the surface of the cell near to 150 times (Handelsman et al., 2018). The LDLR just on exterior of the liver cell binds to released PCSK9, which causes the LDLR absorbed and broken down in the lysosomes. This decreases the number of LDLRs just on cell exterior. The availability of LDLRs on the cell exterior should consequently increase in response to inhibition of released PCSK9, as well as the uptake of LDL-C into the cell. Inhibiting PCSK9 therefore provides a new therapeutic approach for reducing LDL-C levels (Handelsman et al., 2018). The metabolic alterations brought on by statin medication and anti-PCSK9 antibody therapy, however are not the same. While both methods increase HDL cholesterol, lower LDL-C, triglycerides, and apolipoprotein B, statins also lower C-reactive protein (CRP) (Sabatine, 2019). A mouse model revealed that the anti-atherosclerotic actions of PCSK9 antibodies may entail anti-inflammatory properties (Schuster et al., 2019).

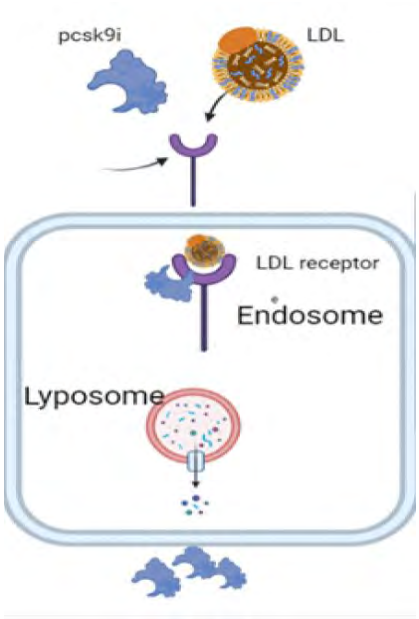

Figure 1: Interaction between PCSK9i and LDL-R. The LDL receptor binds to PCSK9. Degradation of LDL receptor occurs following incorporation of the LDL receptor linked to PCSK9 (and then an LDL). Adapted from (Katzmann et al., 0001)

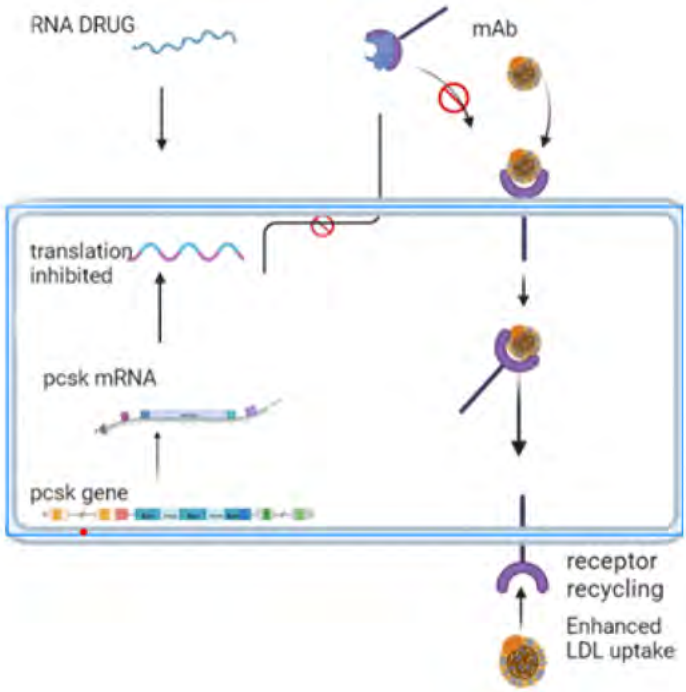

Figure 2: Working mechanism of PCSK9i. Pharmacologically, PCSK9 can be suppressed by mabs that bind and neutralize PCSK9 or by RNA-targeting drugs that comprise an RNA strand ancillary to PCSK9 mRNA and cause the formation of an RNA-induced silencing complex (RISC), which for an extended period of time degrades PCSK9 mRNA and thereby prevents PCSK9 production. Adapted from (Katzmann et al., 0001).

Table 1: Different CVD medicines with actions. Adapted from (Katzmann et al., 0001)

CVD medicine	Inhibit	Action
Statin	3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase	Reduce liver cholesterol production
Ezetimibe	Niemann-Pick C1-like protein 1 (NPC1L1)	Aids cholesterol be absorbed in the gut
PCSK9i	PCSK9 protein	Halt the destruction of LDLR by PCSK9 proteins.

