Diploma thesis

Metabolic effects of lipid-lowering therapies in a tertiary care center – a retrospective study

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Glossary and abbreviations

ABCA1	adenosine triphosphate binding cassette transporter A1
ALT	alanine transaminase
Apo	apolipoprotein
ASCVD	atherosclerotic cardiovascular disease
ASOs	antisense oligonucleotides
AST	aspartate transaminase
ATP	adenosine triphosphate
BAS	bile acid sequestrant
BL	baseline
CHD	coronary heart disease
CK	creatine kinase
CKD	chronic kidney disease
CV	cardiovascular
DM	diabetes mellitus
DNA	deoxyribonucleic acid
EAS	European Atherosclerosis Society
EBBINGHAUS	Evaluating PCSK9 Binding Antibody Influence on Cognitive Health in High Cardiovascular Risk Subjects
EGF-A	epidermal growth factor repeat A
ESC	European Society of Cardiology
FCH	familial combined hyperlipidemia
FCS	familial chylomicron syndrome
FH	familial hypercholesterolemia
GLP1	glucagon- like peptide 1
HbA1c	hemoglobin A1c
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
HeFH	heterozygous familial hypercholesterolemia
HMG-CoA	hydroxymethylglutaryl coenzyme A
HMGCR	hydroxymethylglutaryl coenzyme A reductase
HoFH	homozygous familial hypercholesterolemia
IDL	intermediate-density lipoprotein

IQR	interquartile range
KDIGO	Kidney Disease: Improving Global Outcomes
LCAT	lecithin–cholesterol acyltransferase
LCFA	long-chain fatty acid
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein receptor
LDLR	low-density lipoprotein receptor
LDLRAP1	low-density lipoprotein receptor adaptor protein 1
LP	lipoprotein
Lp (a)	lipoprotein (a)
LPL	lipoprotein-lipase
LTF	lost to follow up
mAbs	monoclonal antibodies
mRNA	messenger ribonucleic acid
NPC1L1	Niemann-Pick C1-like 1
PCSK9	proprotein convertase subtilisin/kexin type 9
PPAR	peroxisome proliferator-activated receptor
PPARα	proliferator-activated receptor α
PREDIMED	Prevención con Dieta Mediterránea
RCTs	randomized controlled trials
RNA	ribonucleic acid
SAMS	statin-associated muscle symptom
SGLT2	sodium-glucose co-transporter 2
siRNA	small interfering ribonucleic acid
T1D	diabetes mellitus type 1
T2D	diabetes mellitus type 2
TG	triglycerides
TIA	transient ischemic attack
VLDL	very-low-density lipoprotein

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Zusammenfassung

Hintergrund

In mehreren Studien konnte der lipidsenkende Effekt sowie die positiven kardiovaskulären Auswirkungen von Proproteinkonvertase Subtilisin/Kexin Typ 9 (PCSK9) -Hemmern gezeigt werden. Aus diesem Grund sind diese Präparate in zunehmender Anwendung in der lipidsenkenden Therapie von Patientinnen und Patienten mit einem hohen kardiovaskulären Risiko. Daten aus dem klinischen Alltag sind jedoch weiterhin rar, besonders bei Patientinnen und Patienten mit Diabetes mellitus Typ 2. Ziel dieser retrospektiven Analyse war die Wirksamkeit von PCSK9-Hemmern in der Senkung von Low-Density Lipoprotein Cholesterin (LDL-C) unter Routinebedingung in einer Lipidstoffwechsel-Spezialambulanz zu untersuchen.

Methodik

Eine retrospektive Analyse von Daten aus der elektronischen Patientenakte wurde durchgeführt. Patientinnen und Patienten mit verordneter Therapie mit einem PCSK9-Hemmer (Alirocumab oder Evolocumab) zwischen 2016 und 2019 wurden in die Studie einbezogen. Die Patientencharakteristika der Studienpopulation, der Effekt auf LDL-C und HbA1c sowie das Auftreten von kardiovaskulären Ereignissen über einen Zeitraum von bis zu 18 Monaten wurden analysiert.

Ergebnisse

237 Patientinnen und Patienten mit einer Erstverordnung eines PCSK9-Hemmers zwischen Jänner 2016 und September 2019 wurden ermittelt. Nahezu alle Patientinnen und Patienten (97,5%) erhielten PCSK9-Hemmer zur Sekundärprävention. 26,2% der Gesamtpopulation hatten Diabetes mellitus als Begleiterkrankung. Häufig wurde von einer Unverträglichkeit von Statinen (83,1%), von Ezetimib (44,7%) oder beider Arzneistoffe (42,6%) berichtet. Drei Monate nach Einleitung einer PCSK9-Hemmer Therapie erreichten 61,2% der Patientinnen und Patienten LDL-C Werte <70 mg/dl und 44,1% Werte unter <55 mg/dl. Der mediane LDL-C Spiegel konnte von 141 mg/dl zur Baseline, auf 60 mg/dl nach 3 Monaten und auf 66 mg/dl nach 12 Monaten gesenkt werden, bedeutend einer LDL-C Senkung von 57,5% nach 3 Monaten und von 53,6% nach 12 Monaten.

Nach 3 Monaten zeigte sich in Patientinnen und Patienten mit Diabetes mellitus Typ 2 ein höherer Anteil mit LDL-C Werte im Zielbereich im Vergleich zu Nicht-Diabetikern (51% vs. 41,5% mit Werten <55 mg/dl, 69,4% vs. 58,5% <70 mg/dl). Dieser höhere Prozentsatz bei Diabetes mellitus Typ 2 zeigte sich nach 12 Monaten unter Therapie noch deutlicher (58,8% vs. 30,1% mit Werten <55 mg/dl; 70,6% vs. 49,6% <70 mg/dl). Patientinnen und Patienten mit Diabetes mellitus Typ 2 und unzureichend

kontrolliertem Blutzuckerwerten (HbA1c >54 mmol/mol) zeigten eine höhere LDL-C Reduktion, hatten jedoch ein höheres Risiko eines kardiovaskulären Ereignisses.

Schlussfolgerung

Eine signifikante Reduktion von LDL-C und ein hoher Prozentsatz von Patientinnen und Patienten mit LDL-C Werten im empfohlenen Zielbereich konnte beobachtet werden. In Patientinnen und Patienten mit Diabetes mellitus Typ 2 erreichte ein höherer Anteil die LDL-C Ziele als in jenen ohne Diabetes mellitus. Trotz verordnetem PCSK9-Hemmer konnten in einige Patientinnen und Patienten nicht die Therapieziele der aktuellen Leitlinien erreichen. Ein besonderes Augenmerk sollte auf diese Personen gelegt werden, um die Therapieziele zu erreichen und kardiovaskuläre Ereignisse zu verhindern.

Abstract

Background

The lipid-lowering and positive cardiovascular effect of proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors was shown in several studies, hence, they are more widely used in the lipid-lowering management of individuals with high cardiovascular risk. As real-world data are still scarce, specifically in people with type 2 diabetes (T2D), the aim of this retrospective analysis was to investigate the efficacy of PCSK9 inhibitors in lowering low-density lipoprotein cholesterol (LDL-C) in an outpatient clinic of a tertiary care center in routine care.

Methods

A retrospective analysis of data extracted from the electronic patient record was performed. Patients who were routinely prescribed with PCSK9 inhibitor therapy (alirocumab or evolocumab) during the years 2016 and 2019 were included in the analysis. Characteristics of the patient population, the effects on LDL-C and HbA1c levels as well as subsequent cardiovascular events were assessed over an observation period of 18 months.

Results

We identified 237 patients treated with PCSK9 inhibitors between January 2016 and September 2019. Almost all patients (97.5%) received PCSK9 inhibitors for secondary prevention. 26.2% of the population had a concomitant diabetes diagnosis. Intolerance to statins (83.1%), ezetimibe (44.7%) or both agents (42.6%) was reported frequently. Three months after initiation of PCSK9 inhibitor therapy, 61.2% of the patients achieved LDL-C levels <70 mg/dl, and 44.1% LDL-C levels <55 mg/dl. The median LDL-C was lowered from 141 mg/dl at baseline, to 60 mg/dl after 3 months and 66 mg/dl after 12 months indicating a reduction of LDL-C as follows: 57.5% after 3 months and 53.6% after 12 months. After 3 months of observation, target achievement of LDL- C was higher in patients with T2D compared to non-diabetes patients; <55 mg/dl: 51% vs. 41.5%; <70 mg/dl 69.4 vs. 58.5%. After 12 months even more pronounced target LDL achievement in T2D was demonstrated <55 mg/dl: 58.8% vs. 30.1%; <70 mg/dl 70.6% vs. 49.6%. Patients with insufficiently controlled T2D (HbA1c >54 mmol/mol) had a higher reduction in LDL-C but still were more likely to subsequent cardiovascular events.

Conclusions

Significant reductions in LDL-C and a high percentage of patients achieving recommended treatment targets were observed. The percentage of patients with T2D meeting recommended LDL-C targets was

higher than in those without T2D. Still some patients did not achieve LDL-C levels as recommended in current guidelines. Special attention to the characteristics of these patients is required in the future to enable achievement of treatment goals and avoid adverse cardiovascular outcomes.

1 INTRODUCTION

1.1. The lipid metabolism

Lipids play vitally important roles for the human body. A total fat intake between 20% and 35% of the energy intake is recommended for adults. Two of the main functions are the source of energy for the cell and the energy storage in adipose tissue. On the other hand, cholesterol and fatty acids serve as component in the cell membrane and are precursors of signaling molecules. Moreover, the intake of essential nutrients, like fat-soluble vitamins and the two essential fatty acids linoleic acid and α-linolenic acid, is indispensable through the fact that these substances cannot be synthesized in the body. [1]

90% of the dietary fats are ingested as triglycerides, which are composed of glycerol and long-chain fatty acids (LCFAs). The other 10% are taken in as phospholipids, sphingolipids and esterified or unesterified cholesterol. A special mechanism is needed to fully absorb the poorly water-soluble lipids in the gastrointestinal tract and for the transportation in the blood plasma. In the digestive system fats are hydrolyzed (broken down) into a simpler form by enzymes (lipases). Lipases are secreted by the sublingual salivary glands, the gastric chief cells in the stomach and most importantly by the pancreas. To optimize their function, the surface-to-volume ratio is increased by mechanical emulsification through mastication and gastrointestinal motility. 10 – 30% of the dietary fats are hydrolyzed in the stomach, 70 – 90% in the duodenum and the upper jejunum. [2-4]
While the gastric mucosa can already absorb medium- and short-chain fatty acids, the major part of the decomposed products is absorbed in the duodenum and the upper jejunum. In the small intestine, micelles form in the presence of bile salts. These micelles contain monoglycerides, free fatty acids (FFAs), cholesterol and bile salts on their surface, while apolar lipids are encased inside the particle. The micelles allow close contact to the intestinal mucosa and increase the solubility for lipophilic compounds. This is crucial for the absorption of long-chain fatty acids. [3]

While medium- and short-chain fatty acids are able to passively diffuse in the enterocytes, LCFAs are resorbed by transport proteins. For the transportation in the blood, lipids have to be packed in special molecular complexes called lipoproteins (LP). The spherical-shaped LP contain hydrophobic fats (triglycerides, esterified cholesterol) and apolipoproteins (Apo) inside of it, while amphiphilic lipids (cholesterol, phospholipids) form the outer surface. In the enterocytes, the absorbed fats are processed into LP (chylomicrons) and secreted into the lymph. Through the lymphatic system the

chylomicrons get into the blood, where lipoprotein-lipases (LPL) hydrolyze their containing triglycerides into FFAs. The released FFAs are primarily resorbed by muscle- and fat tissue. After this first cycle, the liver endocytoses the remaining LP-complex (chylomicron remnant), using apolipoprotein E (Apo E) binding with low-density lipoprotein (LDL) receptors and LDL-related receptors. Absorbed in the hepatocytes, the remaining components of the chylomicron remnants are degraded. The imported or by the liver synthesized triglycerides and cholesterol get back into the circulatory system in the form of very-low-density lipoproteins (VLDL). Again, high-energy FFAs are transported to peripheral tissue. One part of the residual VLDL returns to the liver as intermediate-density lipoproteins (IDL), while the other part is processed into LDL. The LDL particles contain a higher relative content of cholesterol and transport it to hepatic and extrahepatic tissue. The endocytosis of LDL is mediated by the binding of Apo B-100 on the LDL particle with LDL- receptors on the cell surface. Cholesterol is not only a vital component for the structure of the cell wall, but is also chemical precursor for steroid hormones and bile acid. Redundant cholesterol in the periphery and in extrahepatic cells is incorporated in high-density lipoproteins (HDL) to transport it back to the liver, where it is excreted with the bile or repacked in lipoproteins. [3-5]

Lipoprotein	Size (nm)	Major Lipids	Major Apolipoproteins
Chylomicron	75 – 1200	Triglycerides	Apo B-48, Apo C, Apo E, Apo A-I, A-II, A-IV
Chylomicron remnant	30 – 80	Triglycerides Cholesterol	Apo B-48, Apo E
VLDL	30 – 80	Triglycerides	Apo B-100, Apo E, Apo C
IDL	25 – 35	Triglycerides Cholesterol	Apo B-100, Apo E, Apo C
LDL	18 – 25	Cholesterol	Apo B-100
HDL	5 – 12	Cholesterol Phospholipids	Apo A-I, Apo A-II, Apo C, Apo E
Lp (a)	~30	Cholesterol	Apo B-100, Apo (a)

Table 1. *Characteristics of the lipoprotein classes [6]. VLDL = very-low-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; HDL = high-density lipoprotein; Lp (a) = Lipoprotein (a).*

1.2. Dyslipidemia

Pathological elevated levels of lipids are caused by primary (familial) or secondary (acquired) disorder of the lipid metabolism. Often there is a combination of both, given that lipid levels are influenced by genetic and environmental factors. [7]

1.2.1. Familial dyslipidemias

Familial hyperlipidemias are traditionally categorized according to the Fredrickson classification. Five phenotypes are classified based on the lipoprotein pattern in the serum (Table 2). [8]

Type	Increased Lipids		Increased Lipoproteins			
	Triglycerides	Cholesterol	Chylomicrons	LDL	VLDL	IDL
I	↑		↑			
IIa		↑		↑		
IIb	↑	↑		↑	↑	
III	↑	↑				↑
IV		↑			↑	
V	↑	↑	↑		↑	

Table 2. *Fredrickson classification of hyperlipidemias [9]. VLDL = very-low-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein.*

Today, classification based on the patients' lipid profile or on the etiology is more common in the clinical practice. However, the categorization of these disorders is often nonuniform and is getting more complex through progressing genetic understanding of these diseases.

1.2.2. Familial monogenetic hypercholesterolemia

Gene mutations in the LDL receptor (LDLR), LDL receptor adaptor protein 1 (LDLRAP1), Apo B or proprotein convertase subtilisin/kexin 9 (PCSK9) have been identified as cause of familial hypercholesterolemia (FH). Over 95% of the causing mutations are in the LDLR gene, 2-5% in the Apo B, <1% in the LDLRAP1 and <1% in the PCSK9. [10]

These proteins are involved in the functioning clearance of LDL cholesterol (LDL-C) from the bloodstream. Hence, a dysfunction in this system (caused by the mutation) leads to consequently increased LDL-C levels in the plasma. The on the cell surface located LDLR binds Apo B-100 of the LDL

particle. Involving LDLRAP1, the complex is then endocytosed, LDL degraded, and the receptor recycles to the cell surface (illustrated in Figure 1). Due to a loss-of-function mutation in one of the genes of these proteins, the cellular uptake of LDL is reduced, and therefore the LDL plasma level increases. [10] The function of PCSK9, a member of subtilisin serine protease family, was originally discovered in studies in mice. Circulating PCSK9 binds to the LDLR and promotes its lysosomal degradation in the cell (Figure 1). While loss-of-function mutations in the PCSK9 gene lead to lowered plasma LDL-C concentration, a gain-of-function mutation induces genetically determined hypercholesterolemia. Due to an increased activity of PCSK9, more LDLR are degraded. Less LDL-C is absorbed as consequence of the reduced number of recycled receptors, leading to an attenuated LDL-C clearance. [11]

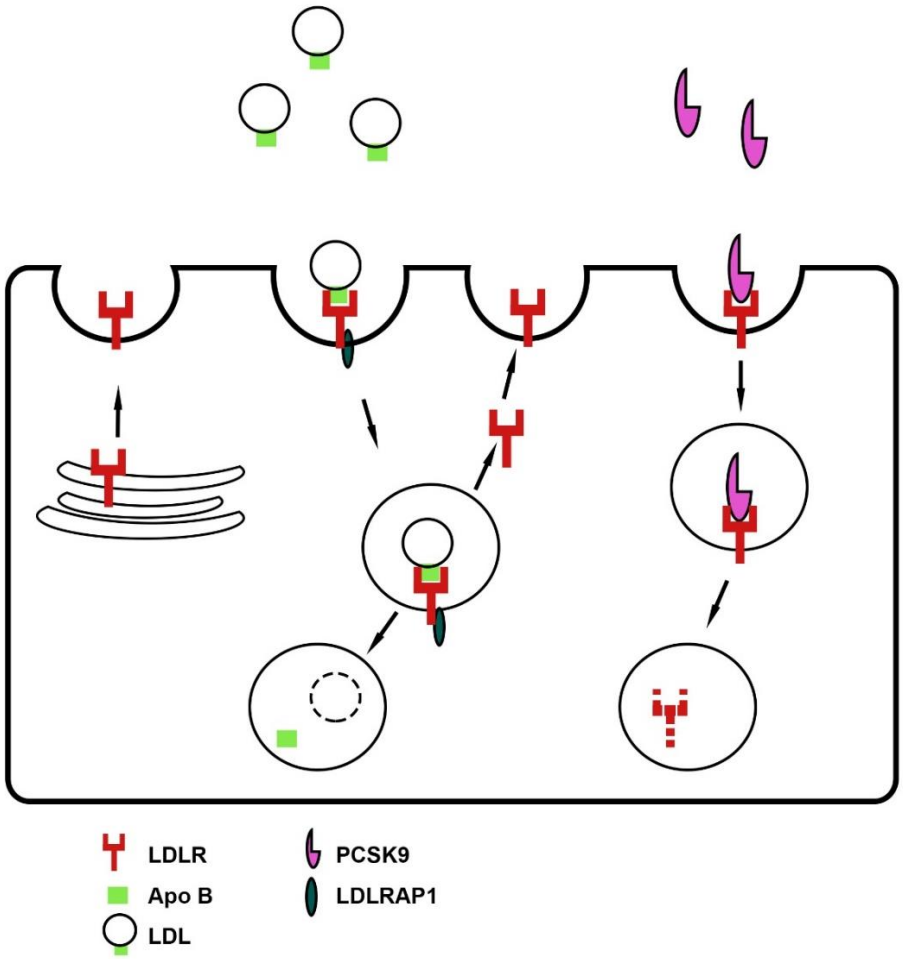


Figure 1. *Cellular uptake of low-density lipoprotein, low-density lipoprotein receptor circulation and function of Proprotein convertase subtilisin/kexin type 9.*

With an estimated prevalence of 1 in 250 individuals, heterozygous familial hypercholesterolemia (HeFH) is the more common form of monogenetic hypercholesterolemia. [12] The homozygous form is more rare (estimated prevalence of 1 in 160 000 – 300 000 people), but a more severe condition.

Patients with homozygous familial hypercholesterolemia (HoFH) can present with massively increased lipid levels. Untreated, these patients develop atherosclerotic cardiovascular disease (ASCVD) before the age of 20 years and often die in their thirties. [10]

1.2.3. **Familial combined hyperlipidemia**

It is estimated that between 0.5 and 2% of the general population is affected by familial combined hyperlipidemia (FCH), which is therefore the most common form of familial dyslipidemia. With an inter-individual variability, characteristics of this mixed form of dyslipidemia are elevated levels of VLDL, LDL, triglycerides, Apo B and decreased HDL levels. Due to the complicity of the various pathways in the lipid metabolism, numerous associated genetic aberrations can play a role in the development of FCH. Both genetic and environmental factors are involved in the pathogenesis of this complex disorder. Usually, the genetic cause of FCH is not linked to just one gene, but rather polygenetic. Mutations leading to reduced lipid-turnover in the adipose tissue, increased lipogenesis in the liver, disturbance in the lipid hydrolyzation or defects in the peripheral clearance of lipoproteins can be part in the development of FCH. [13]

1.2.4. **Familial monogenetic and polygenetic hypertriglyceridemia**

Similar to FCH, the genetic basis of the polygenetic form of familial hypertriglyceridemia is associated with variants in several genes. Carriers of the various mutations are more likely to show hypertriglyceridemia, but many have normal triglyceride levels. A wide range from normal to exceedingly high triglyceride plasma concentration is observed even within carrier families. In patients with secondary causes of hypertriglyceridemia (such as metabolic syndrome, hypothyroidism, renal disease, pregnancy, poor diet or alcohol abuse) a genetic component often plays an additional role in the development of the disorder. [14]

Patients with severe elevated levels of triglyceride (triglyceride concentration >885 mg/dl) are more likely to have a monogenetic form of hypertriglyceridemia, but the disease is rare (prevalence of approximately 1 in 1 000 000). This autosomal recessive disorder usually manifests in childhood or adolescence. In patients with such severe hypertriglyceridemia, prompt therapeutic interventions are needed to reduce the risk of acute pancreatitis. [14]

1.2.5. **Familial dysbetalipoproteinemia**

Familial dysbetalipoproteinemia is caused by mutations in the Apo E gene, which leads to impaired binding capacity for the apolipoprotein to the LDLR. Because of the now defective endocytosis of VLDL and IDL by hepatocytes, plasma concentration of triglycerides and cholesterol increases. However, for the manifestation of familial dysbetalipoproteinemia an additional disorder in the lipid metabolism is required and usually does not develop before adulthood. The disease is more common in men. Because of the protective effect of estrogens, women are usually not affected before menopause. Typical symptoms of dysbetalipoproteinemia are xanthomatous skin lesions on the extremities, but are not present in all patients. [15]

1.2.6. **Familial chylomicron syndrome**

Familial chylomicron syndrome (FCS), also known as familial lipoprotein lipase (LPL) deficiency, is a rare autosomal recessive disorder in the chylomicron pathway. Mutations in the LPL gene, or sometimes in genes coding for cofactors and other proteins interacting with LPL (e.g. Apo C-II), lead to an impaired activity of the enzyme. The normal function of LPL is the hydrolyzation of the in lipoproteins containing triglycerides into FFAs. Due to the impaired clearance of chylomicrons, as a result of the dysfunction of LPL, triglyceride levels can be massively elevated in patients with FCS. Similar to familial hypertriglyceridemia, people affected by the disorder are at risk for acute or recurrent pancreatitis and may show typical eruptive xanthomas. [16]

While patients with other forms of dyslipidemia are at higher risk for cardiovascular events, FCS is not associated with the development of atherosclerosis. At present, the drug therapy options are insufficient in the treatment of FCS. Patients depend on a lifelong very low-fat diet to prevent acute pancreatitis and long-term consequences. [16]

1.2.7. **Familial high-density lipoprotein deficiency**

Low levels of HDL are mostly caused by multifactorial origin (i.e. environmental and polygenetic influences) or in the course of other dyslipidemia phenotypes. While family studies showed strong association between HDL plasma levels and genetic determinism, pure monogenetic HDL disorders are rare. Mutations in genes of three proteins (ATP binding cassette transporter A1 (ABCA1), lecithin–cholesterol acyltransferase (LCAT) and Apo A-1) are known as cause for monogenetic HDL deficiency. [17]

1.3. Role of lipids and lipoproteins in atherosclerosis

1.3.1. Role of low-density lipoprotein in the development of atherosclerosis

There is still a lot unclear about the exact pathophysiology of atherosclerosis development. In the most prevalent hypothesis, the key pathogenic event of lipoproteins causing atheromatous plaques is the retention of Apo B-containing lipoproteins (especially LDL) in the arterial wall and their consequential modification through immuno-inflammatory response. [18]

Apo B-lipoproteins <70 nm in diameter (chylomicron remnants, VLDL, IDL, LDL, Lp (a); see Table 1) are able to cross the vascular endothelium. The large 75 – 1200 nm sized chylomicrons cannot pass the endothelial barrier, which explains why patients with LPL deficiency are usually not affected by ASCVD. The trans-endothelial flux of LP in and out of the arterial wall is found in intact intima, but is increased over atherosclerotic lesions. The higher permeability and hence elevation of Apo B-lipoprotein concentration in the arterial wall, is not the sole underlying cause of the LP-retention. In the extracellular matrix of the intima, Apo B lipoproteins bind with proteoglycans of the arterial wall. Genetic alterations of the binding sites influence their affinity and thereby the accumulation of lipid particles. These findings demonstrate, that the composition of the involved proteins seem to play a key role in the atherogenicity of LDL. [19]

Retained LDL in the arterial wall triggers complex cascades, leading to development of atherosclerotic lesions. Trapped LDL particles are oxidized, whereupon activated endothelial cells induce an immuno-inflammatory reaction. Released chemokines and adhesion molecules mediates chemotaxis of monocytes, which differentiate into macrophages. The migrated and activated macrophages boost the oxidation process and bind to LDL using their specific scavenger receptors. The modified LDL is then phagocytized by the immune cells, which transform into cholesterol- containing foam cells. These foam cells are not exclusively formed by macrophages. Smooth muscle cells of the tunica media are also able to absorb LDL, transforming into macrophage-like cells and later into foam cells. This phenomenon is especially found in advanced atheromatous lesions. On the one hand, the complex inflammatory process is sustained through release of pro-inflammatory and pro-thrombotic mediators by immune cells (i.e. macrophages, mast cells, T- and B-cells). On the other hand, some of the cholesterol-loaded macrophages induce the formation of cholesterol crystals, likewise causing an inflammasome reaction. [18]