3.4 Non-LDL-C-Lowering Effects of PCSK9 Inhibition

Apolipoprotein B and VLDL cholesterol have been reported to be decreased by various PCSK9 inhibitors, in addition to raising LDLR concentrations and decreasing LDL-C concentrations. Additionally, apolipoprotein A-I and HDL-C are somewhat elevated by PCSK9 inhibitors (apoA-I). Lipoprotein(a) levels have also been observed to be decreased by PCSK9 inhibitors of various types. Statins however, have little impact on Lp (a). Reduced apoB may limit the quantity of cholesterol moved from HDL to particles carrying apoB, which is one explanation for this impact (Stoekenbroek et al., 2019). According to one theory, Lp(a) doesn't effectively compete with LDL for LDLR interaction. Therefore, Lp(a) reduces when PCSK9 inhibitors enhance LDLR expression, especially when LDL is low (Romagnuolo et al., 2015) There is no evidence that therapeutic PCSK9 inhibition causes diabetes to rise. Without influencing other bodily regions where PCSK9 is generated, inhibition takes place primarily inside the liver. The incidence of diabetes has been observed to slightly increase as a result of PCSK9 function loss mutations (A. Ference et al., 2016). Plasma glucose levels rise and so does the risk of type 2 diabetes as a result of genetic PCSK9 deficiency. In a research, using PCSK9 knockout (KO) mice, it was discovered that total loss of PCSK9 caused higher glucose intolerance and decreased insulin production, but had no impact on pancreatic β-cell insulin resistance (Da Dalt et al., 2019). Last but not least, decreased levels of thrombosis have been linked to lower PCSK9 levels. FeCl3 due to injuries carotid artery thrombosis observed to be significantly reduced in PCSK9 KO mice (Paciullo, Momi and Gresele, 2019). In contrast, individuals

suffering from acute coronary syndrome who have greater PCSK9 levels also have increased platelet reactivity (ACS). PCSK9 concentrations and platelet reactivity from ACS patients taking daily doses of either prasugrel or ticagrelor were examined in the PCSK9-REACT Study. Every participant had a direct relationship with circulating PCSK9 concentrations and platelet reactivity, which resulted in those with greater serum PCSK9 having more atherothrombotic occurrences at the 1-year mark (Navarese et al., 2017). So, we can say that non-LDL-C-Lowering Effects of PCSK9 Inhibition is present.

Chapter 4

Monoclonal Antibodies PCSK9 Inhibitors

4.1 Evolocumab

Evolocumab is a PCSK9i which is approved by FDA. Human PCSK9 is selectively bound by evolocumab, a human monoclonal immunoglobulin G2, to lower LDL-C (Kasichayanula et al., 2018). Intensive statin therapy that lowers LDL levels slows the development of coronary atherosclerosis in accordance to the LDL-C reductions. However, the effects of these medications on coronary atherosclerosis have not been studied. PCSK9 inhibitors cause incremental LDL-C decrease in people using statins. 968 participants presenting for coronary angiography were enrolled in the GLAGOV multicenter, placebo-controlled, double-blind, completely random clinical trial, which took place at 197 community and academic hospitals in North America, South America, Europe, South Africa Asia and Australia from May 3, 2013, to January 12, 2015 (Nicholls et al., 2016). Evolocumab (420 mg) or placebo (n = 484) was administered subcutaneously once every month for 76 weeks to people with angiographic coronary disease who also took statins. By using serial intravascular ultrasonography (IVUS) imaging, the minimum change in percent atheroma volume (PAV) from beginning to week 78 served as the primary effectiveness measure. The nominal variation in total atheroma volume (TAV) that was standardized and the proportion of patients who experienced plaque regression were used as secondary effectiveness metrics. There was also an evaluation of tolerance and safety (Nicholls et al., 2016). In clinical trials including high-risk patients, Evolocumab and other PCSK9i antibodies decreased unfavorable cardiovascular events throughout a median treatment period of about 3 years (Koren et al., 2019). Evolocumab has an effective half-life of 11 to 17 days. Evolocumab's pharmacodynamic effects on PCSK9 are swift, reaching their peak within 4 hours. At steady state, the peak LDL-C reduction occurs around a week after receiving a subcutaneous dose of 140 mg in Q2W and two weeks after a subcutaneous dose of 420 mg once a month and the level gradually returns to normal over the course of the dosing interval. These evolocumab doses decreased LDL-C by roughly 55-75% compared to placebo in multiple clinical studies. In clinical investigations, evolocumab also decreased levels of lipoprotein(a) and increased levels of other lipids (Kasichayanula et al., 2018). In the longest-lasting PCSK9 inhibitor study to date, the OSLER-1 trial repeatedly showed outstanding LDL-C-lowering effectiveness, tolerance, and safety with evolocumab while detecting no neutralizing antibodies (Koren et al., 2019).

4.2 Alirocumab

Alirocumab (Praluent®; Sanofi), a completely human monoclonal antibody against PCSK9, was approved by the Food and Drug Administration and the European Medicines Agency in 2015 for the treatment of hypercholesterolemic patients unable to meet LDL-C targets as an adjunct to diet in addition to or instead of lipid lowering treatment (Della Pepa et al., 2017). Large plaque burden, thin fibrous caps and high lipid content are characteristics of coronary atherosclerotic plaque that are vulnerable to rupture or rather result in severe cardiac events. The advancement of coronary atherosclerosis can be stopped by statin medication. However, little is known about how adding the PCSK9i Alirocumab to statin therapy may affect the load and makeup of the plaque. Clinical trial PACMAN-AMI, which was double-blinded, randomized, and placebo-controlled (enrollment was on 9th May 2017, through 7th October 2020 and final follow-up: 13th October 2021) 300 patients were enrolled who were having acute myocardial infarction treated with percutaneous coronary intervention at 9 academic hospitals in Europe (Räber et al., 2022). In addition to high-intensity statin medication, patients were randomly assigned to take weekly twice subcutaneous alirocumab (150 mg; n = 148) or placebo (n = 152) that was started less than 24 hours following urgent percutaneous coronary intervention of the culprit lesion (rosuvastatin, 20 mg) (Räber et al., 2022). At start and 52 weeks later, serial intravascular ultrasonography (IVUS), optical coherence tomography and near-infrared spectroscopy procedures were carried out in the 2 non-infarct-related coronary arteries. The change in baseline to week 52 atheroma volume as determined by IVUS served as the major efficacy end objective. Changes in the maximal lipid core load index within 4 mm, as determined by near-infrared spectroscopy were two powered secondary end points (high numbers shows greater lipid presence) and the lowest fibrous cap thickness determined by optical coherence tomography from beginning to week 52 (lower value shows thin-capped, susceptible plaques) (Räber et al., 2022). The reductions in initial lipoprotein(a) and adjusted LDL-C levels and the risk of MACE following recent ACS were predicted by alirocumab. Alirocumab lowers lipoprotein(a), which suggests that lipoprotein(a) might be a separate therapy goal following ACS. Lipoprotein(a) is an individual influencer to MACE reduction (Bittner et al., 2020).

Chapter 5

Summary of Clinical Trials

In 2008 a trial named 0653A-091 was done on Hyperlipidemia of type IIa or IIb patients with Ezetimibe/statin vs. niacin vs. ezetimibe/statin/niacin for 64weeks in the USA. After 6years in 2014 DESCARTES trial took place in multinational with hypercholesterolemia patients with Control versus Evolocumab 420 mg every 4 week and followed up to 52 weeks. In USA IMPROVE-IT, ODYSSEY COMBO I, ODYSSEY OUTCOMES were done whereas GLAGOV and FOURIER was in Europe and ODYSSEY COMBO II, ODYSSEY FH I, ODYSSEY LONG TERM took place in different nation. IMPROVE-IT (2015) with Hypercholesterolemia and a current hospitalization for acute coronary syndrome (within the last 10 days) and treatment was simvastatin 40 mg with placebo versus Ezetimibe 10 mg with simvastatin 40 mg versus for 52 weeks. In the same year ODYSSEY COMBO I in(USA) and ODYSSEY COMBO II(multinational) with Alirocumab 75mg to 150 mg every 2 Week vs. control for Hypercholesterolemia with CHD or CHD-risk equivalent was done for 52weeks. Alirocumab 75mg to 150 mg every 2week vs. control was given to patients with FH in ODYSSEY FH1 in the same year 2015 but followed up for 78 weeks. ODYSSEY LONG TERM was for Heterozygous familial hypercholesterolemia, coronary heart disease, or its risk equivalent patients with Alirocumab 150 mg every 2 Week vs. control and followed up for 78weeks. Angiographic coronary disease patients participated in GLAGOV and given Evolocumab 420mg monthly vs. control and 76weeks follow up. In 2017 FOURIER trial worked with Atherosclerotic CVD with clinical proof and Evolocumab either 140mg every 2 Week or 420 mg every month vs. control given for follow up of 168weeks. ODYSSEY OUTCOMES (2018) was with Acute coronary syndrome hospitalization within the last 52 weeks using Alirocumab 75mg to 150 mg every 2 Week vs. control for 48months (table2).

Table 2: Summary table of trials in different regions. Adapted from (Chiu et al., 2020)

Name of the trial	Year	Country	Treatment	Result
0653A-091	December 2005-February 2008	USA	Ezetimibe/statin vs. niacin vs. ezetimibe/statin/niacin	Analyze the Effectiveness and Safety of Co-Administrating niacin and Ezetimibe/Simvastatin (Extended Release Tablet) in Patients With Type IIa or Type IIb Hyperlipidemia
DESCARTES	January 2012-October 2013	Multinational	Control versus Evolocumab 420 mg every 4 week	Analyze AMG 145's (Evolocumab) Long-Term Tolerance and Lasting Effectiveness on Bad cholesterol in Hyperlipidemic Patients
IMPROVE-IT	October 2005-September 2014	USA	Simvastatin 40 mg with placebo versus Ezetimibe 10 mg with simvastatin 40 mg	In elevated patients presenting with acute coronary syndrome, compare the clinical benefits and tolerability of Vytorin (ezetimibe/simvastatin tablet) to simvastatin monotherapy.