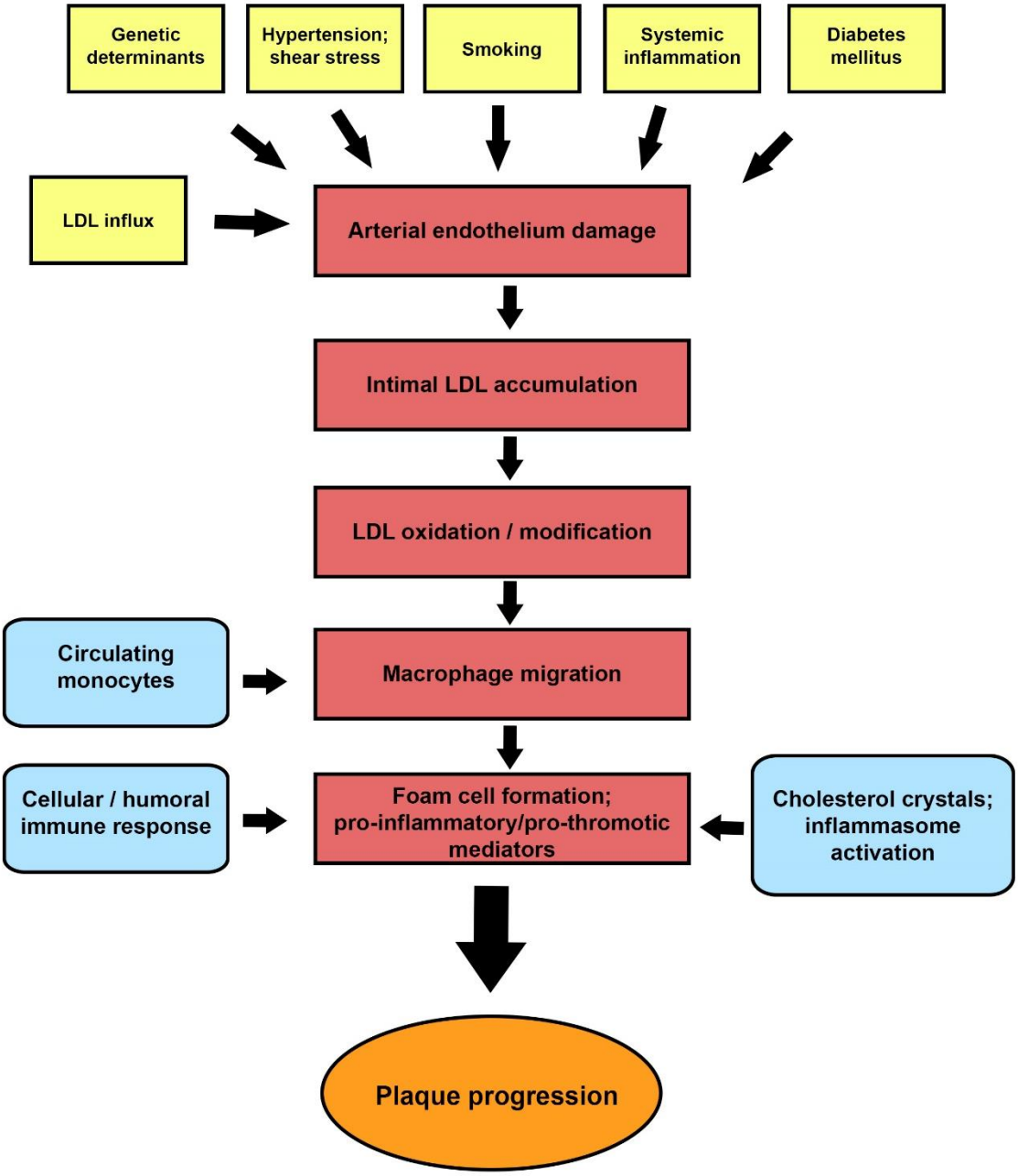


Figure 2. *Pathophysiological role of LDL in atherosclerosis. Adapted from* [20]

Early atheromatous lesions are already found in children and young adults. Classic cardiovascular risk factors, such as smoking, hypercholesterolemia, hypertension, diabetes mellitus and systemic inflammation, contribute to the progression of atherosclerotic plaques, or are the primary triggers for endothelial damages. Cumulative infiltration of lipids, immune cells, smooth muscle cells and connective tissue leads to the growth of early plaques, which gradually develop into a more vulnerable atheroma. Rupture of these plaques can result in acute stenosis due to thrombosis or intramural hemorrhage. Based on calcifying or fibrous remodeling of the lesion, the ruptured plaque can then lead to constriction of the artery lumen, leading to ischemia. [21]

1.3.2. **Causality between low-density lipoprotein and risk for atherosclerotic cardiovascular disease**

Increased plasma concentration of cholesterol-rich Apo B lipoproteins is a well-established risk factor in the development of ASCVD. LDL is the most extensively studied LP and under normal conditions, around 90% of the Apo B lipoproteins in the plasma are in form of LDL. Multiple studies showed unequivocal evidence that LDL causes ASCVD and that reducing LDL-C levels, lowers the risk for cardiovascular events. In clinical practice, concentration of circulating LDL particles is usually not measured directly, but rather calculated from the cholesterol contained in plasma LDL (LDL cholesterol, short LDL-C). Essentially, the risk of LDL causing ASCVD depends on the cumulative exposure to it, which is proportional to the LDL-C plasma concentration and the duration of exposure (age). [22]

Evidence from epidemiologic, genetic and intervention studies emphasize the causality of increased LDL-C concentration and the development of ASCVD. If untreated, people with genetic lipid disorders (e.g. HeFH or HoFH) have markedly elevated levels of LDL-C. In these patients, the risk for cardiovascular events is significantly higher and occur already in younger ages. Comparisons between carriers of such gene mutation and their unaffected sibling showed considerable differences in the lifetime risk of ASCVD, which indicates a causal connection between increased LDL-C and atherosclerotic diseases. These findings are supported by results from mendelian randomization studies. Carrier of gene variants associated with lower LDL-C levels are also at lower risk for coronary heart disease (CHD) development, while people who inherited alleles associated with elevated LDL-C show correspondingly a higher lifetime risk. These studies underline the causal relationship. [22]

These conclusions can be drawn from clinical intervention studies as well and may show the most compelling evidence. Randomized controlled trials (RCTs) of LDL-lowering therapies demonstrated the reduction of cardiovascular event rates. Several studies on well-investigated cholesterol-lowering drugs (e.g. statins, ezetimibe, bile acid sequestrant or PCSK9 inhibitors) were able to show the beneficial effect of reducing plasma LDL-C on the risk for major cardiovascular events. [22]

1.3.3. **Association between high-density lipoprotein and cardiovascular risk reduction**

While the association between low HDL plasma concentration and an increased risk for ASCVD was demonstrated in several studies, the detailed understanding of the role of HDL in the pathophysiology of atherosclerosis is still limited. [18]

HDL is a heterogenous group of lipoproteins, which differ in structure, composition and apparently biological activity. The well-known function of HDL is the transportation of redundant cholesterol from

peripheral tissue to the liver, but experimental research showed additional biological activities of HDL. Not only does HDL inhibit the formation of oxygen radicals and prevents the oxidation of LDL particles, but additionally it features direct protection of endothelial cells from apoptosis. Furthermore, the anti-inflammatory activity of HDL attenuates the complex inflammatory process in atherosclerotic lesions, including the inhibition of the adhesion and activation of monocytes. Moreover, HDL may also influence the vascular occlusion due to inhibition of platelet aggregation and clotting factors, or respectively by its vasodilatory effect. These further activities might play a relevant role in the anti-atherosclerotic effect of HDL. [23]

The experimental findings of the potential anti-atherosclerotic functions of HDL are in line with the epidemiological observation that low levels of plasma HDL (usually measured from their contained cholesterol (HDL-C)) are associated with higher cardiovascular risk. Data from clinical trials emphasize the causal evidence by showing that in patients under LDL-lowering therapy, low HDL-C concentration is an independent risk factor for ASCVD. [24]

This knowledge of low HDL-C as cardiovascular risk factor, originated the concept of therapeutically increasing HDL concentration in patients at risk. Although therapeutic approaches targeting HDL (e.g. cholesteryl ester transfer protein or infusion of HDL mimetics) were able to markedly increase HDL-C plasma levels, recent results could not demonstrate a beneficial effect of preventing cardiovascular events. [25]

1.4. Treatment of hypercholesterolemia

1.4.1. **Diet and lifestyle modifications**

To improve the overall lipid profile, a balanced diet is recommended which on one side accentuates a high intake of fruits, vegetables, whole-grain cereals, legumes, low-fat dairy products, nuts, fish and poultry, and a low consumption of red or processed meats, sweets, and sugar-sweetened beverages on the other side. The total fat consumption should mainly be in the form of unsaturated fatty acids, whereas the intake of trans fat and saturated fat should be limited. To maintain a lasting effect, the diet should be in line with the personal and cultural preferences of the patient. [26] The traditional Mediterranean diet emphasizes these nutrition recommendations and has been associated with a significantly lower risk of ASCVD. [27] The in Spain conducted multicenter trial Prevención con Dieta Mediterránea (PREDIMED) compared two forms of Mediterranean diet (one supplemented with extra-virgin olive oil, and one supplemented with nuts) with a reduced-fat control diet. 7447 patients in primary prevention of cardiovascular disease were randomly assigned to one of the three dietary groups and were invited to frequent dietary-training sessions to assess their adherence. Compared with the control group, participants assigned to a Mediterranean diet were less likely to have a major cardiovascular event (hazard ratio of 0.69 for a Mediterranean diet with olive oil, and of 0.72 with nuts), which shows the benefit of this dietary pattern in the prevention of ASCVD. [28]

Numerous studies over the past several decades clearly showed the inverse association between physical activity and cardiovascular mortality. [29] While sole weight loss has a beneficial effect on parameters of cardiovascular risk factors, no study on lifestyle intervention was able to demonstrate a clear reduction of cardiovascular events so far. [30] Aerobic exercise and weight reduction seem to have only a marginal influence on the lipid profile [26, 31], but their positive effect is likely to be attributed to the influence on other risk factors like hypertension and diabetes mellitus. [32-34]

1.4.2. **Statins**

Along with healthy lifestyle interventions, statins are the cornerstone in the lipid-lowering therapy and have been established for decades.

1.4.2.1. Mechanism of action

The pharmacological mechanism of these drugs is based on the inhibition of hydroxymethylglutaryl coenzyme A reductase (HMGCR). HMGCR catalyzes the conversion of hydroxymethylglutaryl coenzyme A (HMG-CoA) to mevalonic acid. Statins competitively bind to the catalytic domain of HMGCR and inhibit this rate-limiting step in the biosynthesis of cholesterol. As a result of the reduced synthesis, the intracellular concentration of cholesterol declines, inducing an increased expression of LDLR on the surface of hepatocytes. Because of the upregulation of LDLR, the clearance of VLDL and LDL from the blood enhances, consequently leading to a reduction of triglycerides and LDL-C in the plasma. [35]

At present, the most prominent statins on the market are rosuvastatin, atorvastatin, simvastatin, lovastatin, pravastatin and fluvastatin. The LDL-C lowering potency of statins varies between the different agents and their dosage. Regarding the potential LDL-C reduction, statin therapy schemes can be divided into intensity categories (low-, moderate-, high-intensity), illustrated in Table 3. [36]

Low intensity	**Moderate intensity**	**High intensity**
LDL-C reduction <30%	LDL-C reduction 30 – 49%	LDL-C reduction ≥50%
Simvastatin 10 mg Pravastatin 10 – 20 mg Lovastatin 20 mg Fluvastatin 20 – 40 mg	Atorvastatin 10 mg (20 mg) Rosuvastatin (5 mg) 10 mg Simvastatin 20 – 40 mg Pravastatin 40 mg (80 mg) Lovastatin 40 mg (80 mg) Fluvastatin XL 80 mg Fluvastatin 40 mg twice/day Pitavastatin 1 – 4 mg	Atorvastatin (40 mg) 80 mg Rosuvastatin 20 mg (40 mg)

Table 3. *Statin agents, dosage and average LDL-C reduction* [36]

1.4.2.2. Effects of statin therapy on cardiovascular morbidity and mortality

Numerous studies were able to show the positive effect of statin therapy on the reduction of cardiovascular event rates. Data from a large-scale meta-analysis, conducted by the Cholesterol Treatment Trialists Collaborators, confirmed the benefit of statin treatment [37], representing just one example for scientific evidence of the effectiveness of statins. This study analyzed results of 14 RCTs, including 90.056 patients in either statin intervention or control group. In statin users, a proportional

reduction of 12% in all-cause mortality and 21% in the incidence of major vascular events (defined as myocardial infarction, death from CHD, stroke or coronary revascularization) per 1 mmol/l LDL-C reduction (≙ 39 mg/dl) was found during a 5 years follow-up. [37]

Another meta-analysis, focusing specifically on the benefit of statin therapy in patients at low cardiovascular risk (5-years risk of major vascular event <10%), confirmed evidence for the value of statins in primary prevention. In patients at minor risk, statin users showed significant lower risk for said events (reduced incidence of 11 per 1.000 per mmol/l LDL-C-reduction). Even people without a history of ASCVD profited from statin therapy regarding cardiovascular morbidity and mortality. [38]

1.4.2.3. Adverse effects of statins

In general, statin therapy is well-tolerated in most patients, but side effects are one of the main reasons for poor adherence or treatment discontinuation.

Effects on muscle are the most common adverse reaction of statin therapy. Between 7 - 29% of statin users report statin-associated muscle symptoms (SAMS). However, the clinical presentation of these symptoms is heterogeneous and might also be caused by other origin in some cases. In patients suffering from muscle symptoms, but without markedly elevated levels of creatine kinase (CK), evidence of statins as the causal trigger remains unclear. The fact that in blinded RCTs the adverse event rates of SAMS are similar between placebo and intervention groups, may underline the uncertain causality in such cases. A temporal association between therapy adjustments (treatment initiation/increase in dosage) and occurrence of muscle symptoms or CK elevation are signs, that indicate statins as cause for muscle symptoms. [39]

While the incidence of SAMS with markedly increased CK (levels of >10 times upper normal) is estimated to be between 1 : 1.000 and 1 : 10.000 per year, life-threatening rhabdomyolysis as adverse effect of statins occurs in approximately one of 100.000 drug user per year. Patients suffering of rhabdomyolysis typically show massive high levels of CK and are at risk for renal failure. [39]

Clinical trials and genetic studies on the effect on glucose homeostasis showed a slightly increased risk for new-onset diabetes in statin users (approximately 1 : 1.000 per year of exposure). Patients with additional risk factors of development of diabetes mellitus (e.g. age, prediabetes, metabolic syndrome) are more likely to be affected by this adverse effect. However, the prevention of cardiovascular events by statin therapy generally outweighs the small increase of risk for new-onset diabetes. In clinical practice, indicated statin treatment should not be withheld for the reason of increased risk of diabetes. [40]

1.4.3. **Ezetimibe**

The addition of ezetimibe to statin therapy is recommended in patients who do not reach their LDL-C goal despite taking the maximum tolerated statin dose or in cases of statin intolerance. The combination of statins plus ezetimibe is therefore second-line therapy in hypercholesterolemia. [25]

1.4.3.1. Mechanism of action

The lipid-lowering effect of ezetimibe is based on a reduction of cholesterol absorption in the small intestine. Ezetimibe blocks Niemann-Pick C1-like 1 (NPC1L1), an important transport protein involved in the intracellular cholesterol uptake in the enterocytes. As a result of the reduced resorption of dietary cholesterol, less cholesterol is transported to the liver. Hepatocytes respond with an increased expression of LDLR, leading to enhanced LDL-clearance from the plasma. [41]

1.4.3.2. Effects of ezetimibe on lipids and prevention of cardiovascular events

Ezetimibe is primarily used as an add-on drug to statin therapy. Although studies were able to show the lipid-lowering effect of ezetimibe monotherapy, regarding the prevention of cardiovascular events, evidence of the efficacy of ezetimibe is unclear. In one meta-analysis, ezetimibe as sole drug treatment reduced LDL-C by 18.58% and triglycerides by 8.06% in patients with primary hypercholesterolemia. This study findings suggest the potential benefit of ezetimibe monotherapy in patients who do not tolerate statins, but at present, there is not enough evidence supporting the efficacy of ezetimibe for preventing ASCVD. [42, 43]

The add-on of ezetimibe to ongoing statin therapy has additional lipid-lowering effects. A pooled analysis of data from 27 clinical trials showed that adding ezetimibe additionally reduced LDL-C by 23%, triglycerides by 12% and increased HDL-C by 2%, allowing more patients reaching recommended lipid treatment goals. [44] Furthermore, adding ezetimibe to statin therapy moderately lowers the risk for non-fatal myocardial infarction or non-fatal stroke, according to current scientific findings. However, recent studies could not demonstrate the benefit on all-cause or cardiovascular mortality of the addition of ezetimibe to statins. [42]

1.4.3.3. Adverse effects of ezetimibe

Ezetimibe therapy is usually well-tolerated, but data on long-term safety is still scarce. Most common side effects associated with ezetimibe include gastrointestinal or musculoskeletal symptoms, but severe adverse events are rare. At present, possible influence of ezetimibe on statin-related side effects (e.g. SAMS or elevation of hepatic enzymes) is unclear. [42, 43]

One RCT of lipid-lowering therapy with combination of simvastatin and ezetimibe found significantly increased rates of cancer compared to placebo group (105 patients vs 70 patients) [45], leading to concerns about the safety of ezetimibe. However, this findings on cancer as possible adverse effect of ezetimibe was not confirmed by other studies and further investigation is needed. [42]

1.4.4. **Bile acid sequestrants**

Like ezetimibe, bile acid sequestrants (BAS) are most commonly used as an add-on or an alternative pharmacological option to first-line therapy. However, due to their unfavorable tolerability and several drug interactions, BAS is an less attractive LDL-C lowering solution than ezetimibe. [46]

1.4.4.1. Mechanism of action

The in the liver produced bile acids are end products in the cholesterol metabolism. On the one hand, the from cholesterol synthesized bile acids are crucial for the fat digestion, on the other hand, its intestinal secretion is the main elimination pathway of excess cholesterol in the body. About 95% of the secreted bile acids are reabsorbed and transported back to the liver, the remaining 5% are excreted with the feces. BAS increase the fraction of excreted bile acids by forming nonabsorbable complexes, consequently leading to reduced re-transportation of cholesterol molecules. In addition, the function of bile acids to solubilize dietary fats is impaired by the formation of the complex. Due to this interference in the enterohepatic circulation, the body reacts with an enhanced bile acid synthesis. To cover the demand of cholesterol, LDLR on hepatocytes are upregulated, resulting in an increased LDL-C clearance from the plasma. [47]

1.4.4.2. Effects of bile acid sequestrants on lipids and prevention of cardiovascular event

Overall, treatment with BAS agents (e.g. cholestyramine, colestipol or colesevelam) can reduce plasma LDL-C levels by about 15-30%, but do not show a marked effect on HDL-C (increase of approximately 3-5%) and can even cause elevation of triglyceride levels by up to 10%. [48]

While study findings suggest a potential efficacy of BAS in the prevention of cardiovascular events, at present, there is a lack of recent large-scale trials to confirm such evidence. [49]

1.4.4.3. Adverse effects of bile acid sequestrants

Therapy with BAS is frequently accompanied by gastrointestinal side effects (including constipation, nausea, abdominal pain, flatulence, bloating and fullness), which are often a reason for therapy discontinuation. [50] In addition, patients taking BAS are at risk for triglyceride elevation. Due to their unfavorable tolerability and common potential drug interactions, BAS do not have significance as first-line agents. However, in patients not tolerating standard treatment and who are not eligible for PCSK9 inhibitor therapy, BAS may be a therapy option. Besides, since BAS are not systemically absorbed, they may be considered as lipid-lowering therapy during pregnancy and breastfeeding. [25]

1.4.5. **Fibrates**

Fibrates are the most potent drugs to reduce high plasma triglyceride levels and additionally increase HDL-C levels. [51] Due to the triglyceride-lowering effect, they are first-line drugs to prevent pancreatitis in patients with severe hypertriglyceridemia. [14]

1.4.5.1. Mechanism of action

Fibrates are synthetic ligands for peroxisome proliferator-activated receptor α (PPARα). This nuclear receptor is one of three known subtypes (PPAR α, δ, γ), which act as transcription factors for the expression of various genes, including genes of proteins involved in the lipid metabolism. PPARα is mainly expressed in tissues with pronounced fat metabolism (e.g. liver, muscle, heart, kidney) and is physiologically activated by fatty acids, hormones or vitamins. Fibrates structurally resemble short-chain fatty acids and activate the signaling cascade on a pharmacological way. After activation, PPARα binds with another nuclear receptor (retinoid X receptor). This receptor complex can bind to specific regions of the DNA, which initiates gene transcription. [51]

That PPARα mediated activation leads to a couple of modifications in the lipid metabolism. Induction of lipoprotein lipolysis is one of the main mechanisms, resulting in a reduction of triglyceride plasma concentration. This is primary induced trough an augmented LPL activity, due to increased LPL gene expression and a decreased hepatic production of Apo C-III, which acts as an inhibitor for LPL. The accelerated turnover of triglycerides in lipoproteins, conduces to their enhanced clearance from the

blood. In addition, activation of the PPARα promotes the uptake of fatty acids by the liver, while also inhibiting its performance of triglyceride synthesis. [51]

Treatment with fibrates also has a beneficial effect on HDL-C levels. Activated PPARα promotes the gene expression of major apolipoproteins of HDL particles (Apo A-I and Apo A-II), resulting in an increased HDL production and consequently elevated HDL-C plasma concentration. [51]

1.4.5.2. Effects of fibrates on lipids and prevention of cardiovascular event

The expected changes of plasma lipid levels in patients treated with fibrates, depend on their initial lipid profile, and likewise is influenced by other individual factors. Fibrates are most effective in the reduction of triglyceride plasma concentration. In patients with moderate hypertriglyceridemia (<500 mg/dl) fibrates lower triglyceride levels by 30-50%, but show even more efficacy in severely increased levels with a potential reduction of more than 50%. Fibrates also have a beneficial effect on HDL-C levels, which usually raise between 10% to 35%. Regarding LDL-C, therapy with fibrates may reduce patients' levels (up to 35%), may has no effect, or may even increases LDL-C levels. The latter is frequently observed in patients with severe hypertriglyceridemia with low LDL-C levels and is the result of the fibrate-mediated enhanced lipolysis of triglyceride-rich lipoproteins. [51]

Studies on fibrates in primary prevention for ASCVD showed a reduction in the risk for cardiovascular death, myocardial infarction, and stroke. However, in these patients at risk, but without established ASCVD, the five-years risk for any of these events changed from 5.0% to 4.3%. Thereby the beneficial effects in this setting seem to be marginal and a benefit on overall mortality could not be confirmed. [52]

Regarding the prevention for cardiovascular events in patients with established cardiovascular diseases, there is no evidence of the benefit of monotherapy of current available fibrates. [53]

Furthermore, trials that investigated fibrates as add-on drug to statin therapy could not demonstrate an overall additional effect on cardiovascular outcomes, but specifically suggest a potential benefit in patients with marked high triglyceride and marked low HDL-C levels. These findings have led to today's uncertainties of fibrates in routine ASCVD prevention and require further investigation. [52, 54]

1.4.5.3. Fibrates in the prevention of hypertriglyceridemia-induced pancreatitis

Patients suffering from severe hypertriglyceridemia (triglyceride plasma concentration >10 mmol/l ≙ >885 mg/dl) are at risk for acute pancreatitis. An immediate and radical decrease of triglyceride levels is required to reduce that risk. Due to their potent triglyceride-lowering effect, fibrates are the first-

line pharmacological therapy in the management to prevent hypertriglyceridemia-induced pancreatitis and should be considered as an addition to mandatory strict dietary interventions. [14]

1.4.5.4. Adverse effects of fibrates

Fibrates may elevate creatinine levels in some patients. This increase seems to be a drug class effect, but is less common in treatment with gemfibrozil. The reversible creatine elevation is rather caused by a PPAR-mediated increased production, than by an impaired renal clearance and does not represent renal damages. However, fibrates are primarily eliminated renally and may be impaired in patients with kidney dysfunctions.[55] Due to the lack of evidence in the matter of safety and of the clinical benefit, fibrates are not recommend in patients with chronic kidney disease in the guidelines from the Kidney Disease: Improving Global Outcomes (KDIGO). [56]

Although it is unclear if all fibrate members cause gallbladder disease, findings suggest that fibrates are cholelithogenic and may increase the risk for cholelithiasis. [55]

Muscle symptoms are seen in the therapy with all fibrate agents, but is especially associated with gemfibrozil. In addition, gemfibrozil alters the pharmacokinetics of statins, using the same glucuronidation pathway and inhibits certain cytochrome P450 enzymes. Depending on the statin, gemfibrozil can significantly increase their plasma concentration, overall leading to a higher risk for SAMS. Fenofibrate does not influence the pharmacokinetic of statins and is therefore preferred in statin-fibrate combination, but can independently cause muscle symptoms, although less frequently than gemfibrozil. [55]

Fibrate therapy is associated with an increase of homocysteine levels, but the mechanism of action for this effect is unknown and the clinical relevance is unclear. Especially fenofibrate can elevate homocysteine relatively rapidly. Some studies also suggested a small increase of risk for thromboembolic events in the treatment with fenofibrate, which may be associated with the homocysteine elevation. However, the exact cause for the increased risk for thromboembolic disease is still unclear and other studies did not show higher event rates in fibrate users. [55]

1.4.6. **PCSK9 inhibitors**

The in recent years emerging PCSK9 inhibitors are recommended to reduce LDL-C of very-high risk patients who do not achieve their target LDL-C levels despite maximal tolerated standard lipid-lowering therapy, or patients with proven statin intolerance. [25]

1.4.6.1. Mechanism of action

The LDL-C lowering effect of PCSK9 inhibitors results from altered interaction between correspondent protein and the LDLR. The physiological pathway of individual LDLR starts with its synthesis by ribosomes and folding in the endoplasmic reticulum. After glycosylation in the Golgi apparatus the receptor is transported to the cell surface, where it binds circulation LP. Apo B100 of LDL particles is the most relevant ligand for LDLR, but the receptor also binds other LP like VLDL or IDL using their Apo E as domain. The LP-LDLR-complex is then endocytosed by forming clathrin-coated vesicles and is transported to endosomes, where the acidic environment dissolves the ligand-receptor-bond. Whereas the LP is degraded by lysosomes, the LDLR recycles back to the cell surface.[57]

PCSK9 interferes with the recycling procedure of LDLR and consequently with the uptake of LP, mainly LDL. PCSK9 is expressed in the liver, where its interaction with LDLR leads to characterized influence in the lipid metabolism, but also in the intestine, the kidney and the central nervous system. The protein is produced in the endoplasmic reticulum and is composed of a prodomain, a catalytic domain and a C-terminal domain. After secretion, the catalytic domain of PCSK9 binds to the LDLR (on its epidermal growth factor repeat A (EGF-A) domain), initializing a clathrin-mediated endocytosis of the complex, similar to process after binding with LDL. However, the endocytosed PCSK9-LDLR-complex is then degraded, leading to a reduced recycling of LDLR. This results in a lower number of available receptors on the cell surface and a decreased uptake of LDL particles. [58]

The discovery and research of this pathway led the development of PCSK9-targeted therapies. By inhibiting the PCSK9-mediated degradation of LDLR, the intracellular absorption of LDL is increased, resulting in an enhanced hepatic LDL-C clearance. At present, two monoclonal antibodies (mAbs) selectively binding and hence inhibiting PCSK9 are approved. The fully human mAbs alirocumab and evolocumab are already well implemented in the clinical practice. The mechanism of action of PCSK9-targeted mAbs therapy is illustrated in Figure 3.