ODYSSEY COMBO I	July 2012- April 2014	USA	Alirocumab 75mg to 150 mg every 2 Week vs. control	Analyze the Safety and Effectiveness of SAR236553/REGN727 in Patients with High Cardiovascular Risk and Hypercholesterolemia That Is Not Sufficiently Controlled by Their Lipid-Modifying Treatment
ODYSSEY COMBO II	August 2012- July 2015	Multinational	Alirocumab 75mg to 150 mg every 2 Week vs. ezetimibe 10 mg	Analyze the Safety and Effectiveness of SAR236553/REGN727 Compared to Ezetimibe in Patients with High Cardiovascular Risk and Hypercholesterolemia That Is Not Sufficiently Controlled by Their Statin Treatment
ODYSSEY FH I	July 2012- December 2014	Multinational	Alirocumab 75mg to 150 mg every 2week vs. control	Determine the effectiveness and safety of SAR236553/REGN727 in treatment with heterozygous high cholesterol who are not well controlled by their lipid-modifying therapy.
ODYSSEY LONG TERM	January 2012-	Multinational	Alirocumab 150 mg every 2 Week vs. control	Long-term Efficacy and Biocompatibility of SAR236553 (REGN727) in Patients with High

	November 2014			Cardiovascular Risk and Hypercholesterolemia Not Adequately Managed by Current Lipid Modifying Treatment
GLAGOV	April 2013- July 2016	Europe	Evolocumab 420mg monthly vs. control	Assess the Impacts of Evolocumab (AMG 145) Therapy on ASC Disease Burden as Determined by Intravascular Ultrasonography in Patients Undergoing Coronary Catheterization
FOURIER	February 2013- November 2016	Europe	Evolocumab either 140mg every 2 Week or 420 mg every month vs. control	Evaluating the Effect of Further LDL-Cholesterol Lowering on Major Cardiovascular Events
ODYSSEY OUTCOMES	October 2012- January 2018	USA	Alirocumab 75mg to 150 mg every 2 Week vs. control	Analyze the Impact of Alirocumab on Cardiovascular Event Incidence in Patients With Recent Acute Coronary Syndrome

Chapter 6

Discussion

PCSK9 controls the breakdown of LDLR in the liver, which is the principal mechanism by which it controls cholesterol metabolism. The main mechanism by which blood LDL cholesterol levels in people are controlled is by LDL) receptors on the outside of liver hepatocytes. By destroying LDL receptors, PCSK9 indirectly controls serum LDL cholesterol. When there are fewer LDL receptors, LDL-C levels in the blood rise, but when PCSK9 is inhibited or bound, there are more LDL receptors and LDL-C levels fall. The two PCSK9 inhibitors alirocumab and Evolocumab are now licensed for use. They both bind unbound PCSK9 and are completely human monoclonal antibodies. The most effective doses can result in a substantial drop in LDL-C of up to 70%. Different trials have been done on human in different region to know the working mechanism of PCSK9i in people of different region. Because of both high cost and the challenges created by regulatory and health authorities, PCSK9i are not as commonly used as may be anticipated.

Although the majority of patients receiving the monoclonal antibody PCSK9i have the anticipated 50–60% drop in LDL-C(Qamar et al., 2019). There are two main kinds of theoretical reasons behind PCSK9i hypo-responsiveness (15% drop in LDL-C): Reduced monoclonal antibody entrance into the bloodstream and physiological dysfunction as once monoclonal antibody is received. Reduced circulation of a PCSK9i could be caused by Poor PCSK9i therapy adherence is one cause inaccurate PCSK9i delivery, faulty dermatological variables that affect systemic drug absorption, inappropriate antibody disposal (Warden, Fazio and Shapiro, 2020). Other reason for hypo-responsiveness are alterations to the binding site of antibody on circulation of PCSK9, anti-drug antibody deployed against PCSK9i, increased PCSK9 secretion, changes to or malfunction of LDLR, apoB, or apoE (Warden, Fazio and Shapiro, 2020). However, after starting a PCSK9i therapy, stopping concurrent lipid-lowering treatments (such as statins) is by far the most frequent source of apparent PCSK9i resistance(Bays et al., 2018). Numerous trials have demonstrated the safety and efficacy of PCSK9 inhibitors, including in diabetic individuals who are intolerant to statins. There is no proof that using this class of medications will cause any major side effects (Grześk et al., 2022). Although patients with only modestly raised baseline TG levels do not benefit from PCSK9 inhibitor, those with mild to severe hypertriglyceridemia do (Peng, Chen and Zheng, 2020).

Chapter 7

Future Directions

Whether a target LDL-C level should be used in practice is a topic of intense discussion. Regardless of baseline LDL-C levels, a 38.6 mg/dl (1 mmol/l) reduction in LDL-C was linked to a 21% reduction in ASCVD events in Cholesterol Treatment Trialists meta-analysis (Rosenson et al., 2018). In randomized comparisons of various statins or dosage of the same statin, lower event rates have been observed, so the reduce achieved LDL-C provides a justification for lowering the target. For instance, the achieved LDL-C values were 95 and 63 mg/dl, respectively, in Pravastatin or Atorvastatin Evaluation and Infection Therapy experiment, which compared routine versus intensive statin therapy (Cannon et al., 2004). As PCSK9 inhibitors are now accessible, it will be crucial that busy physicians do not underprescribe statins or become discouraged from searching for a dosage and statin drug that is acceptable by the patient. Despite these challenges, PCSK9 inhibitors have revolutionized lipid lowering therapy and are an attractive drug for lowering LDL-C hyperlipidemia. HOPE 3 trial (Heart Outcomes Prevention Evaluation-3), which found that statin medication lowered baseline LDL-C from 127 mg/dl to 90 mg/dl, supports the latter goal (Yusuf et al., 2016). PCSK9i do not show efficacy on triglyceride. Further research need to be conducted regarding this.