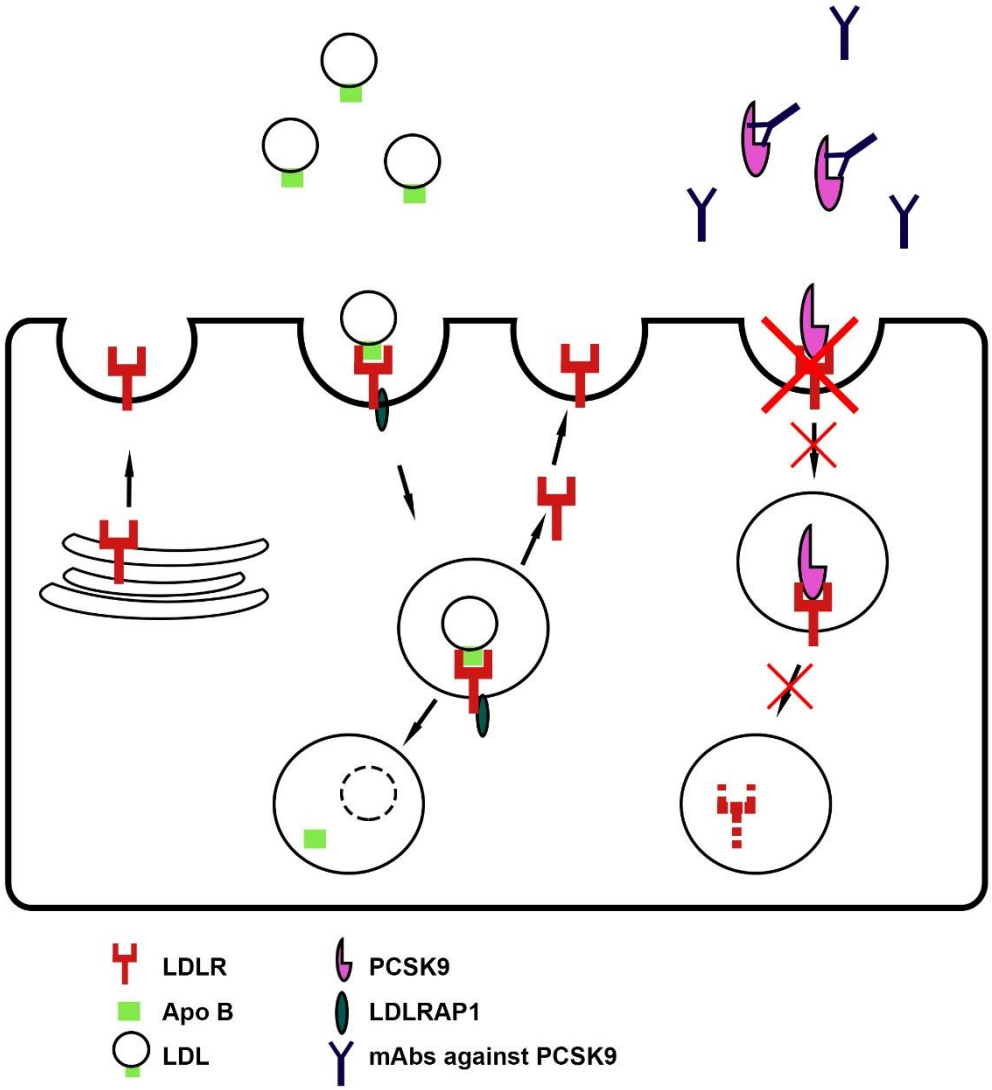


Figure 3. *Mechanism of action of Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors.*

1.4.6.2. Effects of PCSK9 inhibitors on low-density lipoprotein cholesterol

Several clinical trials demonstrated the potent lipid-lowering effect of PCSK9 inhibitors. RCTs comparing participants to placebo might best illustrate the efficacy of this new drug class. An overview of their outcomes on lipid parameters are shown in Table 4.

The phase 3 study program ODYSSEY evaluated the performance of alirocumab across different patient populations. ODYSSEY FH I, ODYSSEY FH II and ODYSSEY HIGH FH focused specifically on subjects with HeFH. HeFH patients receiving statins who did not reach their LDL-C goals recommended in guidelines at that time (<100 mg/dl in primary prevention, <70 mg/dl in secondary prevention), respectively markedly high LDL-C levels (≥160 mg/dl) in the ODYSSEY HIGH FH, were included in these RCTs. After 24 weeks, LDL-C of patients in the intervention group changed from 148 mg/dl at baseline to 71 mg/dl

in ODYSSEY FH I, from 135 mg/dl to 68 mg/dl in ODYSSEY FH II, and from 196 mg/dl to 107 mg/dl in ODYSEEY HIGH FH; meaning a placebo-corrected reduction of 58%, 51%, and 39%. [59, 60]

Other ODYSSEY trials (ODYSSEY COMBO I, ODYSSEY Long Term, ODYSSEY OUTCOMES) ascertained the efficacy of alirocumab in patients at risk for cardiovascular events, accompanied with high LDL-C despite lipid-lowering therapy (mainly statins). ODYSSEY Long Term also included subjects with HeFH with or without ASCVD risk. Representing a major group of people for the indication of lipid-lowering treatment, PCSK9 inhibitor therapy showed positive effects on LDL-C levels. Compared to placebo LDL-C significantly decreased by 46% in ODYSSEY COMBO I and 62% in ODYSSEY Long Term after 24 weeks.[61, 62] Demonstrating the long-term potency of alirocumab, ODYSSEY Long Term and ODYSSEY OUTCOMES showed sustained decreased LDL-C levels after a longer duration (-56% after 78 weeks and -55% after 48 months), but the reduction was less than at shorter period after baseline (-62% after 24 weeks in ODYSSEY Long Term; -63% after 4 months in ODYSSEY OUTCOMES). The investigators explained this increase in LDL-C over time due to the intention-to-treat setting, reflecting cases of treatment discontinuation or adjustments.[62, 63] However, studies of PCSK9 inhibitors on even longer terms, or respectively lifelong treatment, are still missing due to the novelty of this drug class.

The second mAbs against PCSK9 approved today is evolocumab. Similar to the alirocumab trials, the the effect of eveolcumab in different patient subgroups were evaluated evaluated in the PROFICIO study program.

The lipid-lowering outcome of evolocumab in patients suffering from familial hypercholesterolemia were investigated in the RUTHERFORD-2 (on HeFH patients) and TESLA study (on HFH patients). Subjects with stable but elevated levels of LDL-C (>100 mg/dl in RUTHERFORD-2; >131 mg/dl in TESLA), despite lipid-lowering treatment, were included. [64, 65]

In the TESLA trial evolocumab overall reduced LDL-C levels by 31% compared to placebo in the intervention group. However, the study showed that the response to PCSK9 inhibitors in HoFH patients correlates with the underlying mutation. Two patients did not respond to evolocumab, resulting in no reduction of LDL-C concentration at 12 weeks. One of these was carrier of a LDLR-negative mutation in both alleles and the other subject had an autosomal recessive form of HoFH. LDL-C levels of these patients even slightly increased by 10% and 4%. [65]

The RUTHERFORD-2 compared two different dose regimens of evolocumab in patients with HeFH. At 12 weeks, 140 mg of the PCSK9 inhibitor every two weeks led to a placebo-corrected LDL-C reduction of 59%, 420 mg monthly a placebo-corrected reduction of 61%. Mean LDL-C levels changed from 162 mg/dl at baseline to 66 mg/dl, and from 155 mg/dl to 70 mg/dl in the respective subgroups. [64]

The GLAGOV, the FOURIER and the YUKAWA trial of evolocumab enrolled patients with evident ASCVD, respectively people at high risk for cardiovascular events in YUKAWA. Also in these studies, the

enrolled subjects required elevated cholesterol levels under statin therapy at baseline (LDL-C ≥80 or ≥60 + cardiovascular risk factors in GLAGOV; LDL-C ≥70 or non-HLD-C ≥100 in FOURIER; LDL-C ≥116 in YUKAWA). [66-68]

The GLAGOV trial reported an absolute LDL-C reduction of 56 mg/dl (from mean LDL-C of 93 mg/dl to 37 mg/dl) in the intervention group. Similar LDL-C lowering results showed FOURIER with LDL-C change of 92 mg/dl at baseline to 30 mg/dl, meaning a placebo-corrected reduction of 59% after 48 weeks. The PCKS9 inhibitor was able to lower LDL-C levels of ≤70 mg/dl in 87% of the patients, ≤40 mg/dl in 67% and ≤25 mg/dl in 42%. [66, 67]

The phase 2 YUKAWA trial compared different dose regimes of evolocumab in cardiovascular high-risk patients in Japan. The subjects in the intervention subgroups either received the mAbs dose every 2 weeks (70 mg or 140 mg) or monthly (280 mg or 420 mg). At 12 weeks, LDL-C plasma concentrations changed from 143 mg/dl to 58 mg/dl in patients taking 70 mg every 2 weeks, and from baseline LDL-C of 139 mg/dl to 35mg/dl, 58 mg/dl and 46 mg/dl in the other subgroups. evolocumab markedly decreased LDL-C in all intervention subgroups, ranging from 53% to 69% compared to placebo. In patients receiving 140 mg every 2 weeks, 96% achieved LDL-C levels of <70 mg/dl. [68] This dose of evolocumab is standard at present.

The DESCARTES study analyzed the long-term efficacy of evolocumab in patients with different lipid-lowering background therapy at baseline. Overall, in patients receiving the PCSK9 inhibitor drug LDL-C concentration decreased by 57% compared to placebo at 52 weeks. The subgroup analysis showed slightly different results on LDL-C reduction. In evolocumab participants with diet alone as background therapy LDL-C changed from 112 mg/dl at baseline to 54 mg/dl (placebo-corrected LDL-C reduction of 56%); diet plus 10 mg of Atorvastatin from 101 mg/dl to 45 mg/dl (placebo-corrected reduction of 62%); diet plus 80 mg of Atorvastatin from 95 mg/dl to 50 mg/dl (placebo-corrected reduction of 57%); diet plus 80 mg of Atorvastatin from 117 mg/dl to 64 mg/dl (placebo-corrected reduction of 49%). The investigators explained that these findings may be due to a slightly less capacity of further LDL-C reduction of PCSK9 inhibitors in patients with already intensified lipid-lowering therapy. [69]

All in all, these trials of PCSK9 inhibitors demonstrated a potent LDL-C reduction between 39% and 69%, depending on the clinical background.

Trial	PCSK9-i group	Time from baseline	Effects on LDL-C
Alirocumab			
ODYSSEY FH I [59]	n=323	24 weeks	▪ LDL-C changed from 145 mg/dl at BL to 71 mg/dl ▪ LDL-C: -49% ▪ LDL-C: -58% compared to placebo ▪ LDL-C <70 mg/dl in 60% of the patients
ODYSSEY FH II [59]	n=167	24 weeks	▪ LDL-C changed from 135 mg/dl at BL to 68 mg/dl ▪ LDL-C: -49% ▪ LDL-C: -51% compared to placebo ▪ LDL-C <70 mg/dl in 68% of the patients
ODYSEEY HIGH FH [60]	n=72	24 weeks	▪ LDL-C changed from 196 mg/dl at BL to 107 mg/dl ▪ LDL-C: -46% ▪ LDL-C: -39% compared to placebo ▪ LDL-C <70 mg/dl in 32% of the patients
ODYSSEY COMBO I [61]	n=209	24 weeks	▪ LDL-C changed from 100 mg/dl at BL to 50 mg/dl ▪ LDL-C: -48% ▪ LDL-C: -46% compared to placebo
ODYSSEY Long Term [62]	n=1553	24 weeks	▪ LDL-C changed from 123 mg/dl at BL to 48 mg/dl ▪ LDL-C: -61% ▪ LDL-C: -62% compared to placebo ▪ LDL-C: - 56% after 78 weeks compared to placebo ▪ LDL-C <70 mg/dl at BL in 79% of the patients
ODYSSEY OUTCOMES [63]	n=9.462	4 / 12 / 48 months	▪ LDL-C changed from 92 mg/dl at BL to 38 mg/dl / 42 mg/dl / 53 mg/dl after 4 / 12 / 48 months ▪ LDL-C: -63% / -61% / -55% compared to placebo
Evolocumab			
TESLA [65]	n=33	12 weeks	▪ LDL-C: -23% ▪ LDL-C: -31% compared to placebo Homozygous LDLR- negative mutation: ▪ LDL-C: +10% autosomal recessive HoFH: ▪ LDL-C: +4%

RUTHERFORD-2 [64]	n=220	12 weeks	Evolocumab 140 mg every 2 weeks: ▪ LDL-C changed from 162 mg/dl at BL to 66 mg/dl ▪ LDL-C: -61% ▪ LDL-C: -59% compared to placebo Evolocumab 420 mg monthly: ▪ LDL-C changed from 155 mg/dl at BL to 70 mg/dl ▪ LDL-C: -56% ▪ LDL-C: - 61% compared to placebo
GLAGOV [66]	n=484	76 weeks	▪ LDL-C changed from 93 mg/dl at BL to 37 mg/dl ▪ Absolute LDL-C reduction: 56 mg/dl
FOURIER [67]	n=13.784	48 weeks	▪ LDL-C changed from 92 mg/dl at BL to 30 mg/dl ▪ LDL-C: -59% compared to placebo ▪ LDL-C ≤70 mg/dl in 87% of the patients; ≤40 mg/dl in 67%; ≤25 mg/dl in 42%
YUKAWA [68]	n=207	12 weeks	Evolocumab 70 mg every 2 weeks: ▪ LDL-C changed from 143 mg/dl at BL to 58 mg/dl ▪ LDL-C: -53% compared to placebo ▪ LDL-C <100 mg/dl in 94% of the patients; LDL-C <70 mg/dl in 66% Evolocumab 140 mg every 2 weeks: ▪ LDL-C changed from 139 mg/dl at BL to 35 mg/dl ▪ LDL-C: -69% compared to placebo ▪ LDL-C <100 mg/dl in 98% of the patients; LDL-C <70 mg/dl in 96% Evolocumab 280 mg monthly: ▪ LDL-C changed from 139 mg/dl at BL to 58 mg/dl ▪ LDL-C: -58% compared to placebo ▪ LDL-C <100 mg/dl in 94% of the patients; LDL-C <70 mg/dl in 80% Evolocumab 420 mg monthly: ▪ LDL-C changed from 139 mg/dl at BL to 46 mg/dl ▪ LDL-C: -64% compared to placebo ▪ LDL-C <100 mg/dl in 96% of the patients; LDL-C <70 mg/dl in 82%

DESCARTES [69]	n=599	52 weeks	All Evolocumab subjects: ▪ LDL-C changed from 104 mg/dl at BL to 60 mg/dl ▪ LDL-C: -50% ▪ LDL-C: -57% compared to placebo ▪ LDL-C <70 mg/dl in 82% of the patients Background therapy diet alone: ▪ LDL-C changed from 112 mg/dl at BL to 54 mg/dl ▪ LDL-C: -58% compared to placebo Background therapy diet + Atorvastatin 10 mg: ▪ LDL-C changed from 101 mg/dl at BL to 45 mg/dl ▪ LDL-C: -62% compared to placebo Background therapy diet + Atorvastatin 80 mg: ▪ LDL-C changed from 95 mg/dl at BL to 50 mg/dl ▪ LDL-C: -57% compared to placebo Background therapy diet + Atorvastatin 80 mg + Ezetimibe: ▪ LDL-C changed from 116 mg/dl at BL to 64 mg/dl ▪ LDL-C: -49% compared to placebo

Table 4. *Effects of PCS9 inhibitors on low-density lipoprotein cholesterol in clinical trials. PCSK9-i = proprotein convertase subtilisin/kexin type 9 inhibitor; LDL-C = low-density lipoprotein cholesterol; BL = baseline.*

1.4.6.3. Effects of PCS9 inhibitors on other lipid parameters

The above-mentioned trials (except ODYSSEY OUTCOMES, GLAGOV and FOURIER) did also provide data on other lipid parameters in comparison to placebo, which are listed in Table 5.

In these RCTs, PCSK9 inhibitors demonstrated their cholesterol-lowering efficacy by a total cholesterol reduction between 25% and 46%, respectively by reducing non-HDL cholesterol between 36% and 63%. [59-62, 64, 65, 68, 69]

While the ODYSSEY COMBO I and the TESLA study showed no significant changes in triglyceride levels [61, 65], the other trials reported a decrease between 9% and 20% at their primary endpoint. [59, 60, 62, 64, 68, 69]

Except in TESLA, PCSK9 inhibitor therapy raised HDL-C between 4% and 16% compared to placebo treatment, but also levels of lipoprotein (a) decreased by 12 - 32% in these clinical trials [59-62, 64, 65, 68, 69], which might have an additional influence on the cardiovascular benefit of PCSK9 inhibition.

Trial	**Total cholesterol**	**Triglycerides**	**Lp (a)**	**HDL-C**	**Non-HDL-C**
ODYSSEY FH I [59]	-	-16%	-18%	+8%	-52%
ODYSSEY FH II [59]	-	-11%	-20%	+7%	-46%
ODYSEEY HIGH FH [60]	-28%	-9%	-15%	+4%	-36%
ODYSSEY COMBO I [61]	-25%	-1%	-15%	+7%	-38%
ODYSSEY Long Term [62]	-38%	-17%	-26%	+5%	-52%
TESLA [65]	-	0%	-12%	+0%	-
RUTHERFORD-2 [64] (140 mg Q2W)	-	-20%	-32%	+9%	-55%
RUTHERFORD-2 [64] (420 mg QM)	-	-12%	-28%	+9%	-55%
YUKAWA [68] (70 mg Q2W)	-36%	-17%	-	+6%	-50%
YUKAWA [68] (140 mg Q2W)	-46%	-17%	-	+9%	-63%
YUKAWA [68] (280 mg QM)	-40%	-17%	-	+16%	-58%
YUKAWA [68] (420 mg QM)	-42%	-18%	-	+13%	-60%
DESCARTES [69]	-37%	-12%	-22%	+5%	-50%

Table 5. *Effects of PCSK9 inhibitors on other lipid parameters in clinical trials. Lp (a) = lipoprotein (a); HDL-C = high-density lipoprotein cholesterol; Q2W = every 2 weeks; QM = monthly;*

1.4.6.4. Effects of PCSK9 inhibitors on cardiovascular morbidity and mortality

The ODYSSEY OUTCOMES study of alirocumab and the FOURIER study of evolocumab evaluated the effects of PCSK9 inhibitor drugs on cardiovascular events and mortality. Both multicenter, double-blind RCTs randomly assigned a large number of patients at high cardiovascular risk (18.924 patients in ODYSSEY OUTCOMES; 27.564 patients in FOURIER) with elevated cholesterol levels. [63, 67]

During the median follow-up of 2.8 years in ODYSSEY OUTCOMES, the primary end point (defined as CHD death, nonfatal myocardial infarction, ischemic stroke, or hospitalization for unstable angina) occurred in significant less patients treated with alirocumab than in the placebo group (9.5% vs 11.1% of the subjects; hazard ratio: 0.85). [63]

Treatment with evolocumab showed a comparable reduction of major cardiovascular events in the FOURIER trial (median follow-up of 2.2 years). The PCSK9 inhibitor significantly reduced the event rate of myocardial infarction, stroke and coronary revascularization. Evolocumab users were at lower risk for the composition of cardiovascular death, myocardial infarction, stroke hospitalization for unstable angina, or coronary revascularization as the primary end point of the study (9.8% in the evolocumab group vs 11.3% in the placebo group; hazard ratio: 0.85). [67]

However, both studies did not demonstrate a significant reduction in cardiovascular mortality, and there was no effect on the rates of death from any cause in the FOURIER trial. [63, 67]

1.4.6.5. Adverse effects of PCSK9 inhibitors

1.4.6.5.1. Adverse events reported in clinical trials

Adverse effects of alirocumab and evolocumab compared to placebo in RCTs of the ODYSSEY and the PROFICO program are listed in Table 6. Only studies providing data of statistical significance of between-group comparison are included in the list.

In the ODYSSEY LONG TERM, the ODYSSEY OUTCOMES and the FOURIER study, in patients receiving a PCSK9 inhibitor any type of adverse event occurred in about 80%, and serious adverse events was reported in 19%, 23% and 25%, but were similar to the placebo group. [62, 63, 67]

In 13% of diabetics in ODYSSEY LONG TERM and in 19% in ODYSSEY OUTCOMES, worsening of diabetes was observed in the alirocumab group. In the group of PCSK9 inhibitor patients without a history of diabetes mellitus, 2% in ODYSSEY LONG TERM, 10% in ODYSSEY OUTCOMES, and 8% in FOURIER were newly diagnosed with the disorder during the study. [62, 63, 67]

The rate of allergic reaction was up to 10% (ODYSSEY LONG TERM), but lower in the other studies. [62] In all three studies elevation of aminotransferases or CK >3 times upper normal range were rare. Neurocognitive disorders were reported in 1-2%, ophthalmologic events or cataract in 1-3% of the subjects. [62, 63, 67]

However, compared to the placebo group, statistically significant higher adverse event rates were only observed of injection-site reactions in ODYSSEY OUTCOMES (3.8 % vs 2.1%) and in FOURIER (2.1% vs 1.6%), and of myalgia in ODYSSEY LONG TERM (5.4% vs 2.9%). [62, 63, 67]

Adverse event	**ODYSSEY LONG TERM [62]**	**ODYSSEY OUTCOMES [63]**	**FOURIER [67]**
Any adverse event	81%	76%	77%
Serious adverse event	19%	23%	25%
Injection-site reaction	6%	**4%** **(P <0.001)**	**2%** **(P <0.001)**
Allergic reaction	10%	8%	3%
Myalgia/ muscle-related event	**5%** **(P 0.006)**	-	5%
Ophthalmologic event/ cataract	3%	1%	2%
Neurocognitive disorder	1%	2%	2%
New-onset diabetes	2%	10%	8%
Worsening diabetes	13%	19%	-
Aminotransferase >3× ULN	-		2%
ALT >3× ULN	2%	2%	-
AST >3× ULN	1%	2%	-
CK >3× ULN	4%	1%	1%

Table 6. *Adverse events of PCKS9 inhibitor therapy reported in clinical trials. ULN = Upper limit of normal; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK = creatine kinase.*

1.4.6.5.2. PCSK9 inhibitors and the risk of new-onset diabetes

Although the exact mechanism is not fully understood, in vitro and in vivo studies in animals and humans showed a mutual influence of PCSK9 and the glucose homeostasis. Experiments demonstrated a contradicting effect of insulin on PCSK9 expression. These findings suggest that insulin can likewise induce and inhibit PCSK9 production. Under physiological condition, the inhibitory effect of insulin on

PCSK9 appears to be more dominant. In the state of insulin resistance and hyperinsulinemia PCSK9 expression is increased, while animal studies demonstrated a markedly decreased PCSK9 production in rodents with deficient insulin production. Even tough studies showed an association between PCSK9 plasma concentration and glycemic parameters, the potential role of PCSK9 or its drug-induced inhibition in the development of diabetes mellitus is still a topic of recent research. [70] One mendelian randomization study investigating the relation of PCSK9 gene variants and glycemic parameters, suggesting a potential risk for new-onset diabetes in PCSK9 inhibitor treatment. Mimicking the pharmacological effects of PCSK9 inhibition, this study found increased glucose concentrations and an increased risk of type 2 diabetes mellitus in carriers of PCSK9 variants associated with low LDL-C levels. However, these findings represent the life-long effect of these gene variants and may not reflect the pharmacological PCSK9-targeted intervention later in life. [71] Besides, data from RCTs did not show increased rates of new-onset diabetes mellitus compared to placebo, but long-term trials are still scarce. [72]

1.4.6.5.3. PCSK9 inhibitors and neurocognitive function

Cholesterol is an important part of cellular components and is essential for a normal neuronal function. These facts repeatedly led to hypothesis of the potential impact on neurocognitive function of lipid-lowering therapies. Due to the potent LDL-C lowering effect of PCSK9 inhibitors and to the possible specific function of PCSK9 in the brain, these concerns were evoked once again. Although it is unclear to what extent PCSK9 is involved in the nervous system, animal studies and in vitro experiments demonstrated its potential role in the brain development. PCSK9 was found in embryonic neurons of mice and its overexpression was associated with increased neuronal cell differentiation. Studies suggest that PCSK9 may regulate LDLR during neurogenesis. Furthermore, PCSK9 may modulate the controlled neuronal apoptosis during development of the nervous system. [73]

Despite these experimental findings, PCSK9 knockout mice did not show alterations in brain development and PCSK9 mutations in human are not associated with neurocognitive dysfunction. [73]

Even though a few trials reported slightly increased incidences of adverse neurocognitive effects during PCSK9 inhibitor therapy, recent studies did not show evidence to confirm these concerns. The Evaluating PCSK9 Binding Antibody Influence on Cognitive Health in High Cardiovascular Risk Subjects (EBBINGHAUS) trial prospectively investigated possible influence of add-on evolocumab to statin therapy on cognitive functions. Using standardized neuropsychological testing, the study did not find differences in changes of executive function, memory or psychomotor speed between the intervention and the placebo group. The study showed no association between LDL-C and cognitive changes. However, patients with known neurocognitive dysfunctions were excluded and the 19 months median follow-up might be too short to represent evidence for long-term adverse effects. [74]

1.4.7. **Future perspectives of PCSK9-targeted therapies**

1.4.7.1. Natural and synthetic compounds

Several natural or synthetic compounds were found to inhibit PCSK9 gene expression, protein synthesis or interaction with LDLR. The goal of this research is to develop an orally available drug and to replace expensive mAbs. However, at present these drugs are still in early development stages. [75]

1.4.7.2. Adnectins

The synthetic adnectins are non-antibody proteins, which are able to bind to PCSK9 and blocking its interaction with LDLR. Investigators of an open label extension phase 2 study of the adnectin LIB003 reported a marked LDL-C reduction of approximately 60% in patients on maximally tolerated statin therapy. The efficacy and safety of the adnectin LIB003 compared to evolocumab is currently under investigation in a phase 3 clinical trial.[76, 77]

1.4.7.3. Gene silencing

Antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) are two approaches of inhibiting PCSK9 synthesis. Both methods target the mRNA and induces its degradation before PCSK9 is produced, therefore silencing the gene expression. Three ASOs reached early phase clinical trials. However, the development of two of them was discontinued, one due to safety issues. [75]

The development of siRNA against PCSK9 is in a more advanced stage. The first siRNA agent inclisiran has already been approved by the European Medicines Agency in December 2020. [78] The two placebo-controlled RCTs ORION10 and ORION11 trials assessed the efficacy and safety of inclisiran in patients at high risk for ASCVD. 1561 and 1617 patients having elevated LDL-C levels despite lipid-lowering therapy (except PCSK9 inhibitor mAbs) were enrolled in these phase 3 trials. Therapy with the siRNA every 6 months, resulted in a LDL-C reduction of about 50% at the end point of 510 days. [79] Although the benefit on cardiovascular outcomes is still under investigation, due to the easier manufacturing process and simpler drug regime PCSK9-targeted therapy with siRNA might be a promising alternative to mAbs or even replace them in the future.

1.4.7.4. Vaccination

Active immunization against PCSK9 is another concept for a long-lasting option of lipid-lowering treatment. An animal study of the anti-PCSK9 vaccine AT04A reported a up to 30% reduction of total

cholesterol and up to 50% of LDL-C in vaccinated mice. In addition, AT04A showed beneficial impacts on the development of atherosclerotic lesions in the immunized rodents. A phase 1 clinical trial of the vaccine was conducted, but no results published at present. [75, 80]

2 MATERIAL AND METHODS

2.1. Patients and outcome measures

This study (titled: "Real-world data on metabolic effects of PCSK9 inhibitors in a tertiary care center in patients with and without diabetes mellitus" [81]) was a retrospective data analysis approved by the Medical University of Graz, Austria (EK number 32-018 ex 19/20) and included patients at elevated cardiovascular high who were prescribed with PCSK9 inhibitor therapy within routine conditions, considering the national reimbursement criteria (LDL >100 mg/dl despite maximal tolerated statin/ezetimibe therapy, well controlled hypertension, HbA1c <64 mmol/mol, having received nutritional advice by a dietologist and intensive motivation to stop smoking). Data of the electronic patient records of the University hospital were screened for eligible patients from January 2016 to September 2019. Electronic records of adult patients with current or past PCSK9 inhibitor treatment in routine care at the outpatient clinic of the Division of Endocrinology and Diabetology were searched and included in the analysis if they met the inclusion criteria. Inclusion criteria were as follows: age >18 years, treatment with locally available PCSK9 inhibitors (alirocumab 75 or 150 mg, or evolocumab 140 mg) in routine care, available laboratory reports on LDL-C levels at first prescription and LDL-C during a follow-up period longer than 3 months.

In eligible patients the following parameters were drawn from the electronic patient record: age, sex, lipid-lowering therapy at baseline (i.e. statins, ezetimibe, fibrates), cause of prescription of PCSK9 inhibitor (intolerance to lipid-lowering medications at baseline, failure to achieve individual LDL-C levels; primary or secondary prevention as indication), type of PCSK9 inhibitor (i.e. alirocumab or evolocumab), PCSK9 inhibitor therapy adjustments (agent, discontinuation). Baseline macrovascular (coronary heart disease, stroke, transient ischemic attack, peripheral artery disease, carotid artery disease) and microvascular (retinopathy, nephropathy) comorbidities, previous cardiovascular interventions (i.e. percutaneous coronary revascularization or coronary artery bypass grafting) and further cardiovascular risk factors (i.e. smoker status, hypertension, diabetes mellitus) were assessed. The following laboratory parameters were extracted: LDL-C, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), total cholesterol, lipoprotein (a) (Lp[a]), and HbA1c at baseline and during PCSK9 inhibitor therapy for up to 18 months whenever collected in routine care. Number of patients who were lost to follow-up (missing documentation regarding PCSK9 inhibitor therapy ≥1 year) or patients who discontinued therapy (i.e. adverse drug effects towards PCSK9; noncompliance) were also determined. Baseline was defined as the day of the first PCSK9 inhibitor application. Patients enrolled were followed up for subsequent cardiovascular events within the first 18 months after PCSK9 inhibitor

initiation. MACE was defined as occurrence of non-fatal myocardial infarction or stroke or cardiovascular death. All-cause cardiovascular events included the following: any acute coronary syndrome, scheduled coronary intervention, aortic dissection, any cerebral ischemic event (transitory ischemic attack [TIA] or stroke), any peripheral vascular intervention or diagnosis/worsening of peripheral artery disease, newly diagnosed carotid artery disease, surgical carotid intervention, necessity of hemodialysis or acute decompensated heart failure.

2.2. Statistical methods

The follow-up of LDL-C, HDL-C, TG, Lp(a) and HbA1c were analyzed at months 3, 6, 9 and 12, and up to 18 months from baseline, including data from the most current measurement after the defined time points. A last observation carried forward analysis was performed for laboratory parameters, imputing data of the latest measurement of maximum 3 months prior. If PCSK9 inhibitor therapy was discontinued, further observations were not included in the analyses.

Wilcoxon signed-rank test was used to compare parameters between baseline and follow-up time points within a subgroup, Mann-Whitney-U test was used to evaluate between-group differences of two different subgroups. Kruskal-Wallis test was used to analyze differences of LDL-C levels and LDL-C reduction across subgroups of concomitant lipid-lowering therapy. Univariate Cox-regression analysis was used to calculate hazard ratios for events of patients with T2D compared to patients without T2D. P-values of ≤0.05 were considered statistically significant. Statistical analysis was performed with IBM SPSS Statistics version 26.

3 RESULTS

3.1. Patient characteristics

We identified 237 eligible patients (47.7% female) who received alirocumab or evolocumab (104 and 133 patients) of whom the median age was 65.2 years (interquartile range [IQR] 57.8 – 71.5). Almost all patients (97.5%; n=231) received PCSK9 inhibitors for secondary prevention. Apart from hypercholesterolemia, more than half of the study population (53.6%) had two or more additional cardiovascular risk factors (arterial hypertension, chronic kidney disease, age ≥ 65 years, diabetes mellitus, smoker). Medical history included coronary heart disease (74.7%), at least one percutaneous coronary intervention (46.4%), coronary artery bypass graft (CABG) (16%), stroke or transient ischemic attack (13.5%), carotid artery stenosis (30.8%), peripheral artery disease (18.1%), and chronic kidney disease (9.7%). Any type of familial hypercholesterolemia (mostly diagnosed using clinical criteria, no one identified with homozygous FH) was present in 21.5% of the patients. Diabetes mellitus was present in 62 patients of whom the majority had type 2 diabetes (T2D) (n=54). Further relevant comorbidities at baseline were: arterial hypertension (68.8%) and active smoker status (5.9%).

Intolerance to other lipid-lowering agents was frequently present at baseline: 83.1% reported intolerance to at least one statin, 44.7% reported side-effects to ezetimibe. 42.6% indicated statin and ezetimibe intolerance. The full characteristics of the patient cohort are listed in Table 7. The more detailed summary of cardiovascular risk factor profile is shown in Table 8. Due to the retrospective design of this study, we were not able to ascertain laboratory data at the predefined time-points from all patients (i.e. every 3 months after therapy initiation) due to loss to follow-up, discontinuation of treatment or infrequent outpatient clinic visits. Ten patients (4.2%) discontinued PCSK9 inhibitor therapy (five after 3 months from baseline, one after 6 months, one after 9 months, three after 12 months) and were not further included in the analysis after discontinuation. 27 patients (11.4%) were lost to follow-up (missing documentation regarding PCSK9 inhibitor therapy ≥1 year).

Characteristics	Total (n=237)	Alirocumab (n=104)	Evolocumab (n=133)	Without diabetes mellitus (n=175)	With diabetes mellitus type 2 (n=54)
Age (years)	65.2 (57.8 – 71.5)	65.0 (57.8 – 71.5)	65.4 (57.9 – 71.6)	65.0 (57.8 – 70.8)	68.9 (63.1 – 73.8)
Female sex n (%)	113 (47.7)	53 (51.0)	60 (45.1)	88 (50.3)	22 (40.7)
Indication for PCSK9 inhibitor treatment n (%)					
Primary prevention	6 (2.5)	4 (3.8)	2 (1.5)	4 (2.3)	2 (3.7)
Secondary prevention	231 (97.5)	100 (96.2)	131 (98.5)	171 (97.7)	52 (96.3)
Medical history n (%)					
Coronary heart disease	177 (74.7)	80 (76.9)	97 (72.9)	134 (76.6)	38 (70.4)
Percutaneous coronary intervention	110 (46.4)	49 (47.1)	61 (45.9)	86 (49.1)	21 (38.9)
Coronary artery bypass graft	38 (16.0)	16 (15.4)	22 (16.5)	27 (15.4)	8 (14.8)
Stroke or transient ischemic attack	32 (13.5)	12 (11.5)	20 (15.0)	24 (13.7)	8 (14.8)
Carotid artery disease	73 (30.8)	37 (35.6)	36 (27.1)	51 (29.1)	20 (37.0)
Peripheral artery disease	43 (18.1)	15 (14.4)	28 (21.1)	25 (14.3)	15 (27.8)
Arterial hypertension	163 (68.8)	74 (71.2)	89 (66.9)	112 (64.0)	46 (85.2)
Familial hypercholesterolemia (heterozygous)	51 (21.5)	19 (18.3)	32 (24.1)	41 (23.4)	8 (14.8)
Retinopathy	10 (4.2)	5 (4.8)	5 (3.8)	4 (2.3)	3 (5.6)
Chronic kidney disease	23 (9.7)	6 (5.8)	17 (12.8)	10 (5.7)	12 (22.2)
Current tobacco smoker	14 (5.9)	9 (8.7)	5 (3.8)	12 (6.9)	1 (1.9)
Diabetes mellitus any type	62 (26.2)	25 (24.0)	37 (27.8)	-	-
Diabetes mellitus type 1	6 (2.5)	2 (1.9)	4 (3.0)	-	-
Diabetes mellitus type 2	54 (22.8)	22 (21.2)	32 (24.1)	-	54 (100)
Other types of diabetes	2 (0.8)	1 (1.0)	1 (0.8)	-	-
Intolerances/side effects to lipid-lowering medication n (%)					
Statins	197 (83.1)	83 (79.8)	114 (85.7)	147 (84.0)	43 (79.6)
Ezetimibe	106 (44.7)	36 (34.6)	70 (52.6)	76 (43.4)	28 (51.9)
Statins and Ezetimibe	101 (42.6)	34 (32.7)	67 (50.4)	74 (42.3)	25 (46.3)

Table 7. *Baseline characteristics overall as well as per PCSK9 inhibitor used and diabetes status. Patients with type 1 diabetes were not separately analysed due to the small number (n=4). Data are median (interquartile range) or number (%).*

	Coronary heart disease	Stroke/TIA	Carotid artery disease	Peripheral artery disease	Retinopathy	Arterial hypertension	Chronic kidney disease	Age ≥65 years	Diabetes mellitus	Smoking
Coronary heart disease (n=177)	-	17 9.6%	40 22.6%	21 11.9%	8 4.5%	132 74.6%	18 10.2%	95 53.7%	43 24.3%	7 4.0%
Stroke or TIA (n=32)	17 53.1%	-	14 43.8%	6 18.8%	0 0%	23 71.9%	5 15.6%	19 59.4%	8 25.0%	0 0%
Carotid artery disease (n=73)	40 54.8%	14 19.2%	-	19 26.0%	2 2.7%	51 69.9%	6 8.2%	49 67.1%	22 30.1%	4 5.5%
Peripheral artery disease (n=43)	21 48.8%	6 14.0%	19 44.2%	-	3 7.0%	33 76.7%	7 16.3%	23 53.5%	18 41.9%	7 16.3%
Retinopathy (n=10)	8 80.0%	0 0%	2 20.0%	3 30.0%	-	7 70.0%	3 30.0%	5 50.0%	6 60.0%	1 10.0%
Arterial hypertension (n=163)	132 81.0%	23 14.1%	51 31.3%	33 20.2%	7 4.3%	-	19 11.7%	94 57.7%	51 31.3%	8 4.9%
Chronic kidney disease (n=23)	18 78.3%	5 21.7%	6 26.1%	7 30.4%	3 13.0%	19 82.6%	-	18 78.3%	13 56.5%	0 0%
Age ≥65 years (n=123)	95 77.2%	19 15.4%	49 39.8%	23 18.7%	5 4.1%	94 76.4%	18 14.6%	-	36 29.3%	3 2.4%
Diabetes mellitus (n=62)	43 69.4%	8 12.9%	22 35.5%	18 29.0%	6 9.7%	51 82.3%	13 21.0%	36 58.1%	-	2 3.2%
Current smoker (n=14)	7 50.0%	0 0%	4 28.6%	7 50.0%	1 7.1%	8 57.1%	0 0%	3 21.4%	2 14.3%	-

Table 8. *Cardiovascular disease at baseline (by medical condition). Data are number of patients (% from subgroup).*

3.2. Laboratory parameters and concomitant medication at baseline

At baseline, 29.5% of the overall cohort were on statin therapy (mostly rosuvastatin or atorvastatin), 39.7% on ezetimibe and 2.5% on fibrates. 48.9% did not receive lipid-lowering medication (i.e. no statins, ezetimibe or fibrates) at that time. Concomitant lipid-lowering therapy of patients with T2D was comparable to patients without diabetes mellitus. There was only a slight difference in a higher usage of fibrates in the group of patients with diabetes mellitus. Median LDL-C at baseline was 141 mg/dl (117-188), distribution of alirocumab and evolocumab use was 44 and 56%. Median HbA1c in the T2D population was 52 mmol/mol (48-57 mmol/mol; 6.9% [6.5-7.4]). Further laboratory parameters and concomitant medication at baseline according to the prescribed PCSK9 inhibitor and diabetes status are shown in Table 9.

Characteristics	**Total (n=237)**	**Alirocumab (n=104)**	**Evolocumab (n=133)**	**Without diabetes mellitus (n=175)**	**With diabetes mellitus type 2 (n=54)**
LDL-C (mg/dl)	141 (117 – 188) n=237	135 (114 – 181) n=104	149 (118 – 191) n=133	141 (117 – 188) n=175	135 (110 – 178) n=54
Total cholesterol (mg/dl)	229 (198 – 268) n=216	210 (190 – 259) n=94	240 (202 – 272) n=122	230 (200 – 268) n=163	216 (194 – 261) n=47
HDL-C (mg/dl)	54 (45 – 65) n=226	52 (44 – 63) n=100	55 (46 – 67) n=126	54 (46 – 67) n=169	49 (42 – 60) n=49
Triglycerides (mg/dl)	138 (99 – 215) n=227	138 (102 – 255) n=102	138 (97 – 197) n=138	124 (97 – 199) n=167	185 (134 – 249) n=52
Lp(a) (mg/dl)	65 (25 – 101) n=47	87 (71 – 114) n=13	55 (18 – 90) n=34	65 (23 – 114) n=37	67 (46 – 89) n=10
HbA1c (mmol/mol)	41 (37 – 49) n=139	40 (37 – 49) n=61	43 (37 – 50) n=78	38 (36 – 40) n=81	52 (48 – 57) n=51
Statins (n; %)	70 (29.5)	38 (36.5)	32 (24.1)	52 (29.7)	16 (29.6)
Fluvastatin	3 (1.3)	2 (1.9)	1 (0.8)	2 (1.1)	1 (1.9)
Pravastatin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Simvastatin	5 (2.1)	1 (1.0)	4 (3.0)	5 (2.9)	0 (0)
Rosuvastatin	29 (12.2)	15 (14.4)	14 (10.5)	22 (12.6)	5 (9.3)
Atorvastatin	35 (14.8)	21 (20.2)	14 (10.5)	25 (14.3)	10 (18.5)
Ezetimibe	94 (39.7)	46 (44.2)	48 (36.1)	67 (38.3)	22 (40.7)
Fibrates	6 (2.5)	2 (1.9)	4 (3.0)	2 (1.1)	4 (7.4)
No lipid-lowering medication	116 (48.9)	44 (42.3)	72 (54.1)	89 (50.9)	24 (44.4)
Antidiabetic treatment					
Metformin	24 (10.1)	13 (12.5)	11 (8.3)	0 (0)	23 (42.6)
SGLT2 inhibitors	5 (2.1)	2 (1.9)	3 (2.3)	0 (0)	4 (7.4)
DPP-4 inhibitors	14 (5.9)	7 (6.7)	7 (5.3)	0 (0)	14 (25.9)
GLP-1 receptor agonists	4 (1.7)	0 (0)	4 (3.0)	0 (0)	4 (7.4)
Sulfonylureas	1 (0.4)	0 (0)	1 (0.8)	0 (0)	1 (1.9)
Pioglitazone	2 (0.8)	1 (1.0)	1 (0.8)	0 (0)	2 (3.7)
Insulin therapy	23 (9.7)	9 (8.7)	14 (10.5)	0 (0)	16 (29.6)
Diet only	49 (20.7)	22 (21.2)	27 (20.3)	0 (0)	14 (25.9)

Table 9. *Laboratory parameters, concomitant lipid-lowering and anti-hyperglycemic therapy at baseline according to prescribed PCSK9 inhibitor and diabetes status. Data are median (interquartile range) or number (%). SGLT2: Sodium-glucose co-transporter 2. GLP-1: Glucagon-like Peptide 1. DPP-4: Dipeptidyl-peptidase 4*

3.3. Effect on LDL-C levels

Median baseline LDL-C of the total population at baseline was 141 mg/dl and decreased to 60, 59, 61 and 66 mg/dl after 3, 6, 9 and 12 months of observation, respectively. During the course of treatment, a substantial proportion of patients achieved LDL-C levels <70 mg/dl (61.2% after 3 months and 56.2% after 12 months) or <55 mg/dl (44.1% after 3 months and 38.6% after 12 months). LDL-C remained above 100 mg/dl in 17.6% at month 3 months and in 16.3% at month 12. LDL-C reduction >50% was achieved in 64.3% and 59.5% at month 3 and 12, respectively (Figure 4). Table 10 shows the LDL-reduction over time according to the PCSK9 inhibitor used.

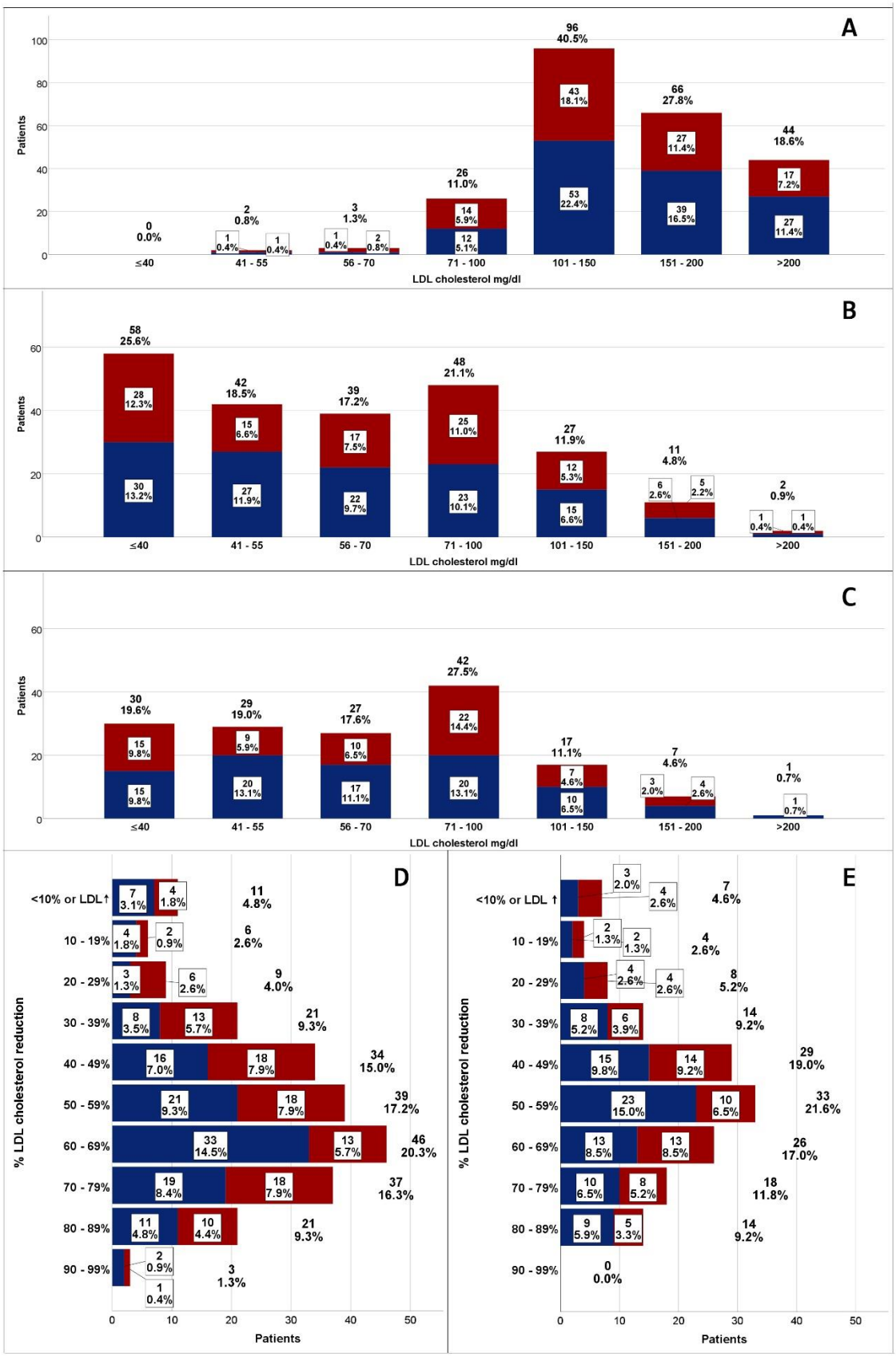


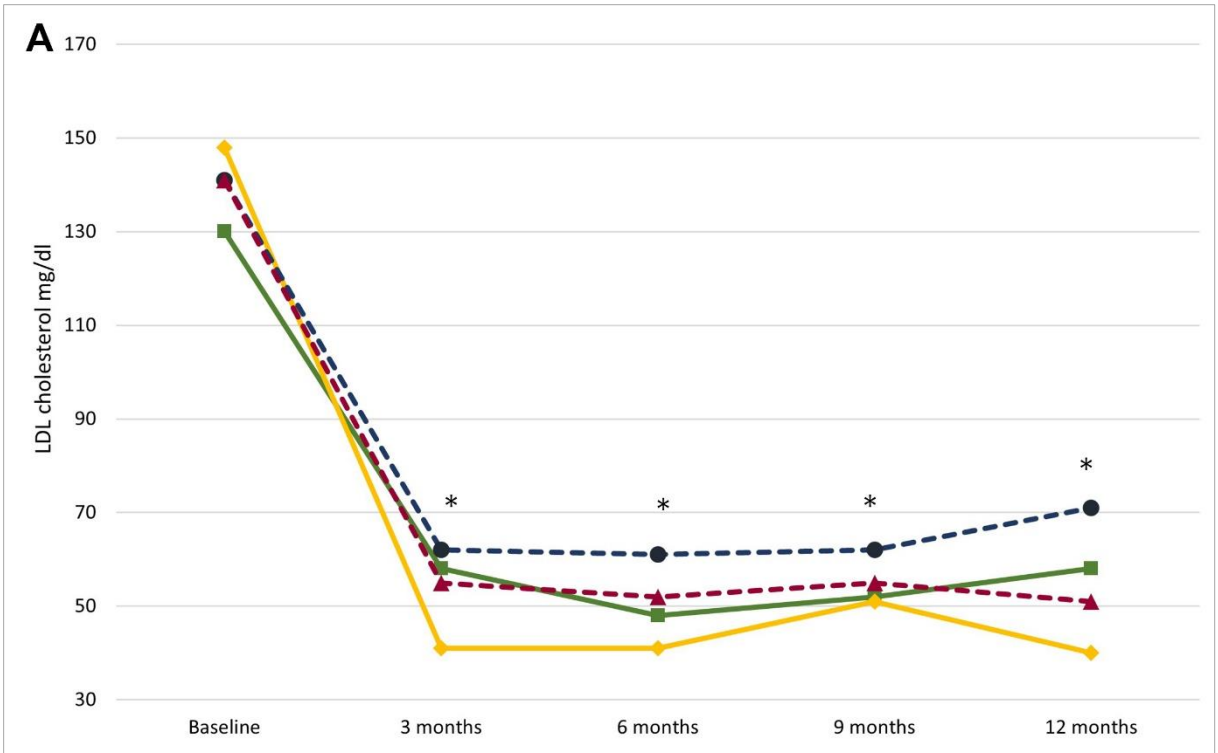
Figure 4. *LDL-C levels and percentage of LDL-C reduction over time. Data are number of patients (n) and percentage (%); red bar = alirocumab, blue bar = evolocumab.*
A*: Baseline LDL-C levels (n=237),* ***B****: LDL-C levels at month 3 (n=227),* ***C****: LDL-C levels at month 12 (n=153);* ***D****: % LDL-C reduction from baseline to month 3 (n=227)* ***E****: % LDL-C reduction from baseline to month 12 (n=153)*

	Overall cohort			Alirocumab			Evolocumab		
	LDL cholesterol (mg/dl)	p-value *	% LDL-C reduction	LDL cholesterol (mg/dl)	p-value *	% LDL-C reduction	LDL cholesterol (mg/dl)	p-value *	% LDL-C reduction
Baseline	141 (117 – 188) (n=237)		n.a.	135 (114 – 181) (n=104)		n.a.	149 (118 – 191) (n=133)		n.a.
Month 3	60 (40 – 83) (n=227)	<0.001	57.5 (42.4 – 70.5)	63 (35 – 87) (n=103)	<0.001	55.3 (40.4 – 71.3)	59 (41 – 82) (n=124)	<0.001	60.8 (45.0 – 70.3)
Month 6	59 (42 – 84) (n=204)	<0.001	57.0 (45.8 – 70.1)	60 (44 – 83) (n=94)	<0.001	54.5 (45.7 – 68.7)	57 (40 – 85) (n=110)	<0.001	60.8 (45.8 – 70.6)
Month 9	61 (44 – 85) (n=178)	<0.001	57.1 (45.5 - 68.7)	61 (44 – 87) (n=79)	<0.001	54.2 (41.9 – 68.3)	60 (43 – 82) (n=99)	<0.001	59.5 (46.6 – 70.3)
Month 12	66 (45 – 86) (n=153)	<0.001	53.6 (42.5 - 66.7)	68 (42 – 88) (n=66)	<0.001	52.5 (40.7 – 67.0)	63 (47 – 60) (n=87)	<0.001	53.6 (43.8 – 66.4)

Table 10. *LDL-C levels over time of the overall cohort and by PCSK9 inhibitor agent. Data are median (IQR). * P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time-point.*

3.3.1. **LDL-C reduction according to diabetes status and quality of glycemic control**

LDL-C levels at baseline of patients with T2D were comparable to those without diabetes. At some points of the analysis (at months 6 and 12), a significantly higher reduction of LDL-C was observed in patients with T2D (Figure 5). Achievement of LDL-C targets <55 mg/dl after 3 months on therapy was numerically but not significantly higher in people with T2D compared to those without: <55 mg/dl: 51% vs. 41.5%; <70 mg/dl 69.4 vs. 58.5% (p=0.119). At 12 months, LDL-C treatment targets were achieved in patients with T2D compared to those without <55 mg/dl: 58.8% vs. 30.1%; <70 mg/dl 70.6 vs. 49.6% (p=0.003). Patients with inadequately controlled T2D (HbA1c > 54 mmol/mol) showed a higher but not statistically significant LDL-C reduction at month 12, than T2D patients with baseline HbA1c ≤54 mmol/mol (p=0.052). Detailed data on LDL-C according to diabetes status are shown in Figure 5 and in Table 11.