Chapter 8

Conclusion

Clinical research demonstrates that PCSK9 inhibitors are well tolerated, significantly lower LDL-C levels, and can prevent or delay further cardiovascular events in people with hyperlipidemia and diabetes mellitus in addition to maximally tolerated statin therapy. This is done without compromising glycemic control or increasing the risk of developing diabetes mellitus in people who do not already have it. The incidence of severe adverse cardiovascular events was reduced with alirocumab than with placebo in patients with a prior acute coronary syndrome and persistently increased levels of atherogenic lipoproteins after statin therapy at a higher dosage or at the maximum tolerable dose. PCSK9 inhibitors are a beneficial supplement to existing LDL-C therapy options. For individuals who are unable to tolerate statins but still need to treat their hyperlipidemia, PCSK9 medication is a welcome alternative. The cost of these recently approved medications and the absence of oral versions are limitations. It is hoped that future research on oral formulations and longer-term clinical trials will increase the use of this new class of pharmacological therapy in the treatment over atherosclerosis and CVD.

References

Bays, Harold E., Robert S. Rosenson, Marie T. Baccara-Dinet, Michael J. Louie, Desmond Thompson, and G. Kees Hovingh. "Assessment of the 1% of Patients with Consistent < 15% Reduction in Low-Density Lipoprotein Cholesterol: Pooled Analysis of 10 Phase 3 ODYSSEY Alirocumab Trials." *Cardiovascular Drugs and Therapy* 32, no. 2 (April 1, 2018): 175–80. https://doi.org/10.1007/s10557-018-6784-z.

Bittner, Vera A., Michael Szarek, Philip E. Aylward, Deepak L. Bhatt, Rafael Diaz, Jay M. Edelberg, Zlatko Fras, et al. "Effect of Alirocumab on Lipoprotein(a) and Cardiovascular Risk After Acute Coronary Syndrome." *Journal of the American College of Cardiology* 75, no. 2 (January 21, 2020): 133–44. https://doi.org/10.1016/j.jacc.2019.10.057.

Cannon, Christopher P., Eugene Braunwald, Carolyn H. McCabe, Daniel J. Rader, Jean L. Rouleau, Rene Belder, Steven V. Joyal, Karen A. Hill, Marc A. Pfeffer, and Allan M. Skene. "Intensive versus Moderate Lipid Lowering with Statins after Acute Coronary Syndromes." *New England Journal of Medicine* 350, no. 15 (April 8, 2004): 1495–1504. https://doi.org/10.1056/NEJMoa040583.

"Cardiovascular Diseases." Accessed January 16, 2023. https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_1.

"Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome | NEJM." Accessed January 19, 2023. https://www.nejm.org/doi/full/10.1056/nejmoa1801174#article_citing_articles.

"Cardiovascular Diseases (CVDs)." Accessed January 25, 2023. https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).

Chiu, Sarah W., Cristina M. Pratt, Richard Feinn, and Saurav Chatterjee. "Proprotein Convertase Subtilisin/Kexin Type 9 Inhibitors and Ezetimibe on Risk of New-Onset Diabetes: A Systematic Review and Meta-Analysis of Large, Double-Blinded Randomized Controlled Trials." *Journal of Cardiovascular Pharmacology and Therapeutics* 25, no. 5 (September 1, 2020): 409–17. https://doi.org/10.1177/1074248420924983.

"Cholesterol Lowering Drugs - Endotext - NCBI Bookshelf." Accessed January 25, 2023. https://www.ncbi.nlm.nih.gov/books/NBK395573/.

"Cholesterol Lowering in Intermediate-Risk Persons without Cardiovascular Disease | NEJM." Accessed February 10, 2023. https://www.nejm.org/doi/full/10.1056/NEJMOA1600176.

Coppinger, Caroline, Mohammad Reza Movahed, Veronica Azemawah, Lee Peyton, James Gregory, and Mehrnoosh Hashemzadeh. "A Comprehensive Review of PCSK9 Inhibitors."

Journal of Cardiovascular Pharmacology and Therapeutics 27 (January 1, 2022): 10742484221100108. https://doi.org/10.1177/10742484221100107.

Cox, Rafael A., and Mario R. García-Palmieri. "Cholesterol, Triglycerides, and Associated Lipoproteins." In *Clinical Methods: The History, Physical, and Laboratory Examinations*, edited by H. Kenneth Walker, W. Dallas Hall, and J. Willis Hurst, 3rd ed. Boston: Butterworths, 1990. http://www.ncbi.nlm.nih.gov/books/NBK351/.

Da Dalt, Lorenzo, Massimiliano Ruscica, Fabrizia Bonacina, Gloria Balzarotti, Ashish Dhyani, Eliana Di Cairano, Andrea Baragetti, et al. "PCSK9 Deficiency Reduces Insulin Secretion and Promotes Glucose Intolerance: The Role of the Low-Density Lipoprotein Receptor." *European Heart Journal* 40, no. 4 (January 21, 2019): 357–68. https://doi.org/10.1093/eurheartj/ehy357.