	Baseline	3 months	6 months	9 months	12 months
Without diabetes mellitus	141 (117 - 188) (n=175)	62 (44 - 83) (n=171)	61 (44 - 85) (n=154)	62 (44 - 87) (n=132)	71 (49 - 91) (n=113)
With diabetes mellitus type 2	135 (110 - 178) (n=54)	55 (34 - 80) (n=49)	48 (36 - 73) (n=45)	53 (39 - 84) (n=40)	52 (39 - 78) (n=34)
HbA1c ≤ 54 (DM2)	130 (104 - 174) (n=34)	58 (35 - 86) (n=32)	48 (37 - 77) (n=29)	52 (39 – 83) (n=26)	58 (40 - 82) (n=22)
HbA1c > 54 (DM2)	148 (119 - 192) (n=17)	41 (30 - 63) (n=14)	41 (30 - 63) (n=13)	51 (34 - 92) (n=11)	40 (30 - 55) (n=9)

B **LDL-C mg/dl**	**Without T2D n (%)**	**T2D HbA1c ≤54 mmol/mol n (%)**	**T2D HbA1c >54 mmol/mol n (%)**
Baseline	n=175 (100)	n=34 (100)	n=17 (100)
<40 mg/dl	0(0)	0(0)	0(0)
<55 mg/dl	2 (1.1)	0(0)	0(0)
<70 mg/dl	4 (2.3)	1(2.9)	0(0)
Month 3	n=171 (100)	n=32 (100)	n=14 (100)
<40 mg/dl	38 (22.2)	9 (28.1)	7 (50.0)
<55 mg/dl	71 (41.5)	15 (46.9)	9 (64.3)
<70 mg/dl	100 (58.5)	22 (68.8)	11(78.6)
Month 12	n=113 (100)	n=22 (100)	n=9 (100)
<40 mg/dl	16 (14.2)	6 (27.3)	5 (55.6)
<55 mg/dl	34 (30.1)	11 (50.0)	7 (77.8)
<70 mg/dl	56 (49.6)	13 (59.1)	9 (100)

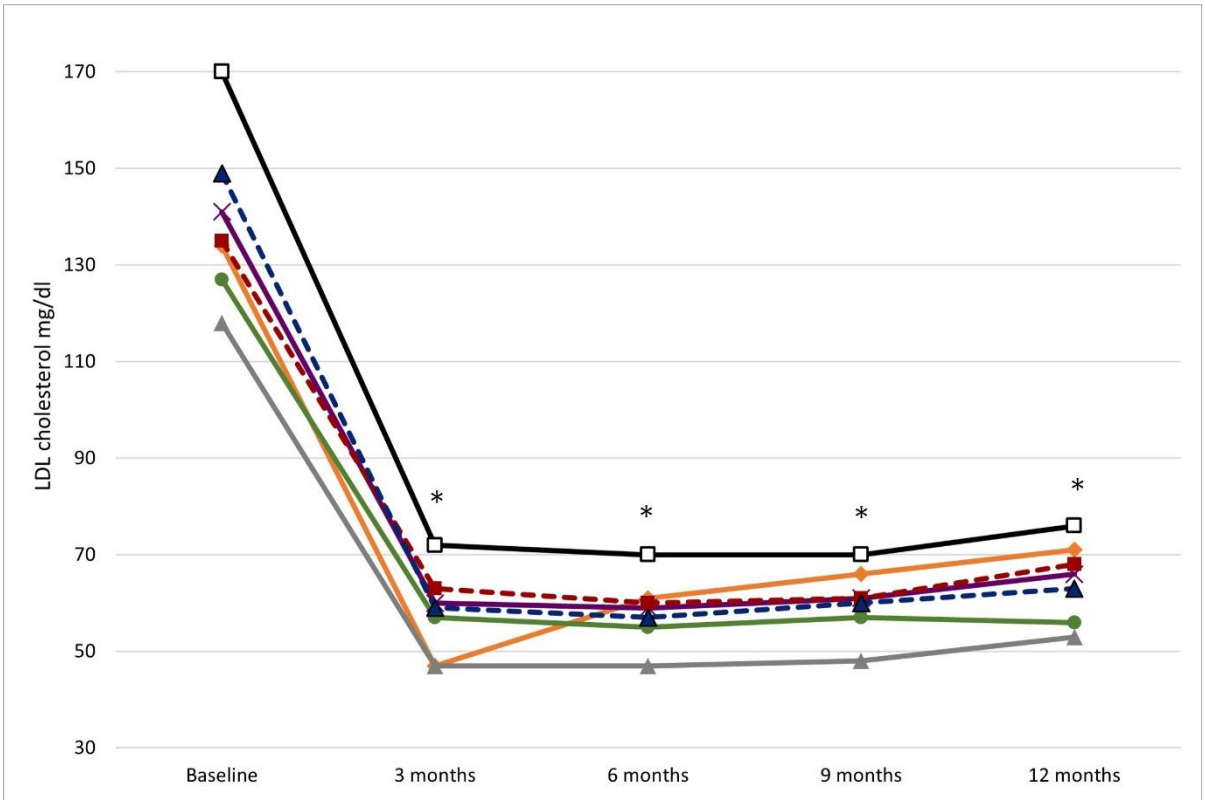
***Figure 5*. A** LDL-C levels of patients without and with T2D 2. **B** Number and percentage of achievement of treatment targets according to diabetes status. Data are median (interquartile range). * Indicates statistical significance when compared to baseline LDL-C levels (for all groups investigated).

	Without diabetes mellitus	T2D	p-value *	T2D HbA1c ≤54	T2D HbA1c >54	p-value *
Baseline	(n=175)	(n=54)		(n=34)	(n=17)	
LDL-C (mg/dl)	141 (117 – 188)	135 (110 – 178)	0.327	130 (104 – 174)	148 (119 – 192)	0.250
Month 3	(n=171)	(n=49)		(n=32)	(n=14)	
LDL-C (mg/dl)	62 (44 – 83)	55 (34 – 80)	0.176	58 (35 – 86)	41 (30 – 63)	0.197
% LDL-C reduction	55.9 (41.3 – 69.4)	62.3 (46.4 – 72.0)	0.239	61.1 (43.0 – 69.4)	70.5 (61.9 – 81.6)	0.025
Month 6	(n=154)	(n=45)		(n=29)	(n=13)	
LDL-C (mg/dl)	61 (44 – 85)	48 (36 – 73)	0.034	48 (37 – 77)	41 (30 – 63)	0.615
% LDL-C reduction	55.4 (44.4 – 68.8)	64.4 (53.9 – 72.0)	0.012	63.8 (54.5 – 68.4)	70.5 (57.7 – 81.3)	0.100
Month 9	(n=132)	(n=40)		(n=26)	(n=11)	
LDL-C (mg/dl)	62 (44 – 87)	53 (39 – 84)	0.215	52 (39 – 83)	51 (34 – 92)	0.740
% LDL-C reduction	55.5 (43.9 – 67.8)	62.6 (48.9 – 71.1)	0.157	62.6 (47.7 – 70.8)	66.4 (54.5 – 75.3)	0.319
Month 12	(n=113)	(n=34)		(n=22)	(n=9)	
LDL-C (mg/dl)	71 (49 – 91)	52 (39 – 78)	0.007	58 (40 – 82)	40 (30 – 55)	0.041
% LDL-C reduction	50.8 (41.3 – 63.6)	64.5 (47.4 – 73.3)	0.010	57.7 (38.2 – 71.1)	66.8 (63.6 – 86.4)	0.041

Table 11. *LDL-C levels over time according to diabetes status. Data are median (IQR). * P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time-point.*

3.3.2. **LDL-C reduction according to adjunct lipid-lowering medication**

Considering additive lipid-lowering medication at baseline, a significant reduction in LDL-C was observed in all groups during all observation points which remained stable over time. The greatest decline was seen in patients who were on concomitant combination therapy of a statin and ezetimibe, while the smallest effect on LDL-C reduction was seen in those who did not have adjunct lipid-lowering therapy apart from the PCSK9 inhibitor therapy (Figure 6).



	Baseline	3 months	6 months	9 months	12 months
PCSK9i only	170 (130 – 203) (n=117)	72 (50 – 100) (n=114)	70 (46 – 101) (n=106)	70 (49 – 98) (n=93)	76 (54 – 104) (n=81)
Statin	134 (115 – 171) (n=26)	47 (32 – 85) (n=26)	61 (39 – 84) (n=23)	66 (46 – 87) (n=19)	71 (42 – 87) (n=13)
Ezetimibe	127 (117 – 158) (n=50)	57 (40 – 74) (n=45)	55 (35 – 69) (n=39)	57 (39 – 68) (n=33)	56 (43 – 68) (n=36)
Statin + ezetimibe	118 (93 – 140) (n=44)	47 (30 – 62) (n=42)	47 (35 – 59) (n=36)	48 (33 – 62) (n=33)	53 (35 – 68) (n=23)
Total	141 (117 – 188) (n=237)	60 (40 – 83) (n=227)	59 (42 – 84) (n=204)	61 (44 – 85) (n=178)	66 (45 – 86) (n=153)
Alirocumab	135 (114 – 181) (n=104)	63 (35 – 87) (n=103)	60 (44 – 83) (n=94)	61 (44 – 87) (n=79)	68 (42 – 88) (n=66)
Evolocumab	149 (118 – 191) (n=133)	59 (41 – 82) (n=124)	57 (40 – 85) (n=110)	60 (43 – 82) (n=99)	63 (47 – 60) (n=87)

Figure 6. LDL-C levels of patients according to lipid-lowering medication at baseline. All patients were on PCSK9 inhibitor therapy; data are median (interquartile range). * Indicates statistical significance when compared to baseline LDL-C levels for all groups investigated.

3.4. Effect on triglyceride levels

Triglyceride (TG) levels slightly decreased from baseline (138 mg/dl) over time: 107 mg/dl (month 3) months, 110 mg/dl (month 6), 111 mg/dl (month 9) and 114 mg/dl (month 12). Median numerical and percentual changes are shown in Table 12 . There was no significance in the TG reduction between alirocumab and evolocumab. Patients with T2D had significantly higher TG levels at baseline compared to those without diabetes (185 and 124 mg/dl; p=0.001), TG reduction during treatment was comparable between patients with and without T2D. Further details related to TG reduction over time considering PCSK9 inhibitor used and diabetes status are demonstrated in Table 12 and in Table 13.

	Overall cohort			**Alirocumab**			**Evolocumab**		
	Triglycerides (mg/dl)	p-value *	% change from baseline	Triglycerides (mg/dl)	p-value *	% change from baseline	Triglycerides (mg/dl)	p-value *	% change from baseline
Baseline	138 (99 – 215) (N=227)		n.a.	138 (102 – 255) (n=102)		n.a.	138 (97 – 197) (n=125)		n.a.
Month 3	107 (77 – 170) (n=218)	<0.001	-19.5 (-36.9 – +1.0)	109 (86 – 169) (n=101)	<0.001	-19.4 (-35.8 – +5.1)	106 (73 – 173) (n=117)	<0.001	-20.0 (-37.9 – 0.0)
Month 6	110 (74 – 181) (n=195)	<0.001	-19.5 (-37.5 – +0.7)	116 (85 – 202) (n=92)	<0.001	-17.7 (-32.3 – 0.0)	106 (70 – 173) (n=103)	<0.001	-22.1 (-41.5 – +2.5)
Month 9	111 (80 – 169) (n=152)	0.004	-16.9 (-38.5 – +3.1)	113 (82 – 189) (n=64)	<0.001	-13.1 (-34.0 – +0.5)	110 (76 – 154) (n=88)	<0.001	-22.6 (-40.2 – +3.8)
Month 12	114 (84 - 176) (n=118)	0.001	-10.4 (-30.8 – +10.3)	121 (73 – 201) (n=41)	0.014	-13.1 (-23.0 – +7.8)	111 (85 – 169) (n=77)	0.019	-8.2 (-31.3 – +11.4)

Table 12. *Triglyceride levels over time of the overall cohort and by PCSK9 inhibitor. Data are median (IQR). * P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time-point.*

	Without diabetes mellitus	T2D	p-value*
Baseline	(n=167)	(n=52)	
Triglycerides (mg/dl)	124 (97 – 199)	185 (134 – 249)	0.001
Month 3	(n=164)	(n=47)	
Triglycerides mg/dl	100 (72 – 160)	161 (106 – 231)	>0.001
% Triglyceride change	-19.7 (-37.0 – -0.8)	-15.3 (-35.3 – +9.8)	0.205
Month 6	(n=146)	(n=44)	
Triglycerides (mg/dl)	101 (69 – 161)	171 (99 – 243)	>0.001
% Triglyceride change	-22.9 (-39.2 – -1.9)	-11.2 (-30.6 – +5.8)	0.073
Month 9	(n=111)	(n=36)	
Triglycerides (mg/dl)	101 (73 – 154)	152 (100 – 216)	0.001
% Triglyceride change	-17.8 (-38.6 – 0.0)	-11.0 (-31.9 – +14.9)	0.140
Month 12	(n=86)	(n=29)	
Triglycerides (mg/dl)	101 (77 – 142)	177 (100 – 231)	>0.001
% Triglyceride change	-13.7 (-31.3 – +5.2)	-1.1 (-25.7 – +27.4)	0.084

Table 13. *Triglyceride levels over time according to diabetes status. Data are median (IQR). * P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time-point.*

3.5. Effect on high-density lipoprotein

In the total population, high-density lipoprotein (HDL) slightly increased from baseline (54 mg/dl) at months 3 and 12 (57 mg/dl), respectively. The HDL increase was not significantly sustained after 12 months in the alirocumab treated group. Patients with T2D had significantly lower HDL levels at baseline when compared to people without T2D. Similar to the overall group, there was a slight increase of HDL in both patients without and with T2D. Further details related to HDL change over time considering PCSK9 inhibitor used and diabetes status are demonstrated in Table 14 and in Table 15.

	Overall cohort			Alirocumab			Evolocumab		
	HDL cholesterol (mg/dl)	p-value *	% change from baseline	HDL cholesterol (mg/dl)	p-value *	% change from baseline	HDL cholesterol (mg/dl)	p-value *	% change from baseline
Baseline	54 (45 – 65) (n=226)		n.a.	52 (44 – 63) (n=100)		n.a.	55 (46 – 67) (n=126)		n.a.
Month 3	57 (48 – 68) (n=217)	<0.001	+6.5 (-4.0 – +16.5)	56 (46 – 67) (n=98)	0.007	+6.1 (-5.0 – +15.2)	59 (50 – 71) (n=119)	<0.001	+6.7 (-2.5 – +18.5)
Month 6	57 (47 – 69) (n=191)	<0.001	+8.9 (-3.5 – +16.3)	55 (45 – 66) (n=87)	<0.001	+9.8 (-3.2 – +16.0)	60 (48 – 71) (n=104)	<0.001	+8.5 (-4.1 – +17.9)
Month 9	58 (48 – 70) (n=154)	<0.001	+8.5 (-3.7 – +18.4)	56 (49 – 68) (n=66)	<0.001	+9.8 (-3.0 – +18.0)	59 (47 – 71) (n=88)	0.001	+8.0 (-4.1 – +20.0)
Month 12	57 (49 – 69) (n=121)	0.010	+3.8 (-4.6 – +16.7)	59 (51 – 68) (n=45)	0.156	+3.4 (-4.5 – +14.2)	55 (48 – 71) (n=76)	0.028	+4.3 (-4.8 – +18.0)

Table 14. *HDL cholesterol levels over time of the overall cohort and by PCSK9 inhibitor agent. Data are median (IQR). * P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time-point.*

	Without diabetes mellitus	T2D	p-value*
Baseline	(n=169)	(n=49)	
HDL-C (mg/dl)	54 (46 – 67)	49 (42 – 60)	0.027
Month 3	(n=166)	(n=44)	
HDL-C (mg/dl)	59 (49 – 71)	52 (46 – 62)	0.008
% HDL change	+7.3 (-3.6 – +16.2)	+4.2 (-6.9 – +19.1)	0.365
Month 6	(n=146)	(n=40)	
HDL-C (mg/dl)	58 (47 – 70)	52 (47 – 67)	0.232
% HDL change	+9.1 (-4.0 – +16.0)	+7.5 (-1.3 – +20.4)	0.600
Month 9	(n=115)	(n=35)	
HDL-C (mg/dl)	58 (48 – 71)	52 (43 – 68)	0.192
% HDL change	+9.5 (-4.3 – +18.4)	+6.5 (-2.3 – +20.5)	0.862
Month 12	(n=91)	(n=28)	
HDL-C (mg/dl)	58 (50 – 69)	53 (43 – 69)	0.200
% HDL change	+2.9 (-5.0 – +15.1)	+5.5 (-1.3 – +17.9)	0.313

Table 15. *HDL cholesterol levels over time according to diabetes status. Data are median (IQR). * P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time-point.*

3.6. Effect on Lipoprotein (a)

Follow-up data on lipoprotein (a) [Lp(a)] was only available in 26 patients, due to infrequent measurements in routine care based on current guideline recommendations (single measurement recommended at least once in a lifetime, not subsequent)[82]. Lp(a) decreased from 67 mg/dl at baseline to 55 mg/dl at the first follow-up measurement (after median 95 days [28 – 190] from baseline) in these patients (Table 16). The significant decrease of Lp(a) shown for patients without diabetes, could not be confirmed in people with T2D (only data from 5 patients available).

	Lp(a) at baseline (mg/dl)	Lp(a) follow-up † (mg/dl)	p-value*	% change from baseline
Overall cohort (n=26)	67 (37 – 127)	55 (34 – 96)	<0.001	-14.7 (-44.8 – -2.6)
Alirocumab (n=7)	105 (90 – 163)	81 (69 - 110	0.028	-28.2 (-43.6 – -3.5)
Evolocumab (n=19)	56 (25 – 80)	51 (17 – 92)	0.004	-14.4 (-48.5 – +0.2)
Without diabetes mellitus (n=21)	67 (43 – 151)	63 (43 – 115)	0.001	-15.0 (-38.0 – -4.9)
Diabetes mellitus type 2 (n=5)	51 (26 – 91)	42 (13 – 66)	0.225	-13.9 (-65.3 – +9.0)

Table 16. *Lipoprotein(a) levels and follow-up measurement of the overall cohort, by PCSK9 inhibitor agent and by diabetes status. Data are median (IQR). *P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time. Only patients with follow-up data of Lp(a) were included in the analysis. † First performed follow-up measurement after initiation of PCSK9 inhibitor therapy was included in the analysis.*

3.7. Effects on HbA1c

The baseline HbA1c of the overall cohort was 41 (37-49) mmol/mol and did not significantly change during the observation period of up to 18 months. Also, in patients with T2D (baseline HbA1c 52 [48-57] mmol/mol) no significant changes of HbA1c were found over time, as illustrated in Table 17.
Adjustments of the antidiabetic therapy during the study period occurred in 15 patients (T2D, n=14, T1D, n=1). Drug treatment intensifications were performed in 9 subjects: new or additional oral antidiabetic drug (OAD) or Glucagon-like peptide 1 receptor agonist (GLP1- RA) in 3 patients, increase of OAD dose (n=1), increase of insulin dose in 4 patients (n=3 in T2D, n=1 in T1D), additional OAD plus increase of insulin dose (n=1). A switch from one class of OAD to another was made in five patients. A reduction of insulin dose occurred in one patient.

	Overall cohort		Without diabetes mellitus		Diabetes mellitus any type		T2D	
	HbA1c (mmol/mol)	p-value *	HbA1c (mmol/mol)	p-value *	HbA1c (mmol/mol)	p-value *	HbA1c (mmol/mol)	p-value *
Baseline	41 (37 – 49) (n=139)		38 (36 – 40) (n=81)		52 (48 – 59) (n=58)		52 (48 – 57) (n=51)	
Month 3	42 (37 – 52) (n=97)	0.876	38 (36 – 40) (n=51)	0.204	52 (47 – 63) (n=46)	0.625	52 (45 – 62) (n=40)	0.539
Month 6	42 (37 – 52) (n=98)	0.540	38 (36 – 40) (n=53)	0.054	53 (47 – 62) (n=45)	0.702	52 (47 – 61) (n=40)	0.826
Month 9	41 (37 – 51) (n=78)	0.110	38 (35 – 39) (n=43)	0.052	52 (47 – 57) (n=36)	0.333	51 (46 – 57) (n=32)	0.454
Month 12	42 (37 – 49) (n=61)	0.083	37 (35 – 39) (n=31)	0.505	49 (45 – 56) (n=30)	0.054	47 (43 – 57) (n=26)	0.215

Table 17. *HbA1c levels over time of the overall cohort, without and with diabetes mellitus at baseline. Data are median (IQR). * P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time-point.*

3.8. Cardiovascular outcomes

A total of 42 cardiovascular events in 36 patients were observed during the 18 months observation period; 12 of those were patients with T2D. A composite of major adverse cardiac events (MACE) including non-fatal myocardial infarction (ST- segment elevation myocardial infarction [STEMI] or non-ST- segment elevation myocardial infarction [NSTEMI]), indication of urgent need for coronary intervention, non-fatal stroke or TIA, and cardiovascular death occurred 15 times in 13 patients, four of those with T2D. Patients with insufficiently controlled T2D (HbA1c >54 mmol/mol) experienced a higher risk of cardiovascular events (HR: 5.1 [2.2-12.4]; p<0.001) when compared to patients without diabetes mellitus. The total event-rate in those with well controlled T2D (HbA1c ≤54 mmol/mol) not was comparable (HR: 1.5 [0.6-4.1; p=0.410]) to those without diabetes mellitus. The MACE did not significantly differ between those with and without T2D (HR: 2.3 [0.6-7.9]; p=0.212). Kaplan-Meier survival curves indicating event-free and MACE-free survival are shown in Figure 7.

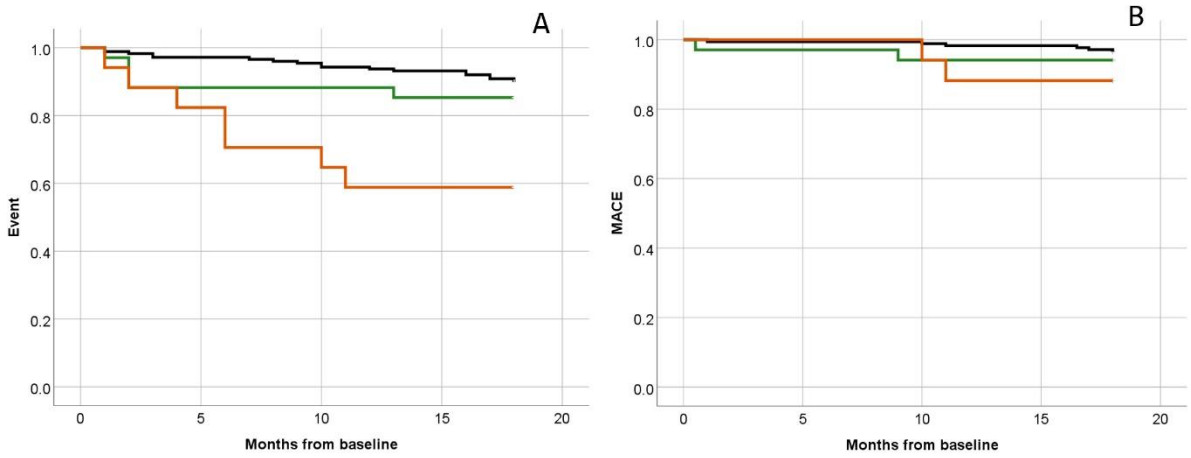


Figure 7. **A** Event-free and **B** MACE-free survival curves of patients without diabetes mellitus (black line), with T2D and baseline HbA1c ≤54 mmol/mol (green line), and with T2D and baseline HbA1c of >54 mmol/mol (orange line). MACE was defined as composite endpoint including non-fatal myocardial infarction (STEMI or NSTEMI), indication of urgent need for coronary intervention, non-fatal stroke or TIA, and cardiovascular death.

3.9. Therapy adjustments, discontinuation, adverse effects

In nine patients the PCSK9 inhibitor agent was altered over time (i.e. change from one agent to the other). Seven patients discontinued PCSK9 inhibitor treatment due to side-effects (mostly because of joint or muscle pain, skin lesions or pruritus, or gastrointestinal symptoms), one because of insufficient response to PCSK9 inhibitor therapy, one did not fulfill the criteria for health insurance coverage of PCSK9 inhibitor treatment (ongoing smoking) and one discontinued by the patient's own request. One patient with T1D reported on elevated insulin requirements and mild ketoacidosis after 4 months of PCSK9 inhibitor therapy leading to cessation of treatment.

Adverse effects of PCSK9 inhibitor therapy were documented in 27 patients. The majority of side effects was reported from patients without concomitant lipid lowering therapy (20 patients; 74%); all of the reported effects were of mild quality. The most frequent side effects were joint or muscle pain, rhinitis, flu-like symptoms, fatigue, skin lesions or pruritus. Side effects associated with the PCSK9 inhibitor therapy and the motivations for therapy adjustments are shown in Table *18*.

Adverse effect documented in patients record	
Joint or muscle pain	10
Rhinitis	5
Flu-like symptoms	5
Fatigue	4
Skin lesions or pruritus	4
Gastrointestinal symptoms	2
Insomnia	1
Elevated insulin requirements	1
Cause leading to PCSK9 inhibitor therapy adjustment (switch of PCSK9 inhibitor agent)	
Adverse effect	8
Joint or muscle pain	6
Flu-like symptoms	3
Fatigue	1
Gastrointestinal symptoms	1
Dose reduction (evolocumab 140 mg to alirocumab 75 mg)	1
Cause leading to PCSK9 inhibitor therapy discontinuation	
Adverse effect	7
Joint or muscle pain	3
Skin lesions or pruritus	2
Gastrointestinal symptoms	1
Adverse effects not specified	1
Elevated insulin requirement	1
Non-responder (clearly documented)	1
Reasons of insurance coverage	1
By patient's request	1

Table 18. Side effects of PCSK9 inhibitor therapy reported by patients, cause of therapy adjustments and discontinuation. Data are number of patients. Patients could have more than one symptom.

4 DISCUSSION

This real-world data analysis evaluated data from patients in a tertiary center who received PCSK9 inhibitors for the treatment of hypercholesterolemia. Within this setting PCSK9 inhibitors were mainly prescribed in secondary prevention for patients with established cardiovascular disease and multiple cardiovascular risk factors. Statin intolerance and side-effects of ezetimibe was reported in the majority of cases (83.1% and 44.7, respectively) and appears to be a main driver for prescription of PCSK9 inhibitor therapy. This frequent intolerance to these first-line agents explains why about half of the patients had no lipid-lowering medication on board at the time of the first prescription of a PCSK9 inhibitor.

In our investigation, the significant reduction of LDL-C levels which was comparable with both agents after only a few weeks underlines the effective potential of PCSK9 inhibitor drugs as lipid-lowering therapy. RCTs like the FOURIER and ODYSSEY OUTCOMES trials showed impressive results in lowering LDL-C. As compared to our study, in the FOURIER trial, substantially more patients achieved LDL-C levels <70 mg/dl and <40 mg/dl (87% and 67% vs. 56.2% and 19.6% in our study). In both RCTs a reduction to even lower LDL-C levels was achieved (LDL-C of 30 mg/dl after 48 weeks in the FOURIER trial; LDL-C of 48 mg/dl after 12 months in the ODYSSEY OUTCOMES trial vs. LDL-C of 66 mg/dl after 12 months in our study). However, it should be considered that the baseline LDL-C was considerably lower in these RCTs, and that a majority of patients were on concomitant statin therapy (FOURIER: 69.3%; ODYSSEY: 88.8%) with or without ezetimibe (FOURIER: 5.2%; ODYSSEY: 2.9%), which may explain the differences to our data[83, 84]. In routine care, and also as shown by our analysis, there is a high number of patients on PCSK9 inhibitor therapy without concomitant lipid-lowering therapy. In many cases this is due to intolerance against the first-line therapy. This condition thus hinders further intensification of lipid-lowering therapy aside from PCSK9 inhibitor therapy. Within clinical studies, nutrition and adherence to prescribed medication are intensely monitored; this might positively impact LDL-C reductions seen in RCTs and might result in a lesser effect LDL-C reduction by PCSK9 inhibitors in everyday use.

Of note, our population differed from the patients in the ODYSSEY OUTCOMES study, as in this study only patients with recent cardiovascular events (<12 months) were included[84]. History of cardiovascular diseases was frequent in our patients, but a recent or previous cardiovascular event was not a mandatory criterion to be eligible for analysis.

Our study showed a similar percentage of LDL-C reduction reported in a systematic review on PCSK9 inhibitors (LDL-C reduction of 53.86% at month 6) [72]. Real-world studies also observed inter-individual

reduction of LDL-C and postulated that LDL-C goals cannot be reached in all patients [85-87]. This should be considered in the PCSK9 inhibitor treatment in clinical practice. Achievement of treatment goals should be regularly monitored in routine care with special attention to treatment adherence and therapy intensification, if possible.

In a prespecified analysis of the ODYSSEY OUTCOMES study comparing the effect of alirocumab on patients with diabetes, prediabetes and normoglycemia, no significant differences in LDL-C reduction were found across these subgroups (median LDL-C reduction of 64 - 65% at month 4)[88]. This finding was confirmed in another sub-cohort of the ODYSSEY collective [89]. In a sub-study of the FOURIER study, no differences in LDL-C reduction were seen when evolocumab was used in patients with and without diabetes (-57% in patients with and - 60% in patients without diabetes) [90]. In our study, PCSK9 inhibitor therapy markedly lowered LDL-C in both patients with and without diabetes irrespective of glycemic control. Both, the FOURIER and the ODYSSEY OUTCOMES trial found a more pronounced absolute risk reduction of a composite of major cardiovascular events in patients with diabetes mellitus receiving PCSK9 inhibitors compared to those without diabetes [88, 90]. Patients with T2D are at an extraordinary increased cardiovascular risk. Therefore, patient with T2D and hypercholesterinemia especially benefit in particular from an intensified lipid-lowering treatment.

Clinical trials and genetic studies have ascertained a potential link between the use of lipid-lowering therapy and an increased risk for deterioration of glucose control and the development of T2D. In statin users the risk to develop T2D was estimated to be approximately 1:1.000 per year of exposure and patients with additional risk factors to develop T2D (e.g. age, prediabetes, metabolic syndrome) were at an even increased risk [40]. A Chinese study reported on the association between elevated circulating PCSK9 levels and an increased risk for T2D in women with prediabetes [91].

Schmidt et al. investigated the relation of PCSK9 gene variants and glycemic parameters in a mendelian study, suggesting a potential risk for new-onset diabetes in patients on PCSK9 inhibitor treatment. Mimicking the pharmacological effects of PCSK9 inhibition, this study found increased glucose concentrations and an increased risk of T2D in carriers of PCSK9 variants associated with low LDL-C levels. However, these findings represent the life-long effect of these gene variants and may not reflect the pharmacological PCSK9-targeted intervention later in life [71].

No significant changes on patients' Hb1Ac levels were found in our study, in both patients with and without preexisting diabetes. A limitation of our study was that only in a small number of patients all investigated parameters were available at all time-points. Nevertheless, the results are consistent with findings from past studies, which showed no relevant changes in glycemic control during PCSK9 inhibitor therapy [92-96].

In our study we observed adjustments and intensifications of diabetes therapy when required. Of note, due to refund claims by the Austrian health insurance only patients with sufficiently controlled diabetes (HbA1c <64 mmol/mol (8.0%)) are eligible for PCSK9 inhibitor therapy. Therefore, this study cannot provide data on potential effects of PCSK9 inhibitor therapy on glucose control, LDL-C and other parameters of the lipid panel as well as cardiovascular outcome in patients with insufficiently controlled diabetes mellitus.

While there is a plenty of observational data available demonstrating a reduction of cardiovascular outcomes by therapeutically reduce LDL-C [97, 98], to the best of our knowledge there is no real-world evidence available investigating these potentially beneficial effects when populations with PCSK9 inhibitor therapy were selected.

It has been shown that low PCSK9 levels are associated with the presence of metabolic syndrome and atherosclerosis in people with coronary heart disease[99] and recently, a study conducted by Peng et al described an important relationship between elevated circulating PCSK9 levels to be associated with higher cardiovascular morbidity and adverse cardiovascular outcomes specifically in people with diabetes[100]. The authors suggest to measure PCSK9 levels in people with diabetes to identify the ones with particularly high cardiovascular risk, indicating that these people might be the ones who might even profit from lower LDL-targets (e.g. <40 mg/dl) established by therapeutic PCSK9 inhibition[100, 101]. Within our population, 42 cardiovascular events of any kind during an 18 months observation, of those 15 events met the criteria to be characterized as a major adverse cardiovascular outcome event (MACE). Noteworthy, we have found significantly more all cause events in patients with T2D at baseline and intriguingly, despite a higher LDL reduction than the non-diabetic population those patients with T2D with HbA1c >54 mmol/mol experienced a 5-fold higher risk of any cardiovascular event compared to those without diabetes. This finding emphasizes diabetes to be a further potent cardiovascular risk factor, specifically when insufficiently controlled which needs to be accordingly paid attention.

In our analysis we observed a reduction in triglyceride levels of about 20% throughout the observational period. This finding has to be interpreted with caution as patients do not mandatorily need to be in a fasting state for blood sampling in our clinic. Still, this result is in-line with previous research indicating a 12-30% reduction of triglycerides due to PCSK9 inhibitor therapy[102, 103]. In our population, this finding might be attributed to various factors: first, due to the national reimbursement criteria, all patients must receive professional nutritional advice prior to initiation of PCSK9 inhibitor therapy. Secondly, previous data suggests that intra- and extracellular PCSK9 might act in a complementary fashion to regulate triglyceride levels, on the one hand, intracellularly, by the ability of PCSK9 to modulate apolipoprotein B (APOB) secretion and on the other hand, extracellularly, by

triggering LDL- receptor mediated catabolism[104]. Further investigations, focusing on the physiologic mechanisms which might explain the positive correlation of PCSK9 inhibitor therapy on triglyceride levels are in progress.

A further well-known cardiovascular risk factor is lipoprotein(a) [Lp(a)]. At present there are no approved medical agents to reduce Lp(a) available. Lp(a) currently can be lowered only by means of lipid apheresis and to some extent by dietary interventions [82]. In our study Lp(a) data over time are only available to a very limited extent. This is caused by current guidelines that suggest only a single Lp(a) determination to determine whether lipid apheresis might be necessary[82]. In secondary analyses of the FOURIER and the ODYSSEY OUTCOMES trials, alirocumab and evolocumab demonstrated only a small reduction (-20%) of Lp(a) [59, 105], which is similar to the results seen in our study. A novel and not yet approved medical approach (antisense-oligonucleotide therapy) might lead to clinically relevant Lp(a) reduction in the future [106].

In our analysis, adverse effects of alirocumab or evolocumab were rare. Since the documented symptoms stated by the patients were mainly unspecific (e.g. joint or muscle pain, rhinitis), it remains speculative whether they were associated with PCSK9 inhibitor therapy. Most of the adverse events leading to discontinuation of PCSK9 inhibitor therapy occurred in patients without concomitant lipid-lowering therapy. Thus, the potential side effect can probably truly be attributed to the PCSK9 inhibitor therapy. The adverse effects described in our study are similar to those found in other studies [61, 69, 83, 84]. Overall, PCSK9 inhibitors appear to be well tolerated, although long-term data on adverse health effects are not available yet.

The strength of this retrospective study is the large sample size of 237 patients. Many real-world studies available have investigated only one of the two agents and were mainly sponsor-initiated studies[107]. Furthermore, real-world evidence is substantially lacking investigating the efficacy of PCSK9 inhibitor therapy on LDL-C reduction in patients with diabetes. In our study we describe for the first time, that patients with insufficiently controlled T2D might have a superior LDL-C reduction by PCSK9 inhibitor therapy compared to patients without diabetes mellitus. This finding needs to be addressed in prospective randomized controlled clinical trials. Additionally, special attention should be given to patients with insufficiently controlled T2D as glycemic control seems to have a relevant strong impact on cardiovascular events despite achieving LDL-C treatment goals. Also, insufficiently controlled T2D as glycemic control seems to have a relevant strong impact on cardiovascular events despite achieving LDL-C treatment goals. This finding might be attributed to the fact that in our population the proportion of patients on sodium glucose co-transporters 2 (SGLT2) inhibitors [108-110] and GLP1-RA [111,

[112] was rather low at baseline. Both agents have demonstrated a beneficial effect on cardiovascular outcome in patients with T2D. The broad uptake of these medications into routine care only occurred in more recent years following guideline recommendations [113]. In the beginning these agents were mainly prescribed by diabetologists and endocrinologists whereas a large proportion of our patients were seen by a GP for diabetes care until the first presentation at our center. Potential beneficial cardiovascular effects might be expected in patients with diabetes receiving both PCSK9 inhibitor therapy as lipid-lowering agent and SGLT2 inhibitors and/or GLP1-RA for diabetes management.

By the retrospective nature of the study several limitations have to be addressed. The major limitation is the varying availability of data, especially laboratory data. This is due to the fact that in routine clinical care, no stringent protocol as in a clinical trial is applied. Many of our patients performed the laboratory analysis with their GP who might only chose a limited laboratory panel than recommended in our patient letters. Additionally, follow-up visits in many cases are performed via telemedical methods (i.e. video call, telephone call) which has a negative impact on availability of laboratory measurements or further baseline characteristics such as BMI or blood pressure.

There was a relatively large number of patients (11.4%; n=27) who were lost to follow-up (LTF). This might result in some selection bias and thus can impact the results. One can assume that more compliant and adherent patients thus are represented by our data. Also, no telephone follow-up was performed due to the nature of the study. So, there might even have been a fatal event in one of patients LTF, as only fatalities within the Styrian hospital cluster (KAGES) and adjacent hospitals would have been noticed.

Additionally, concurrent lipid-lowering medication was ascertained only at baseline and potential adjustments were not considered in the analysis. Further, adherence to lifestyle recommendations were not systematically documented. As all patients who are prescribed with and reimbursed for PCSK9 inhibitor therapy obtain professional nutritional advice, the effect of this training should be evenly distributed among the investigated population.

Another limitation is the lack of continuous documentation of further cardiovascular risk factors such as body weight, body mass index or blood pressure. This is due to the fact that the enrolled patients presented in a specialized clinic for lipid metabolism disorders which focuses their recommendations on metabolic disease (lipids and diabetes); other cardiovascular risk factors are adjusted elsewhere.

5 CONCLUSIONS

In conclusion, significant reductions in LDL-C and a high percentage of patients achieving the recommended treatment targets were observed in a real-world population over the course of 18 months. Interestingly, patients with T2D, and specifically those with insufficiently controlled T2D showed a superior response to PCSK9 inhibitor therapy. Still, they experienced a substantial number of cardiovascular events. Thus, efforts also must be made to achieve better glycemic control. Even when receiving PCSK9 inhibitor therapy, currently the most potent lipid-lowering agent, some patients did not meet LDL-C treatment goals as recommended in current guidelines. Special attention to these patients is required in the future to achieve these goals to avoid subsequent adverse cardiovascular outcomes.

6 REFERENCES

1. Joint F. Fats and fatty acids in human nutrition. Report of an expert consultation, 10-14 November 2008, Geneva. 2010.
2. Leipziger J. Fette. In: Behrends J, Bischofberger J, Deutzmann R, Ehmke H, Frings S, Grissmer S, et al., editors. Duale Reihe Physiologie. 3., vollständig überarbeitete Auflage ed: Georg Thieme Verlag; 2016.
3. Silbernagl S, Despopoulos jr A, Draguhn A. Ernährung, Verdauung. In: Silbernagl S, Despopoulos jr A, Draguhn A, editors. Taschenatlas Physiologie. 9., vollständig überarbeitete Auflage ed: Georg Thieme Verlag; 2018.
4. Gekle M. Dünn- und Dickdarm: Nährstoffverdauung und -absorption. In: Pape H-C, Kurtz A, Silbernagl S, editors. Physiologie. 9., vollständig überarbeitete Auflage ed: Georg Thieme Verlag; 2019.
5. Gekle M. Physiologie der Leber. In: Pape H-C, Kurtz A, Silbernagl S, editors. Physiologie. 9., vollständig überarbeitete Auflage ed: Georg Thieme Verlag; 2019.
6. Feingold KR, Grunfeld C. Introduction to lipids and lipoproteins. endotext [internet]: MDText. com, Inc.; 2018.
7. Heller DA, de Faire U, Pedersen NL, Dahlen G, McClearn GE. Genetic and Environmental Influences on Serum Lipid Levels in Twins. New England Journal of Medicine. 1993;328(16):1150-6.
8. Fredrickson DS, Lees RS. A SYSTEM FOR PHENOTYPING HYPERLIPOPROTEINEMIA. Circulation. 1965;31:321-7.
9. Memorandum W. Classification of hyperlipidemias and hyperlipoproteinemias. Circulation. 1972;45:501-8.
10. Cuchel M, Bruckert E, Ginsberg HN, Raal FJ, Santos RD, Hegele RA, et al. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. European Heart Journal. 2014;35(32):2146-57.
11. Lagace TA. PCSK9 and LDLR degradation: regulatory mechanisms in circulation and in cells. Curr Opin Lipidol. 2014;25(5):387-93.
12. Akioyamen LE, Genest J, Shan SD, Reel RL, Albaum JM, Chu A, et al. Estimating the prevalence of heterozygous familial hypercholesterolaemia: a systematic review and meta-analysis. BMJ Open. 2017;7(9):e016461.
13. Taghizadeh E, Mardani R, Rostami D, Taghizadeh H, Bazireh H, Hayat SMG. Molecular mechanisms, prevalence, and molecular methods for familial combined hyperlipidemia disease: A review. Journal of cellular biochemistry. 2019;120(6):8891-8.
14. Hegele RA, Ginsberg HN, Chapman MJ, Nordestgaard BG, Kuivenhoven JA, Averna M, et al. The polygenic nature of hypertriglyceridaemia: implications for definition, diagnosis, and management. Lancet Diabetes Endocrinol. 2014;2(8):655-66.
15. Smelt A, De Beer F, editors. Apolipoprotein E and familial dysbetalipoproteinemia: clinical, biochemical, and genetic aspects. Seminars in vascular medicine; 2004: Copyright© 2004 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New ….
16. Baass A, Paquette M, Bernard S, Hegele RA. Familial chylomicronemia syndrome: an under-recognized cause of severe hypertriglyceridaemia. Journal of Internal Medicine. 2020;287(4):340-8.
17. von Eckardstein A. Differential diagnosis of familial high density lipoprotein deficiency syndromes. Atherosclerosis. 2006;186(2):231-9.
18. Borén J, Chapman MJ, Krauss RM, Packard CJ, Bentzon JF, Binder CJ, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2020;41(24):2313-30.
19. Borén J, Williams KJ. The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. Curr Opin Lipidol. 2016;27(5):473-83.

20. Borén J, Chapman MJ, Krauss RM, Packard CJ, Bentzon JF, Binder CJ, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2020;41(24):2313-30.
21. Gutstein DE, Fuster V. Pathophysiology and clinical significance of atherosclerotic plaque rupture. Cardiovascular Research. 1999;41(2):323-33.
22. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017;38(32):2459-72.
23. Camont L, Chapman MJ, Kontush A. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. Trends Mol Med. 2011;17(10):594-603.
24. Chapman MJ, Ginsberg HN, Amarenco P, Andreotti F, Borén J, Catapano AL, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. European Heart Journal. 2011;32(11):1345-61.
25. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk: The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). European Heart Journal. 2019;41(1):111-88.
26. Eckel RH, Jakicic JM, Ard JD, Jesus JMd, Miller NH, Hubbard VS, et al. 2013 AHA/ACC Guideline on Lifestyle Management to Reduce Cardiovascular Risk. Journal of the American College of Cardiology. 2014;63(25_Part_B):2960-84.
27. Mente A, de Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Arch Intern Med. 2009;169(7):659-69.
28. Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, et al. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. New England Journal of Medicine. 2018;378(25):e34.
29. Nocon M, Hiemann T, Müller-Riemenschneider F, Thalau F, Roll S, Willich SN. Association of physical activity with all-cause and cardiovascular mortality: a systematic review and meta-analysis. European journal of cardiovascular prevention and rehabilitation. 2008;15(3):239-46.
30. Powell-Wiley TM, Poirier P, Burke LE, Després J-P, Gordon-Larsen P, Lavie CJ, et al. Obesity and cardiovascular disease: a scientific statement from the American Heart Association. Circulation. 2021;143(21):e984-e1010.
31. Poobalan A, Aucott L, Smith WCS, Avenell A, Jung R, Broom J, et al. Effects of weight loss in overweight/obese individuals and long-term lipid outcomes – a systematic review. Obesity Reviews. 2004;5(1):43-50.
32. Cornelissen VA, Fagard RH. Effects of endurance training on blood pressure, blood pressure–regulating mechanisms, and cardiovascular risk factors. Hypertension. 2005;46(4):667-75.
33. Boulé NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of Exercise on Glycemic Control and Body Mass in Type 2 Diabetes MellitusA Meta-analysis of Controlled Clinical Trials. JAMA. 2001;286(10):1218-27.
34. LeBlanc ES, Patnode CD, Webber EM, Redmond N, Rushkin M, O'Connor EA. Behavioral and Pharmacotherapy Weight Loss Interventions to Prevent Obesity-Related Morbidity and Mortality in Adults: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2018;320(11):1172-91.
35. McKenney JM. Pharmacologic characteristics of statins. Clin Cardiol. 2003;26(4 Suppl 3):Iii32-8.
36. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;139(25):e1082-e143.

37. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet. 2005;366(9493):1267-78.
38. Mihaylova B, Emberson J, Blackwell L, Keech A, Simes J, Barnes EH, et al. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. Lancet. 2012;380(9841):581-90.
39. Stroes ES, Thompson PD, Corsini A, Vladutiu GD, Raal FJ, Ray KK, et al. Statin-associated muscle symptoms: impact on statin therapy—European Atherosclerosis Society Consensus Panel Statement on Assessment, Aetiology and Management. European Heart Journal. 2015;36(17):1012-22.
40. Mach F, Ray KK, Wiklund O, Corsini A, Catapano AL, Bruckert E, et al. Adverse effects of statin therapy: perception vs. the evidence – focus on glucose homeostasis, cognitive, renal and hepatic function, haemorrhagic stroke and cataract. European Heart Journal. 2018;39(27):2526-39.
41. Davis HRJ, Tershakovec AM, Tomassini JE, Musliner T. Intestinal sterol transporters and cholesterol absorption inhibition. Curr Opin Lipidol. 2011;22(6):467-78.
42. Zhan S, Tang M, Liu F, Xia P, Shu M, Wu X. Ezetimibe for the prevention of cardiovascular disease and all-cause mortality events. Cochrane Database of Systematic Reviews. 2018(11).
43. Pandor A, Ara RM, Tumur I, Wilkinson AJ, Paisley S, Duenas A, et al. Ezetimibe monotherapy for cholesterol lowering in 2,722 people: systematic review and meta-analysis of randomized controlled trials. J Intern Med. 2009;265(5):568-80.
44. Morrone D, Weintraub WS, Toth PP, Hanson ME, Lowe RS, Lin J, et al. Lipid-altering efficacy of ezetimibe plus statin and statin monotherapy and identification of factors associated with treatment response: A pooled analysis of over 21,000 subjects from 27 clinical trials. Atherosclerosis. 2012;223(2):251-61.
45. Rossebø AB, Pedersen TR, Boman K, Brudi P, Chambers JB, Egstrup K, et al. Intensive Lipid Lowering with Simvastatin and Ezetimibe in Aortic Stenosis. New England Journal of Medicine. 2008;359(13):1343-56.
46. Reiner Z. Combined therapy in the treatment of dyslipidemia. Fundam Clin Pharmacol. 2010;24(1):19-28.
47. Staels B, Kuipers F. Bile Acid Sequestrants and the Treatment of Type 2 Diabetes Mellitus. Drugs. 2007;67(10):1383-92.
48. Jacobson TA, Ito MK, Maki KC, Orringer CE, Bays HE, Jones PH, et al. National lipid association recommendations for patient-centered management of dyslipidemia: part 1--full report. J Clin Lipidol. 2015;9(2):129-69.
49. Ross S, D'Mello M, Anand SS, Eikelboom J, Stewart AF, Samani NJ, et al. Effect of Bile Acid Sequestrants on the Risk of Cardiovascular Events: A Mendelian Randomization Analysis. Circ Cardiovasc Genet. 2015;8(4):618-27.
50. Scaldaferri F, Pizzoferrato M, Ponziani FR, Gasbarrini G, Gasbarrini A. Use and indications of cholestyramine and bile acid sequestrants. Intern Emerg Med. 2013;8(3):205-10.
51. Fazio S, Linton MF. The role of fibrates in managing hyperlipidemia: mechanisms of action and clinical efficacy. Curr Atheroscler Rep. 2004;6(2):148-57.
52. Jakob T, Nordmann AJ, Schandelmaier S, Ferreira-González I, Briel M. Fibrates for primary prevention of cardiovascular disease events. Cochrane Database Syst Rev. 2016;11(11):Cd009753.
53. Wang D, Liu B, Tao W, Hao Z, Liu M. Fibrates for secondary prevention of cardiovascular disease and stroke. Cochrane Database Syst Rev. 2015;2015(10):Cd009580.
54. Ginsberg HN, Elam MB, Lovato LC, Crouse JR, 3rd, Leiter LA, Linz P, et al. Effects of combination lipid therapy in type 2 diabetes mellitus. N Engl J Med. 2010;362(17):1563-74.
55. Davidson MH, Armani A, McKenney JM, Jacobson TA. Safety considerations with fibrate therapy. Am J Cardiol. 2007;99(6a):3c-18c.
56. Eknoyan G, Lameire N, Eckardt K, Kasiske B. KDIGO clinical practice guideline for lipid management in chronic kidney disease. Kidney Int Suppl. 2013;3(3):259-305.
57. Beglova N, Blacklow SC. The LDL receptor: how acid pulls the trigger. Trends Biochem Sci. 2005;30(6):309-17.