Della Pepa, Giuseppe, Lutgarda Bozzetto, Giovanni Annuzzi, and Angela Albarosa Rivellese. "Alirocumab for the Treatment of Hypercholesterolaemia." *Expert Review of Clinical Pharmacology* 10, no. 6 (June 2017): 571–82. https://doi.org/10.1080/17512433.2017.1318063.

Ding, Lingqing, Congqin Chen, Yongkuan Yang, Jie Fang, Longxing Cao, and Yige Liu. "Musculoskeletal Adverse Events Associated with PCSK9 Inhibitors: Disproportionality Analysis of the FDA Adverse Event Reporting System." *Cardiovascular Therapeutics* 2022 (January 25, 2022): 9866486. https://doi.org/10.1155/2022/9866486.

Druce, Irena, Hussein Abujrad, and Teik Chye Ooi. "PCSK9 and Triglyceride-Rich Lipoprotein Metabolism." *Journal of Biomedical Research* 29, no. 6 (November 2015): 429–36. https://doi.org/10.7555/JBR.29.20150052.

"Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes | NEJM." Accessed February 10, 2023. https://www.nejm.org/doi/full/10.1056/NEJMoa1410489.

Feingold, Kenneth R. "Cholesterol Lowering Drugs." In *Endotext*, edited by Kenneth R. Feingold, Bradley Anawalt, Alison Boyce, George Chrousos, Wouter W. de Herder, Ketan Dhatariya, Kathleen Dungan, et al. South Dartmouth (MA): MDText.com, Inc., 2000. http://www.ncbi.nlm.nih.gov/books/NBK395573/.

———. *Cholesterol Lowering Drugs*. *Endotext [Internet]*. MDText.com, Inc., 2021. https://www.ncbi.nlm.nih.gov/books/NBK395573/.

Grundy, Scott M., James I. Cleeman, C. Noel Bairey Merz, H. Bryan Brewer, Luther T. Clark, Donald B. Hunninghake, Richard C. Pasternak, Sidney C. Smith, and Neil J. Stone. "Implications of Recent Clinical Trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines." *Circulation* 110, no. 2 (July 13, 2004): 227–39. https://doi.org/10.1161/01.CIR.0000133317.49796.0E.

Grześk, Grzegorz, Bednarska Dorota, Łukasz Wołowiec, Anna Wołowiec, Joanna Osiak, Mariusz Kozakiewicz, and Joanna Banach. "Safety of PCSK9 Inhibitors." *Biomedicine & Pharmacotherapy* 156 (December 1, 2022): 113957. https://doi.org/10.1016/j.biopha.2022.113957.

Handelsman, Yehuda, and Norman E. Lepor. "PCSK9 Inhibitors in Lipid Management of Patients With Diabetes Mellitus and High Cardiovascular Risk: A Review." *Journal of the American Heart Association* 7, no. 13 (July 3, 2018): e008953. https://doi.org/10.1161/JAHA.118.008953.

Chemical & Engineering News. "How Are Drug Hunters Inhibiting PCSK9? Let Us Count the Ways." Accessed January 24, 2023. https://cen.acs.org/pharmaceuticals/drug-discovery/ways-inhibit-PCSK9/100/i4.

Jellinger, Paul S., Yehuda Handelsman, Paul D. Rosenblit, Zachary T. Bloomgarden, Vivian A. Fonseca, Alan J. Garber, George Grunberger, et al. "American Association of Clinical Endocrinologists and American College of Endocrinology Guidelines for Management of Dyslipidemia and Prevention of Cardiovascular Disease." *Endocrine Practice* 23 (April 1, 2017): 1–87. https://doi.org/10.4158/EP171764.APPGL.

Kasichayanula, Sreeneeranj, Anita Grover, Maurice G. Emery, Megan A. Gibbs, Ransi Somaratne, Scott M. Wasserman, and John P. Gibbs. "Clinical Pharmacokinetics and Pharmacodynamics of Evolocumab, a PCSK9 Inhibitor." *Clinical Pharmacokinetics* 57, no. 7 (July 2018): 769–79. https://doi.org/10.1007/s40262-017-0620-7.

Katzmann, Julius L., Ioanna Gouni-Berthold, and Ulrich Laufs. "PCSK9 Inhibition: Insights From Clinical Trials and Future Prospects." *Frontiers in Physiology* 11 (2020). https://www.frontiersin.org/articles/10.3389/fphys.2020.595819.

Katzmann, Julius L., Chris J. Packard, M. John Chapman, Isabell Katzmann, and Ulrich Laufs. "Targeting RNA With Antisense Oligonucleotides and Small Interfering RNA in Dyslipidemias: JACC State-of-the-Art Review." *Journal of the American College of Cardiology* 76, no. 5 (August 4, 2020): 563–79. https://doi.org/10.1016/j.jacc.2020.05.070.

Koren, Michael J., Marc S. Sabatine, Robert P. Giugliano, Gisle Langslet, Stephen D. Wiviott, Andrea Ruzza, Yuhui Ma, Andrew W. Hamer, Scott M. Wasserman, and Frederick J. Raal. "Long-Term Efficacy and Safety of Evolocumab in Patients With Hypercholesterolemia." *Journal of the American College of Cardiology* 74, no. 17 (October 29, 2019): 2132–46. https://doi.org/10.1016/j.jacc.2019.08.1024.