58. Schulz R, Schlüter KD, Laufs U. Molecular and cellular function of the proprotein convertase subtilisin/kexin type 9 (PCSK9). Basic Res Cardiol. 2015;110(2):4.
59. Kastelein JJ, Ginsberg HN, Langslet G, Hovingh GK, Ceska R, Dufour R, et al. ODYSSEY FH I and FH II: 78 week results with alirocumab treatment in 735 patients with heterozygous familial hypercholesterolaemia. Eur Heart J. 2015;36(43):2996-3003.
60. Ginsberg HN, Rader DJ, Raal FJ, Guyton JR, Baccara-Dinet MT, Lorenzato C, et al. Efficacy and safety of alirocumab in patients with heterozygous familial hypercholesterolemia and LDL-C of 160 mg/dl or higher. Cardiovascular drugs and therapy. 2016;30(5):473-83.
61. Kereiakes DJ, Robinson JG, Cannon CP, Lorenzato C, Pordy R, Chaudhari U, et al. Efficacy and safety of the proprotein convertase subtilisin/kexin type 9 inhibitor alirocumab among high cardiovascular risk patients on maximally tolerated statin therapy: The ODYSSEY COMBO I study. Am Heart J. 2015;169(6):906-15.e13.
62. Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, et al. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. N Engl J Med. 2015;372(16):1489-99.
63. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, et al. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. N Engl J Med. 2018;379(22):2097-107.
64. Raal FJ, Stein EA, Dufour R, Turner T, Civeira F, Burgess L, et al. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. Lancet. 2015;385(9965):331-40.
65. Raal FJ, Honarpour N, Blom DJ, Hovingh GK, Xu F, Scott R, et al. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. Lancet. 2015;385(9965):341-50.
66. Nicholls SJ, Puri R, Anderson T, Ballantyne CM, Cho L, Kastelein JJP, et al. Effect of Evolocumab on Progression of Coronary Disease in Statin-Treated Patients: The GLAGOV Randomized Clinical Trial. JAMA. 2016;316(22):2373-84.
67. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. N Engl J Med. 2017;376(18):1713-22.
68. Hirayama A, Honarpour N, Yoshida M, Yamashita S, Huang F, Wasserman SM, et al. Effects of evolocumab (AMG 145), a monoclonal antibody to PCSK9, in hypercholesterolemic, statin-treated Japanese patients at high cardiovascular risk--primary results from the phase 2 YUKAWA study. Circ J. 2014;78(5):1073-82.
69. Blom DJ, Hala T, Bolognese M, Lillestol MJ, Toth PD, Burgess L, et al. A 52-week placebo-controlled trial of evolocumab in hyperlipidemia. N Engl J Med. 2014;370(19):1809-19.
70. Momtazi AA, Banach M, Pirro M, Stein EA, Sahebkar A. PCSK9 and diabetes: is there a link? Drug Discov Today. 2017;22(6):883-95.
71. Schmidt AF, Swerdlow DI, Holmes MV, Patel RS, Fairhurst-Hunter Z, Lyall DM, et al. PCSK9 genetic variants and risk of type 2 diabetes: a mendelian randomisation study. Lancet Diabetes Endocrinol. 2017;5(2):97-105.
72. Schmidt AF, Pearce LS, Wilkins JT, Overington JP, Hingorani AD, Casas JP. PCSK9 monoclonal antibodies for the primary and secondary prevention of cardiovascular disease. Cochrane Database Syst Rev. 2017;4(4):Cd011748.
73. Mannarino MR, Sahebkar A, Bianconi V, Serban MC, Banach M, Pirro M. PCSK9 and neurocognitive function: Should it be still an issue after FOURIER and EBBINGHAUS results? J Clin Lipidol. 2018;12(5):1123-32.
74. Giugliano RP, Mach F, Zavitz K, Kurtz C, Im K, Kanevsky E, et al. Cognitive Function in a Randomized Trial of Evolocumab. N Engl J Med. 2017;377(7):633-43.
75. Lavecchia A, Cerchia C. Recent advances in developing PCSK9 inhibitors for lipid-lowering therapy. Future Med Chem. 2019;11(5):423-41.
76. Catapano AL, Pirillo A, Norata GD. New Pharmacological Approaches to Target PCSK9. Curr Atheroscler Rep. 2020;22(7):24.

77. Stein EA, Turner T, Kereiakes DJ, Butcher B, Mangu P, Zhou R. Safety, Tolerability and LDL-C Reduction With LIB003 a Novel Anti-PCSK9 Recombinant Fusion Protein: Results of Open-Label Extension Phase 2B Study. Circulation. 2019;140(Suppl_1):A17222-A.
78. Agency EM. Leqvio EPAR 2020 [Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/leqvio.
79. Ray KK, Wright RS, Kallend D, Koenig W, Leiter LA, Raal FJ, et al. Two Phase 3 Trials of Inclisiran in Patients with Elevated LDL Cholesterol. N Engl J Med. 2020;382(16):1507-19.
80. Landlinger C, Pouwer MG, Juno C, van der Hoorn JWA, Pieterman EJ, Jukema JW, et al. The AT04A vaccine against proprotein convertase subtilisin/kexin type 9 reduces total cholesterol, vascular inflammation, and atherosclerosis in APOE*3Leiden.CETP mice. Eur Heart J. 2017;38(32):2499-507.
81. Fischer LT, Hochfellner DA, Knoll L, Pöttler T, Mader JK, Aberer F. Real-world data on metabolic effects of PCSK9 inhibitors in a tertiary care center in patients with and without diabetes mellitus. Cardiovascular Diabetology. 2021;20(1):89.
82. Nordestgaard BG, Chapman MJ, Ray K, Boren J, Andreotti F, Watts GF, et al. Lipoprotein(a) as a cardiovascular risk factor: current status. Eur Heart J. 2010;31(23):2844-53.
83. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. New England Journal of Medicine. 2017;376(18):1713-22.
84. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, et al. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. New England Journal of Medicine. 2018;379(22):2097-107.
85. Zafrir B, Jubran A. Lipid-lowering therapy with PCSK9-inhibitors in the real-world setting: Two-year experience of a regional lipid clinic. Cardiovascular Therapeutics. 2018;36(5):e12439.
86. Stoekenbroek RM, Hartgers ML, Rutte R, de Wijer DD, Stroes ES, Hovingh GK. PCSK9 inhibitors in clinical practice: delivering on the promise? Atherosclerosis. 2018;270:205-10.
87. Saborowski M, Dölle M, Manns MP, Leitolf H, Zender S. Lipid-lowering therapy with PCSK9-inhibitors in the management of cardiovascular high-risk patients: effectiveness, therapy adherence and safety in a real world cohort. Cardiology journal. 2018;25(1):32-41.
88. Ray KK, Colhoun HM, Szarek M, Baccara-Dinet M, Bhatt DL, Bittner VA, et al. Effects of alirocumab on cardiovascular and metabolic outcomes after acute coronary syndrome in patients with or without diabetes: a prespecified analysis of the ODYSSEY OUTCOMES randomised controlled trial. Lancet Diabetes Endocrinol. 2019;7(8):618-28.
89. Ray KK, Leiter LA, Muller-Wieland D, Cariou B, Colhoun HM, Henry RR, et al. Alirocumab vs usual lipid-lowering care as add-on to statin therapy in individuals with type 2 diabetes and mixed dyslipidaemia: The ODYSSEY DM-DYSLIPIDEMIA randomized trial. Diabetes Obes Metab. 2018;20(6):1479-89.
90. Sabatine MS, Leiter LA, Wiviott SD, Giugliano RP, Deedwania P, De Ferrari GM, et al. Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the FOURIER randomised controlled trial. Lancet Diabetes Endocrinol. 2017;5(12):941-50.
91. Shi J, Zhang W, Niu Y, Lin N, Li X, Zhang H, et al. Association of circulating proprotein convertase subtilisin/kexin type 9 levels and the risk of incident type 2 diabetes in subjects with prediabetes: a population-based cohort study. Cardiovasc Diabetol. 2020;19(1):209.
92. Leiter L, Tinahones F, Karalis D, Bujas-Bobanovic M, Letierce A, Mandel J, et al. Alirocumab safety in people with and without diabetes mellitus: pooled data from 14 ODYSSEY trials. Diabetic Medicine. 2018;35(12):1742-51.
93. Leiter LA, Zamorano JL, Bujas-Bobanovic M, Louie MJ, Lecorps G, Cannon CP, et al. Lipid-lowering efficacy and safety of alirocumab in patients with or without diabetes: a sub-analysis of ODYSSEY COMBO II. Diabetes, Obesity and Metabolism. 2017;19(7):989-96.
94. Ray KK, Leiter LA, Müller-Wieland D, Cariou B, Colhoun HM, Henry RR, et al. Alirocumab vs usual lipid-lowering care as add-on to statin therapy in individuals with type 2 diabetes and mixed

dyslipidaemia: the ODYSSEY DM-DYSLIPIDEMIA randomized trial. Diabetes, Obesity and Metabolism. 2018;20(6):1479-89.
95. Blom DJ, Koren MJ, Roth E, Monsalvo ML, Djedjos CS, Nelson P, et al. Evaluation of the efficacy, safety and glycaemic effects of evolocumab (AMG 145) in hypercholesterolaemic patients stratified by glycaemic status and metabolic syndrome. Diabetes, Obesity and Metabolism. 2017;19(1):98-107.
96. Rosenson RS, Daviglus ML, Handelsman Y, Pozzilli P, Bays H, Monsalvo ML, et al. Efficacy and safety of evolocumab in individuals with type 2 diabetes mellitus: primary results of the randomised controlled BANTING study. Diabetologia. 2019;62(6):948-58.
97. Schubert J, Lindahl B, Melhus H, Renlund H, Leosdottir M, Yari A, et al. Low-density lipoprotein cholesterol reduction and statin intensity in myocardial infarction patients and major adverse outcomes: a Swedish nationwide cohort study. Eur Heart J. 2021;42(3):243-52.
98. Chamberlain AM, Gong Y, Shaw KM, Bian J, Song WL, Linton MF, et al. PCSK9 Inhibitor Use in the Real World: Data From the National Patient-Centered Research Network. J Am Heart Assoc. 2019;8(9):e011246.
99. Caselli C, Del Turco S, Ragusa R, Lorenzoni V, De Graaf M, Basta G, et al. Association of PCSK9 plasma levels with metabolic patterns and coronary atherosclerosis in patients with stable angina. Cardiovasc Diabetol. 2019;18(1):144.
100. Peng J, Zhu CG, Li JJ. The predictive utility of circulating PCSK9 levels on diabetes mellitus. Cardiovasc Diabetol. 2021;20(1):45.
101. Peng J, Liu MM, Jin JL, Cao YX, Guo YL, Wu NQ, et al. Association of circulating PCSK9 concentration with cardiovascular metabolic markers and outcomes in stable coronary artery disease patients with or without diabetes: a prospective, observational cohort study. Cardiovasc Diabetol. 2020;19(1):167.
102. Raal FJ, Stein EA, Dufour R, Turner T, Civeira F, Burgess L, et al. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. Lancet. 2015;385(9965):331-40.
103. Robinson JG, Nedergaard BS, Rogers WJ, Fialkow J, Neutel JM, Ramstad D, et al. Effect of evolocumab or ezetimibe added to moderate- or high-intensity statin therapy on LDL-C lowering in patients with hypercholesterolemia: the LAPLACE-2 randomized clinical trial. JAMA. 2014;311(18):1870-82.
104. Dijk W, Le May C, Cariou B. Beyond LDL: What Role for PCSK9 in Triglyceride-Rich Lipoprotein Metabolism? Trends Endocrinol Metab. 2018;29(6):420-34.
105. Raal FJ, Hovingh GK, Blom D, Santos RD, Harada-Shiba M, Bruckert E, et al. Long-term treatment with evolocumab added to conventional drug therapy, with or without apheresis, in patients with homozygous familial hypercholesterolaemia: an interim subset analysis of the open-label TAUSSIG study. Lancet Diabetes Endocrinol. 2017;5(4):280-90.
106. Tsimikas S, Karwatowska-Prokopczuk E, Gouni-Berthold I, Tardif JC, Baum SJ, Steinhagen-Thiessen E, et al. Lipoprotein(a) Reduction in Persons with Cardiovascular Disease. N Engl J Med. 2020;382(3):244-55.
107. Leitner DR, Toplak H, Kedenko L, Steinmaurer T, Graff V, Metzner T, et al. Efficacy and tolerability of alirocumab in Austrian clinical practice - results of the non-interventional PEARL-AT study. Curr Med Res Opin. 2020;36(9):1419-25.
108. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med. 2015;373(22):2117-28.
109. Wiviott SD, Raz I, Sabatine MS. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. Reply. N Engl J Med. 2019;380(19):1881-2.
110. Neal B, Perkovic V, Matthews DR. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med. 2017;377(21):2099.
111. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med. 2016;375(4):311-22.

112. Gerstein HC, Colhoun HM, Dagenais GR, Diaz R, Lakshmanan M, Pais P, et al. Dulaglutide and cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind, randomised placebo-controlled trial. Lancet. 2019;394(10193):121-30.
113. Buse JB, Wexler DJ, Tsapas A, Rossing P, Mingrone G, Mathieu C, et al. 2019 update to: Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia. 2020;63(2):221-8.

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Cardiovascular Diabetology

ORIGINAL INVESTIGATION

Open Access

Real-world data on metabolic effects of PCSK9 inhibitors in a tertiary care center in patients with and without diabetes mellitus

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Abstract

Background: The lipid-lowering and positive cardiovascular effect of proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors was shown in several studies, hence, they are more widely used in the lipid-lowering management of individuals with high cardiovascular risk. As real-world data are still scarce, specifically in patients with type 2 diabetes (T2D), the aim of this retrospective analysis was to investigate the efficacy of PCSK9 inhibitors in lowering low-density lipoprotein cholesterol (LDL-C) in an outpatient clinic of a tertiary care center in routine care.

Methods: A retrospective analysis of data extracted from the electronic patient record was performed. Patients who were routinely prescribed with PCSK9 inhibitor therapy (alirocumab or evolocumab) during the years 2016 and 2019 were included in the analysis. Characteristics of the patient population, the effects on LDL-C and HbA1c levels as well as subsequent cardiovascular events were assessed over an observation period of 18 months.

Results: We identified 237 patients treated with PCSK9 inhibitors between January 2016 and September 2019. Almost all patients (97.5%) received PCSK9 inhibitors for secondary prevention. 26.2% of the population had a concomitant diabetes diagnosis. Intolerance to statins (83.1%), ezetimibe (44.7%) or both agents (42.6%) was reported frequently. Three months after initiation of PCSK9 inhibitor therapy, 61.2% of the patients achieved LDL-C levels < 70 mg/dl, and 44.1% LDL-C levels < 55 mg/dl. The median LDL-C was lowered from 141 mg/dl at baseline, to 60 mg/dl after 3 months and 66 mg/dl after 12 months indicating a reduction of LDL-C as follows: 57.5% after 3 months and 53.6% after 12 months. After 3 months of observation, target achievement of LDL-C was higher in patients with T2D compared to non-diabetes patients; < 55 mg/dl: 51% vs. 41.5%; < 70 mg/dl 69.4 vs. 58.5%. After 12 months even more pronounced target LDL achievement in T2D was demonstrated < 55 mg/dl: 58.8% vs. 30.1%; < 70 mg/dl 70.6 vs. 49.6%. Patients with insufficiently controlled T2D (HbA1c > 54 mmol/mol) had a higher reduction in LDL-C but still were more likely to subsequent cardiovascular events.

Conclusions: Significant reductions in LDL-C and a high percentage of patients achieving recommended treatment targets were observed. The percentage of patients with T2D meeting recommended LDL-C targets was higher than in those without T2D. Still some patients did not achieve LDL-C levels as recommended in current guidelines. Special attention to the characteristics of these patients is required in the future to enable achievement of treatment goals and avoid adverse cardiovascular outcomes.

Keywords: PCSK9 inhibitor therapy, Lipid lowering therapy, Real world data

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Background

Low-density lipoprotein cholesterol (LDL-C) is a well-established risk factor in the development of atherosclerotic cardiovascular disease (ASCVD). Several studies have shown unequivocal evidence that high levels of LDL-C have an unfavorable effect on ASCVD and contribute to cardiovascular death [1–3]. At present, statins are in addition to lifestyle interventions the first-line therapy for LDL-lowering, in addition to lifestyle interventions, in most patients. Further pharmacological lipid-lowering options include combinations of ezetimibe, bile acid sequestrants, fibrates and proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors [4].

Both, the FOURIER trial on evolocumab and the ODYSSEY OUTCOMES trial on alirocumab showed that PCSK9 inhibitors were not only capable to significantly lower LDL-C levels, but also result in a substantial reduction of the cardiovascular event rate without relevant risk of adverse events [5, 6]. As recommended in the guidelines for the management of dyslipidemias by the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS), treatment with a PCSK9 inhibitor is indicated for secondary prevention to reduce plasma LDL-C in very-high risk patients who do not achieve their target LDL-C or even for primary prevention in particular very-high risk patients as those with familial hypercholesterolemia (FH) who do not achieve their LDL-C goal despite maximal tolerated therapy with statins and ezetimibe [4]. In 2015, the PCSK9 inhibitors alirocumab and evolocumab were approved in the European Union by the European Medicines Agency.

Patients with diabetes represent a particular risk group for cardiovascular disease and consecutive events. Evidence gained from randomized controlled trials using PCSK9 inhibitors in secondary prevention suggests a similar efficacy on LDL-C reduction and clinical benefit in patients with diabetes mellitus compared to those without diabetes [7, 8]. However, hardly any observational trials that confirmed these results when investigated during real-world conditions are available.

Since patients who participate in clinical trials are usually seen on a regular and rather frequent basis throughout the course of the study, are provided free of charge with study medication and adherence to medication is closely monitored which can be subsumed as study effect, there might be differences in routine care that are worth investigating. In addition, most of the available real-world studies on PCSK9 inhibitors only investigated small groups of patients and did not specifically focus on patients with diabetes mellitus.

Previous research suggested a potential diabetogenic effect by using statins in the treatment of hyperlipidemia [9]. Also, genetic studies demonstrated that specific PCSK9 variants might increase the risk for the development of diabetes mellitus type 2 (T2D) [10], however this finding could not be evidently verified in randomized controlled trials (RCTs) when PCSK9 inhibitors were exogenously administered [11].

The aim of this retrospective analysis was to investigate the effect of PCSK9 inhibitor therapy on markers of lipid metabolism, to determine patient characteristics, to assess the indications for their use and to evaluate the tolerance of PCSK9 inhibitor therapy in patients with and without T2D in a routine care-setting of an outpatient clinic in a tertiary care center. In addition, we intended to examine potential influence of PCSK9 inhibitor therapy on glycemic control assessed by HbA1c and evaluated whether there were differences in LDL-C reduction efficacy in those with and without T2D. Furthermore, the population was screened for subsequent cardiovascular events within an observation period of 18 months after initiation of PCSK9 inhibitor therapy.

Material and methods

Patients and outcome measures

This study was a retrospective data analysis approved by the Medical University of Graz, Austria (EK number 32-018 ex 19/20) and included cardiovascular high-risk patients who were prescribed with PCSK9 inhibitor therapy within routine conditions, considering the national reimbursement criteria (LDL > 100 mg/dl despite maximal tolerated statin/ezetimibe therapy, well controlled hypertension, HbA1c < 64 mmol/mol, having received nutritional advice by a dietologist and intensive motivation to stop smoking). Data of the electronic patient records of the University hospital were screened for eligible patients from January 2016 to September 2019. Electronic records of adult patients with current or past PCSK9 inhibitor treatment in routine care at the outpatient clinic of the Division of Endocrinology and Diabetology were searched and included in the analysis if they met the inclusion criteria. Inclusion criteria were as follows: age > 18 years, treatment with locally available PCSK9 inhibitors (alirocumab 75 or 150 mg, or evolocumab 140 mg) in routine care, available laboratory reports on LDL-C levels at first prescription and LDL-C during a follow-up period longer than 3 months.

In eligible patients the following parameters were drawn from the electronic patient record: age, sex, lipid-lowering therapy at baseline (i.e. statins, ezetimibe, fibrates), cause of prescription of PCSK9 inhibitor (intolerance to lipid-lowering medications at baseline, failure to achieve individual LDL-C levels; primary or secondary prevention as indication), type of PCSK9 inhibitor (i.e. alirocumab or evolocumab), PCSK9 inhibitor therapy adjustments (agent, discontinuation). Baseline

macrovascular (coronary heart disease, stroke, transient ischemic attack, peripheral artery disease, carotid artery disease) and microvascular (retinopathy, nephropathy) comorbidities, previous cardiovascular interventions (i.e. percutaneous coronary revascularization or coronary artery bypass grafting) and further cardiovascular risk factors (i.e. smoker status, hypertension, diabetes mellitus) were assessed. The following laboratory parameters were extracted: LDL-C, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), total cholesterol, lipoprotein (a) (Lp[a]), and HbA1c at baseline and during PCSK9 inhibitor therapy for up to 18 months whenever collected in routine care. Number of patients who were lost to follow-up (missing documentation regarding PCSK9 inhibitor therapy ≥ 1 year) or patients who discontinued therapy (i.e. adverse drug effects towards PCSK9; noncompliance) were also determined. Baseline was defined as the day of the first PCSK9 inhibitor application. Patients enrolled were followed up for subsequent cardiovascular events within the first 18 months after PCSK9 inhibitor initiation. MACE was defined as occurrence of non-fatal myocardial infarction or stroke or cardiovascular death. All-cause cardiovascular events included the following: any acute coronary syndrome, scheduled coronary intervention, aortic dissection, any cerebral ischemic event (transitory ischemic attack [TIA] or stroke), any peripheral vascular intervention or diagnosis/worsening of peripheral artery disease, newly diagnosed carotid artery disease, surgical carotid intervention, necessity of hemodialysis or acute decompensated heart failure.

Statistical methods

The follow-up of LDL-C, HDL-C, TG, Lp(a) and HbA1c were analyzed at months 3, 6, 9 and 12, and up to 18 months from baseline, including data from the most current measurement after the defined time points. A last observation carried forward analysis was performed for laboratory parameters, imputing data of the latest measurement of maximum 3 months prior. If PCSK9 inhibitor therapy was discontinued, further observations were not included in the analyses.

Wilcoxon signed-rank test was used to compare parameters between baseline and follow-up time points within a subgroup, Mann–Whitney-U test was used to evaluate between-group differences of two different subgroups. Kruskal–Wallis test was used to analyze differences of LDL-C levels and LDL-C reduction across subgroups of concomitant lipid-lowering therapy. Univariate Cox-regression analysis was used to calculate hazard ratios for events of patients with T2D compared to patients without T2D. P-values of ≤ 0.05 were considered statistically significant. Statistical analysis was performed with IBM SPSS Statistics version 26.

Results

Patient characteristics

We identified 237 eligible patients (47.7% female) who received alirocumab or evolocumab (104 and 133 patients) of whom the median age was 65.2 years (interquartile range [IQR] 57.8–71.5). Almost all patients (97.5%; n = 231) received PCSK9 inhibitors for secondary prevention. Apart from hypercholesterolemia, more than half of the study population (53.6%) had two or more additional cardiovascular risk factors (arterial hypertension, chronic kidney disease, Age ≥ 65 years, diabetes mellitus, smoker). Medical history included coronary heart disease (74.7%), at least one percutaneous coronary intervention (46.4%), coronary artery bypass graft (CABG) (16%), stroke or transient ischemic attack (13.5%), carotid artery stenosis (30.8%), peripheral artery disease (18.1%), and chronic kidney disease (9.7%). Any type of familial hypercholesterolemia (mostly diagnosed using clinical criteria, no one identified with homozygous FH) was present in 21.5% of the patients. Diabetes mellitus was present in 62 patients of whom the majority had type 2 diabetes (T2D) (n = 54). Further relevant comorbidities at baseline were: arterial hypertension (68.8%) and active smoker status (5.9%).