"LDLR Gene: MedlinePlus Genetics." Accessed January 28, 2023. https://medlineplus.gov/genetics/gene/ldlr/.

"LDLR Gene: MedlinePlus Genetics." Accessed January 25, 2023. https://medlineplus.gov/genetics/gene/ldlr/.

Liao, Sharon. "PCSK9 Inhibitors for High Cholesterol: Benefits, Risks, Side Effects." WebMD. Accessed January 24, 2023. https://www.webmd.com/cholesterol-management/pcsk9-inhibitors-treatment.

Mahajan, Rajiv. "Novel Lipid-Lowering Agents Proprotein Convertase Subtilisin-Kexin Type 9 Inhibitors: Do They Show Mortality Benefits?" *International Journal of Applied and Basic Medical Research* 8, no. 3 (2018): 135–36. https://doi.org/10.4103/ijabmr.IJABMR_217_18.

Michael M Page, and Gerald F. Watts. "PCSK9 Inhibitors – Mechanisms of Action." *Australian Prescriber* 39, no. 5 (October 2016): 164. https://doi.org/10.18773/austprescr.2016.060.

Navarese, Eliano P., Michalina Kolodziejczak, Max-Paul Winter, Arman Alimohammadi, Irene M. Lang, Antonino Buffon, Gregory Yh Lip, and Jolanta M. Siller-Matula. "Association of PCSK9 with Platelet Reactivity in Patients with Acute Coronary Syndrome Treated with Prasugrel or Ticagrelor: The PCSK9-REACT Study." *International Journal of Cardiology* 227 (January 15, 2017): 644–49. https://doi.org/10.1016/j.ijcard.2016.10.084.

Nicholls, Stephen J., Rishi Puri, Todd Anderson, Christie M. Ballantyne, Leslie Cho, John J. P. Kastelein, Wolfgang Koenig, et al. "Effect of Evolocumab on Progression of Coronary Disease in Statin-Treated Patients: The GLAGOV Randomized Clinical Trial." *JAMA* 316, no. 22 (December 13, 2016): 2373–84. https://doi.org/10.1001/jama.2016.16951.

Paciullo, Francesco, Stefania Momi, and Paolo Gresele. "PCSK9 in Haemostasis and Thrombosis: Possible Pleiotropic Effects of PCSK9 Inhibitors in Cardiovascular Prevention." *Thrombosis and Haemostasis* 119, no. 3 (March 2019): 359–67. https://doi.org/10.1055/s-0038-1676863.

Peng, Yanren, Guojun Chen, and Hua Zheng. "[The triglyceride-lowering effects of PCSK9 inhibitor differ in patients with different baseline triglyceride levels]." *Nan Fang Yi Ke Da Xue Xue Bao = Journal of Southern Medical University* 40, no. 8 (August 30, 2020): 1141–47. https://doi.org/10.12122/j.issn.1673-4254.2020.08.11.

Pirillo, Angela, and Alberico Luigi Catapano. "Inclisiran: How Widely and When Should We Use It?" *Current Atherosclerosis Reports* 24, no. 10 (2022): 803–11. https://doi.org/10.1007/s11883-022-01056-0.

Pokhrel, Binod, Wei C. Yuet, and Steven N. Levine. "PCSK9 Inhibitors." In *StatPearls*. Treasure Island (FL): StatPearls Publishing, 2022. http://www.ncbi.nlm.nih.gov/books/NBK448100/.

Qamar, Arman, Robert P. Giugliano, Anthony C. Keech, Julia F. Kuder, Sabina A. Murphy, Christopher E. Kurtz, Scott M. Wasserman, Peter S. Sever, Terje R. Pedersen, and Marc S. Sabatine. "Interindividual Variation in Low-Density Lipoprotein Cholesterol Level Reduction

With Evolocumab: An Analysis of FOURIER Trial Data." *JAMA Cardiology* 4, no. 1 (January 1, 2019): 59–63. https://doi.org/10.1001/jamacardio.2018.4178.

Quintão, E. C. "[Statins in hypercholesterolemia treatment]." *Revista Da Associacao Medica Brasileira (1992)* 40, no. 1 (1994): 50–58.

Räber, Lorenz, Yasushi Ueki, Tatsuhiko Otsuka, Sylvain Losdat, Jonas D. Häner, Jacob Lonborg, Gregor Fahrni, et al. "Effect of Alirocumab Added to High-Intensity Statin Therapy on Coronary Atherosclerosis in Patients With Acute Myocardial Infarction: The PACMAN-AMI Randomized Clinical Trial." *JAMA* 327, no. 18 (May 10, 2022): 1771–81. https://doi.org/10.1001/jama.2022.5218.

Ray, Kausik K., Stefano Del Prato, Dirk Müller-Wieland, Bertrand Cariou, Helen M. Colhoun, Francisco J. Tinahones, Catherine Domenger, et al. "Alirocumab Therapy in Individuals with Type 2 Diabetes Mellitus and Atherosclerotic Cardiovascular Disease: Analysis of the ODYSSEY DM-DYSLIPIDEMIA and DM-INSULIN Studies." *Cardiovascular Diabetology* 18, no. 1 (November 9, 2019): 149. https://doi.org/10.1186/s12933-019-0951-9.

Romagnuolo, Rocco, Corey A. Scipione, Michael B. Boffa, Santica M. Marcovina, Nabil G. Seidah, and Marlys L. Koschinsky. "Lipoprotein(a) Catabolism Is Regulated by Proprotein Convertase Subtilisin/Kexin Type 9 through the Low Density Lipoprotein Receptor." *The Journal of Biological Chemistry* 290, no. 18 (May 1, 2015): 11649–62. https://doi.org/10.1074/jbc.M114.611988.

Rosenson, Robert S., Robert A. Hegele, Sergio Fazio, and Christopher P. Cannon. "The Evolving Future of PCSK9 Inhibitors." *Journal of the American College of Cardiology* 72, no. 3 (July 17, 2018): 314–29. https://doi.org/10.1016/j.jacc.2018.04.054.

Sabatine, Marc S. "PCSK9 Inhibitors: Clinical Evidence and Implementation." *Nature Reviews. Cardiology* 16, no. 3 (March 2019): 155–65. https://doi.org/10.1038/s41569-018-0107-8.

Sabatine, Marc S., Robert P. Giugliano, Stephen D. Wiviott, Frederick J. Raal, Dirk J. Blom, Jennifer Robinson, Christie M. Ballantyne, et al. "Efficacy and Safety of Evolocumab in Reducing Lipids and Cardiovascular Events." *New England Journal of Medicine* 372, no. 16 (April 16, 2015): 1500–1509. https://doi.org/10.1056/NEJMoa1500858.

"SAGE Journals: Your Gateway to World-Class Journal Research." Accessed January 19, 2023. https://journals.sagepub.com/.

Schuster, Susanne, Sandra Rubil, Matthias Endres, Hans M. G. Princen, Jes-Niels Boeckel, Karsten Winter, Christian Werner, and Ulrich Laufs. "Anti-PCSK9 Antibodies Inhibit pro-Atherogenic Mechanisms in APOE*3Leiden.CETP Mice." *Scientific Reports* 9, no. 1 (July 31, 2019): 11079. https://doi.org/10.1038/s41598-019-47242-0.

Schwartz, Gregory G., P. Gabriel Steg, Michael Szarek, Deepak L. Bhatt, Vera A. Bittner, Rafael Diaz, Jay M. Edelberg, et al. "Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome." *New England Journal of Medicine* 379, no. 22 (November 29, 2018): 2097–2107. https://doi.org/10.1056/NEJMoa1801174.

Seidah, Nabil G., Suzanne Benjannet, Louise Wickham, Jadwiga Marcinkiewicz, Stéphanie Bélanger Jasmin, Stefano Stifani, Ajoy Basak, Annik Prat, and Michel Chrétien. "The Secretory Proprotein Convertase Neural Apoptosis-Regulated Convertase 1 (NARC-1): Liver Regeneration and Neuronal Differentiation." *Proceedings of the National Academy of Sciences* 100, no. 3 (February 4, 2003): 928–33. https://doi.org/10.1073/pnas.0335507100.

Stoekenbroek, Robert M., Gilles Lambert, Bertrand Cariou, and G. Kees Hovingh. "Inhibiting PCSK9 — Biology beyond LDL Control." *Nature Reviews Endocrinology* 15, no. 1 (January 2019): 52–62. https://doi.org/10.1038/s41574-018-0110-5.

Tsao, Connie W., Aaron W. Aday, Zaid I. Almarzooq, Alvaro Alonso, Andrea Z. Beaton, Marcio S. Bittencourt, Amelia K. Boehme, et al. "Heart Disease and Stroke Statistics—2022 Update: A Report From the American Heart Association." *Circulation* 145, no. 8 (February 22, 2022). https://doi.org/10.1161/CIR.0000000000001052.

"Variation in PCSK9 and HMGCR and Risk of Cardiovascular Disease and Diabetes | NEJM." Accessed February 10, 2023. https://www.nejm.org/doi/10.1056/NEJMoa1604304.

Wadhera, Rishi K., Dylan L. Steen, Irfan Khan, Robert P. Giugliano, and JoAnne M. Foody. "A Review of Low-Density Lipoprotein Cholesterol, Treatment Strategies, and Its Impact on Cardiovascular Disease Morbidity and Mortality." *Journal of Clinical Lipidology* 10, no. 3 (May 1, 2016): 472–89. https://doi.org/10.1016/j.jacl.2015.11.010.

Warden, Bruce A., Sergio Fazio, and Michael D. Shapiro. "The PCSK9 Revolution: Current Status, Controversies, and Future Directions." *Trends in Cardiovascular Medicine* 30, no. 3 (April 1, 2020): 179–85. https://doi.org/10.1016/j.tcm.2019.05.007.

White, H., P. Steg, M. Szarek, D. Bhatt, V. Bittner, R. Diaz, J. Edelberg, et al. "Cardiovascular Outcomes With Alirocumab After Acute Coronary Syndrome: Results of the Odyssey Outcomes Trial." *Heart, Lung and Circulation* 27 (January 1, 2018): S305–6. https://doi.org/10.1016/j.hlc.2018.06.585.