Intolerance to other lipid-lowering agents was frequently present at baseline: 83.1% reported intolerance to at least one statin, 44.7% reported side-effects to ezetimibe. 42.6% indicated statin and ezetimibe intolerance. The full characteristics of the patient cohort are listed in Table 1. The more detailed summary of cardiovascular risk factor profile is shown in Additional file 1: Table S1. Due to the retrospective design of this study, we were not able to ascertain laboratory data at the predefined time-points from all patients (i.e. every 3 months after therapy initiation) due to loss to follow-up, discontinuation of treatment or infrequent outpatient clinic visits. Ten patients (4.2%) discontinued PCSK9 inhibitor therapy (five after 3 months from baseline, one after 6 months, one after 9 months, three after 12 months) and were not further included in the analysis after discontinuation. 27 patients (11.4%) were lost to follow-up (missing documentation regarding PCSK9 inhibitor therapy ≥ 1 year).

Laboratory parameters and concomitant medication at baseline

At baseline, 29.5% of the overall cohort were on statin therapy (mostly rosuvastatin or atorvastatin), 39.7% on ezetimibe and 2.5% on fibrates. 48.9% did not receive lipid-lowering medication (i.e. no statins, ezetimibe or

Table 1 Baseline characteristics overall as well as per PCSK9 inhibitor used and diabetes status

Characteristics	Total (n = 237)	Alirocumab (n = 104)	Evolocumab (n = 133)	Without diabetes mellitus (n = 175)	With diabetes mellitus type 2 (n = 54)
Age (years)	65.2 (57.8–71.5)	65.0 (57.8–71.5)	65.4 (57.9–71.6)	65.0 (57.8–70.8)	68.9 (63.1–73.8)
Female sex n (%)	113 (47.7)	53 (51.0)	60 (45.1)	88 (50.3)	22 (40.7)
Indication for PCSK9 inhibitor treatment n (%)					
Primary prevention	6 (2.5)	4 (3.8)	2 (1.5)	4 (2.3)	2 (3.7)
Secondary prevention	231 (97.5)	100 (96.2)	131 (98.5)	171 (97.7)	52 (96.3)
Medical history n (%)					
Coronary heart disease	177 (74.7)	80 (76.9)	97 (72.9)	134 (76.6)	38 (70.4)
Percutaneous coronary intervention	110 (46.4)	49 (47.1)	61 (45.9)	86 (49.1)	21 (38.9)
Coronary artery bypass graft	38 (16.0)	16 (15.4)	22 (16.5)	27 (15.4)	8 (14.8)
Stroke or transient ischemic attack	32 (13.5)	12 (11.5)	20 (15.0)	24 (13.7)	8 (14.8)
Carotid artery disease	73 (30.8)	37 (35.6)	36 (27.1)	51 (29.1)	20 (37.0)
Peripheral artery disease	43 (18.1)	15 (14.4)	28 (21.1)	25 (14.3)	15 (27.8)
Arterial hypertension	163 (68.8)	74 (71.2)	89 (66.9)	112 (64.0)	46 (85.2)
Familial hypercholesterolemia (heterozygous)	51 (21.5)	19 (18.3)	32 (24.1)	41 (23.4)	8 (14.8)
Retinopathy	10 (4.2)	5 (4.8)	5 (3.8)	4 (2.3)	3 (5.6)
Chronic kidney disease	23 (9.7)	6 (5.8)	17 (12.8)	10 (5.7)	12 (22.2)
Current tobacco smoker	14 (5.9)	9 (8.7)	5 (3.8)	12 (6.9)	1 (1.9)
Diabetes mellitus any type	62 (26.2)	25 (24.0)	37 (27.8)	–	–
Diabetes mellitus type 1	6 (2.5)	2 (1.9)	4 (3.0)	–	–
Diabetes mellitus type 2	54 (22.8)	22 (21.2)	32 (24.1)	–	54 (100)
Other types of diabetes	2 (0.8)	1 (1.0)	1 (0.8)	–	–
Intolerances/side effects to lipid-lowering medication n (%)					
Statins	197 (83.1)	83 (79.8)	114 (85.7)	147 (84.0)	43 (79.6)
Ezetimibe	106 (44.7)	36 (34.6)	70 (52.6)	76 (43.4)	28 (51.9)
Statins and ezetimibe	101 (42.6)	34 (32.7)	67 (50.4)	74 (42.3)	25 (46.3)

Patients with type 1 diabetes were not separately analysed due to the small number (n = 4). Data are median (interquartile range) or number (%)

fibrates) at that time. Concomitant lipid-lowering therapy of patients with T2D was comparable to patients without diabetes mellitus. There was only a slight difference in a higher usage of fibrates in the group of patients with diabetes mellitus. Median LDL-C at baseline was 141 mg/dl (117–188), distribution of alirocumab and evolocumab use was 44 and 56%. Median HbA1c in the T2D population was 52 mmol/mol (48–57 mmol/mol; 6.9% [6.5–7.4]%). Further laboratory parameters and concomitant medication at baseline according to the prescribed PCSK9 inhibitor and diabetes status are shown in Table 2.

Effect on LDL-C levels

Median baseline LDL-C of the total population at baseline was 141 mg/dl and decreased to 60, 59, 61 and 66 mg/dl after 3, 6, 9 and 12 months of observation, respectively. During the course of treatment, a substantial proportion of patients achieved LDL-C levels < 70 mg/dl (61.2% after 3 months and 56.2% after 12 months) or < 55 mg/dl (44.1% after 3 months and 38.6% after 12 months). LDL-C remained above 100 mg/dl in 17.6% at month 3 months and in 16.3% at month 12. LDL-C reduction > 50% was achieved in 64.3% and 59.5% at month 3 and 12, respectively (Fig. 1). Additional file 1: Table S2 shows the LDL-reduction over time according to the PCSK9 inhibitor used.

LDL-C reduction according to diabetes status and quality of glycemic control

LDL-C levels at baseline of patients with T2D were comparable to those without diabetes. At some points of the analysis (at months 6 and 12), a significantly higher reduction of LDL-C was observed in patients with T2D (Fig. 3). Achievement of LDL-C targets < 55 mg/dl after 3 months on therapy was numerically but not significantly higher in patients with T2D compared to those without: < 55 mg/dl: 51% vs. 41.5%; < 70 mg/dl 69.4 vs. 58.5% (p = 0.119). At 12 months, LDL-C treatment targets were achieved in patients with T2D compared to

Table 2 Laboratory parameters, concomitant lipid-lowering and anti-hyperglycemic therapy at baseline according to prescribed PCSK9 inhibitor and diabetes status

Characteristics	Total (n = 237)	Alirocumab (n = 104)	Evolocumab (n = 133)	Without diabetes mellitus (n = 175)	With diabetes mellitus type 2 (n = 54)
LDL-C (mg/dl)	141 (117–188) n = 237	135 (114–181) n = 104	149 (118–191) n = 133	141 (117–188) n = 175	135 (110–178) n = 54
Total cholesterol (mg/dl)	229 (198–268) n = 216	210 (190–259) n = 94	240 (202–272) n = 122	230 (200–268) n = 163	216 (194–261) n = 47
HDL-C (mg/dl)	54 (45–65) n = 226	52 (44–63) n = 100	55 (46–67) n = 126	54 (46–67) n = 169	49 (42–60) n = 49
Triglycerides (mg/dl)	138 (99–215) n = 227	138 (102–255) n = 102	138 (97–197) n = 138	124 (97–199) n = 167	185 (134–249) n = 52
Lp(a) (mg/dl)	65 (25–101) n = 47	87 (71–114) n = 13	55 (18–90) n = 34	65 (23–114) n = 37	67 (46–89) n = 10
HbA1c (mmol/mol)	41 (37–49) n = 139	40 (37–49) n = 61	43 (37–50) n = 78	38 (36–40) n = 81	52 (48–57) n = 51
Statins (n; %)	70 (29.5)	38 (36.5)	32 (24.1)	52 (29.7)	16 (29.6)
Fluvastatin	3 (1.3)	2 (1.9)	1 (0.8)	2 (1.1)	1 (1.9)
Pravastatin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Simvastatin	5 (2.1)	1 (1.0)	4 (3.0)	5 (2.9)	0 (0)
Rosuvastatin	29 (12.2)	15 (14.4)	14 (10.5)	22 (12.6)	5 (9.3)
Atorvastatin	35 (14.8)	21 (20.2)	14 (10.5)	25 (14.3)	10 (18.5)
Ezetimibe	94 (39.7)	46 (44.2)	48 (36.1)	67 (38.3)	22 (40.7)
Fibrates	6 (2.5)	2 (1.9)	4 (3.0)	2 (1.1)	4 (7.4)
No lipid-lowering medication	116 (48.9)	44 (42.3)	72 (54.1)	89 (50.9)	24 (44.4)
Antidiabetic treatment					
Metformin	24 (10.1)	13 (12.5)	11 (8.3)	0 (0)	23 (42.6)
SGLT2 inhibitors	5 (2.1)	2 (1.9)	3 (2.3)	0 (0)	4 (7.4)
DPP-4 inhibitors	14 (5.9)	7 (6.7)	7 (5.3)	0 (0)	14 (25.9)
GLP-1 receptor agonists	4 (1.7)	0 (0)	4 (3.0)	0 (0)	4 (7.4)
Sulfonylureas	1 (0.4)	0 (0)	1 (0.8)	0 (0)	1 (1.9)
Pioglitazone	2 (0.8)	1 (1.0)	1 (0.8)	0 (0)	2 (3.7)
Insulin therapy	23 (9.7)	9 (8.7)	14 (10.5)	0 (0)	16 (29.6)
Diet only	49 (20.7)	22 (21.2)	27 (20.3)	0 (0)	14 (25.9)

Data are median (interquartile range) or number (%)

SGLT2 sodium-glucose co-transporter 2, *GLP-1* Glucagon-like peptide 1, *DPP-4* dipeptidyl-peptidase 4

those without < 55 mg/dl: 58.8% vs. 30.1%; < 70 mg/dl 70.6 vs. 49.6% (p = 0.003). Patients with inadequately controlled T2D (HbA1c > 54 mmol/mol) showed a higher but not statistically significant LDL-C reduction at month 12, than T2D patients with baseline HbA1c ≤ 54 mmol/mol (p = 0.052). Detailed data on LDL-C according to diabetes status are shown in Fig. 2 and in Additional file 1: Table S2.

LDL-C reduction according to adjunct lipid-lowering medication

Considering additive lipid-lowering medication at baseline, a significant reduction in LDL-C was observed in all groups during all observation points which remained stable over time. The greatest decline was seen in patients who were on concomitant combination therapy of a statin and ezetimibe, while the smallest effect on LDL-C reduction was seen in those who did not have adjunct lipid-lowering therapy apart from the PCSK9 inhibitor therapy (Fig. 3). When comparing LDL-C levels at different time points, there was no significant difference in the numerical reduction of LDL-C between these groups (Additional file 1: Table S2).

Effect on triglyceride levels

Triglyceride (TG) levels slightly decreased from baseline (138 mg/dl) over time: 107 mg/dl (month 3) months, 110 mg/dl (month 6), 111 mg/dl (month 9) and 114 mg/dl (month 12) (p = 0.001). Median numerical and percental changes are shown in Additional file 1:

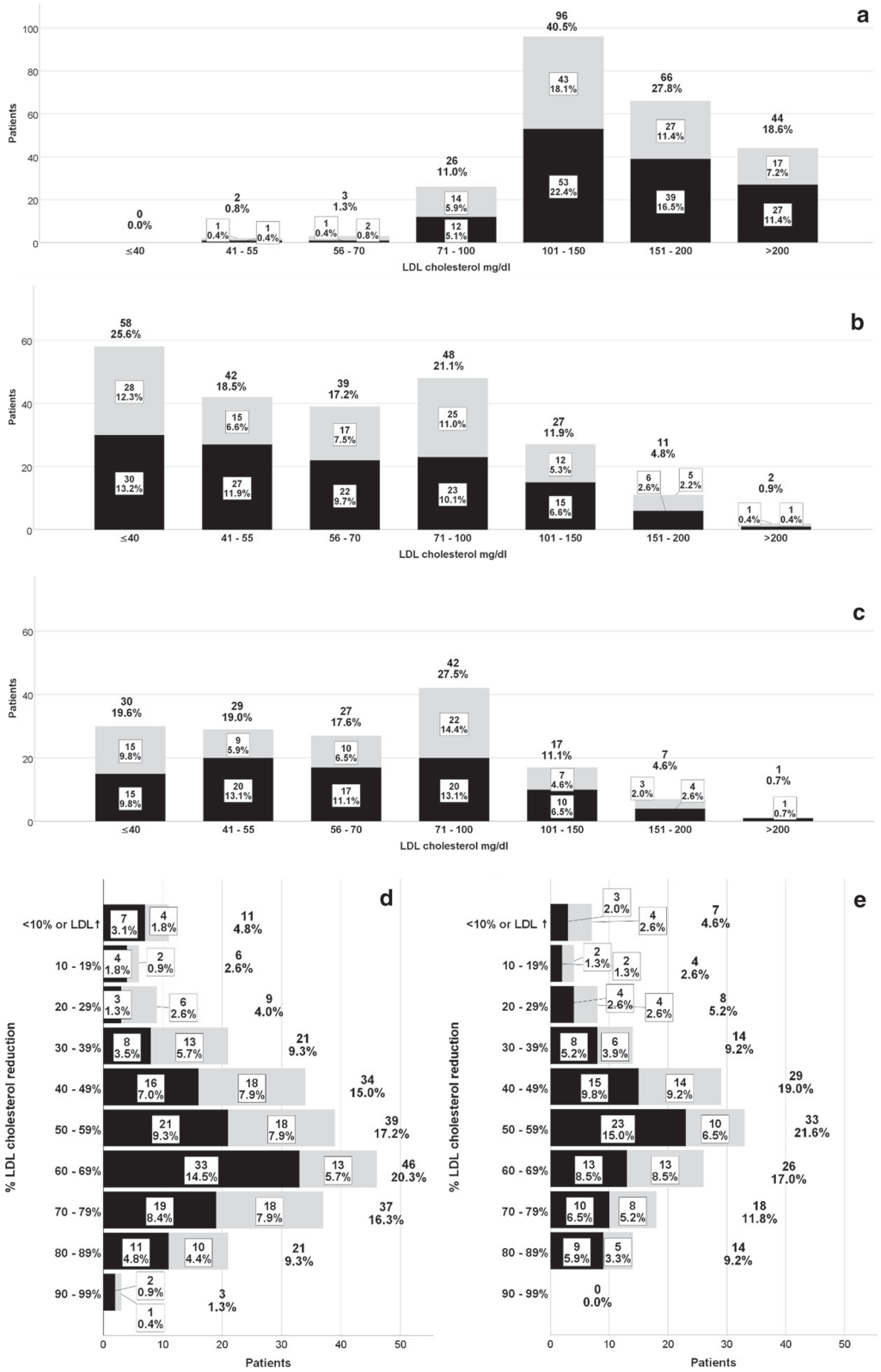
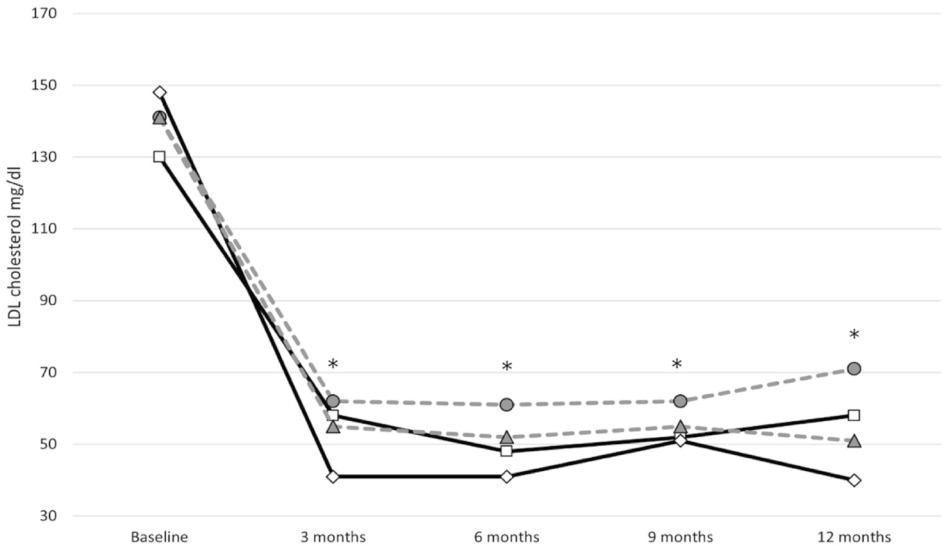


Fig. 1 LDL-C levels and percentage of LDL-C reduction over time; data are number of patients (n) and percentage (%); grey bar = alirocumab, black bar = evolocumab. **a** Baseline LDL-C levels (n = 237), **b** LDL-C levels at month 3 (n = 227), **c** LDL-C levels at month 12 (n = 153); **d** % LDL-C reduction from baseline to month 3 (n = 227) **e** % LDL-C reduction from baseline to month 12 (n = 153)

a

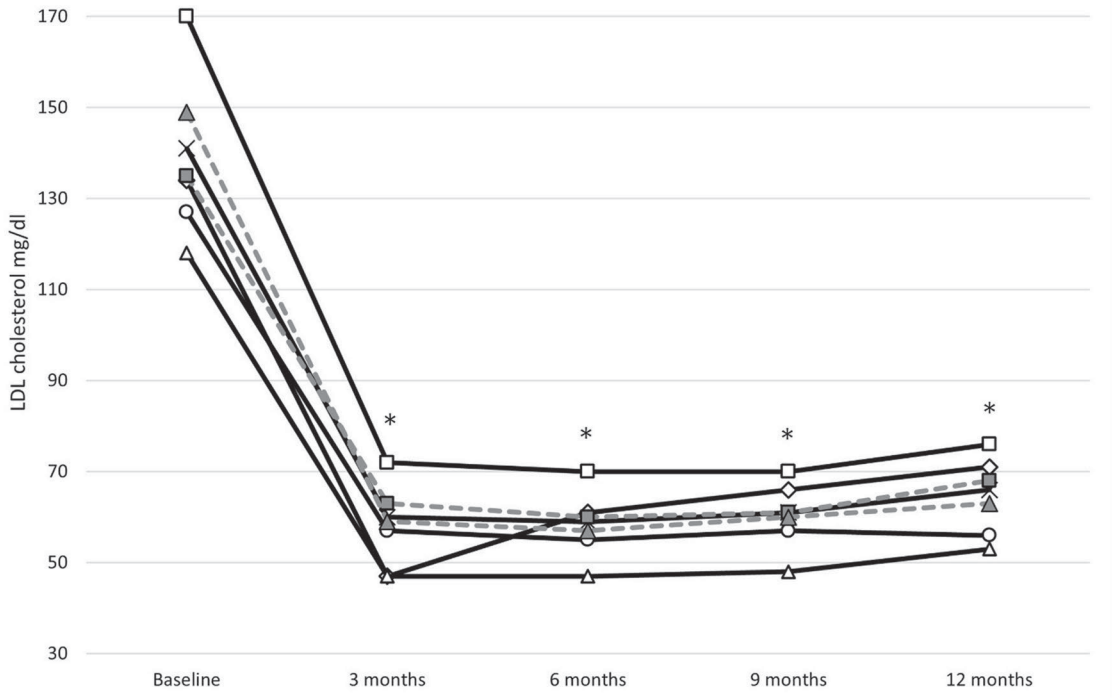


	Baseline	3 months	6 months	9 months	12 months
Without diabetes mellitus	141 (117 - 188) (n=175)	62 (44 - 83) (n=171)	61 (44 - 85) (n=154)	62 (44 - 87) (n=132)	71 (49 - 91) (n=113)
With diabetes mellitus type 2	135 (110 - 178) (n=54)	55 (34 - 80) (n=49)	48 (36 - 73) (n=45)	53 (39 - 84) (n=40)	52 (39 - 78) (n=34)
HbA1c ≤ 54 (DM2)	130 (104 - 174) (n=34)	58 (35 - 86) (n=32)	48 (37 - 77) (n=29)	52 (39 – 83) (n=26)	58 (40 - 82) (n=22)
HbA1c > 54 (DM2)	148 (119 - 192) (n=17)	41 (30 - 63) (n=14)	41 (30 - 63) (n=13)	51 (34 - 92) (n=11)	40 (30 - 55) (n=9)

b

LDL-C mg/dl	Without T2D n (%)	T2D HbA1c ≤54 mmol/mol n (%)	T2D HbA1c >54 mmol/mol n (%)
Baseline	n=175 (100)	n=34 (100)	n=17 (100)
<40 mg/dl	0(0)	0(0)	0(0)
<55 mg/dl	2 (1.1)	0(0)	0(0)
<70 mg/dl	2 (1.1)	1(2.9)	0(0)
Month 3	n=171 (100)	n=32 (100)	n=14 (100)
<40 mg/dl	38 (22.2)	9 (28.1)	7 (50.0)
<55 mg/dl	71 (41.5)	15 (46.9)	9 (64.3)
<70 mg/dl	100 (58.5)	22 (68.8)	11(78.6)
Month 12	n=148 (100)	n=22 (100)	n=9 (100)
<40 mg/dl	16 (14.2)	6 (27.3)	5 (55.6)
<55 mg/dl	34 (30.1)	11 (50.0)	7 (77.8)
<70 mg/dl	56 (49.6)	13 (59.1)	9 (100)

Fig. 2 **a** LDL-C levels of patients without and with T2D 2. **b** Number and percentage of achievement of treatment targets according to diabetes status. Data are median (interquartile range). *Indicates statistical significance when compared to baseline LDL-C levels (for all groups investigated)



	Baseline	3 months	6 months	9 months	12 months
PCSK9i only	170 (130 – 203) (n=117)	72 (50 – 100) (n=114)	70 (46 – 101) (n=106)	70 (49 – 98) (n=93)	76 (54 – 104) (n=81)
Statin	134 (115 – 171) (n=26)	47 (32 – 85) (n=26)	61 (39 – 84) (n=23)	66 (46 – 87) (n=19)	71 (42 – 87) (n=13)
Ezetimibe	127 (117 – 158) (n=50)	57 (40 – 74) (n=45)	55 (35 – 69) (n=39)	57 (39 – 68) (n=33)	56 (43 – 68) (n=36)
Statin + ezetimibe	118 (93 – 140) (n=44)	47 (30 – 62) (n=42)	47 (35 – 59) (n=36)	48 (33 – 62) (n=33)	53 (35 – 68) (n=23)
Total	141 (117 – 188) (n=237)	60 (40 – 83) (n=227)	59 (42 – 84) (n=204)	61 (44 – 85) (n=178)	66 (45 – 86) (n=153)
Alirocumab	135 (114 – 181) (n=104)	63 (35 – 87) (n=103)	60 (44 – 83) (n=94)	61 (44 – 87) (n=79)	68 (42 – 88) (n=66)
Evolocumab	149 (118 – 191) (n=133)	59 (41 – 82) (n=124)	57 (40 – 85) (n=110)	60 (43 – 82) (n=99)	63 (47 – 60) (n=87)

Fig. 3 LDL-C levels of patients according to lipid-lowering medication at baseline; all patients were on PCSK9 inhibitor therapy. Data are median (interquartile range). *Indicates statistical significance when compared to baseline LDL-C levels for all groups investigated

Table S4. There was no significance in the TG reduction between alirocumab and evolocumab. Patients with T2D had significantly higher TG levels at baseline compared to those without diabetes (185 and 124 mg/dl; $p = 0.001$), TG reduction during treatment was comparable between patients with and without T2D (n.s.). Further details related to TG reduction over time considering PCSK9 inhibitor used and diabetes status is demonstrated in Additional file 1: Table S3.

Effect on high-density lipoprotein

In the total population, high-density lipoprotein (HDL) slightly increased from baseline (54 mg/dl) at months 3 and 12 (57 mg/dl), respectively. The HDL increase was not significantly sustained after 12 months in the alirocumab treated group. Patients with T2D had significantly lower HDL levels at baseline when compared to patients without T2D. Similar to the overall group, there was a slight increase of HDL in both patients without and with T2D (n.s.). Further details are shown in Additional file 1: Table S4.

Effect on lipoprotein (a)

Follow-up data on lipoprotein (a) [Lp(a)] was only available in 26 patients, due to infrequent measurements in routine care based on current guideline recommendations (single measurement recommended at least once in a lifetime, not subsequent) [12]. Lp(a) decreased from 67 mg/dl at baseline to 55 mg/dl at the first follow-up measurement (after median 95 days [28–190] from baseline) in these patients. The significant decrease of Lp(a) shown for patients without diabetes, could not be confirmed in patients with T2D (only data from 5 patients available). Further details can be found in Additional file 1: Table S5.

Effects on HbA1c

The baseline HbA1c of the overall cohort was 41 (37–49) mmol/mol and did not significantly change during the observation period of up to 18 months. Also, in patients with T2D (baseline HbA1c 52 [48–57] mmol/mol) no significant changes of HbA1c were found over time. Detailed data on HbA1c can be found in Additional file 1: Table S6.

Adjustments of the antidiabetic therapy during the study period occurred in 15 patients (T2D, n = 14, T1D, n = 1). Drug treatment intensifications were performed in 9 patients: new or additional oral antidiabetic drug (OAD) or Glucagon-like peptide 1 receptor agonist (GLP1-RA) in 3 patients, increase of OAD dose (n = 1), increase of insulin dose in 4 patients (n = 3 in T2D, n = 1 in T1D), additional OAD plus increase of insulin dose (n = 1). A switch from one class of OAD to another was made in five patients. A reduction of insulin dose occurred in one patient.

Cardiovascular outcomes

A total of 42 cardiovascular events in 36 patients were observed during the 18 months observation period; 12 of those were patients with T2D. A composite of major adverse cardiac events (MACE) including non-fatal myocardial infarction (ST—segment elevation myocardial infarction [STEMI] or non-ST—segment elevation myocardial infarction [NSTEMI]), indication of urgent need for coronary intervention, non-fatal stroke or TIA, and cardiovascular death occurred 15 times in 13 patients, four of those with T2D. Patients with insufficiently controlled T2D (HbA1c > 54 mmol/mol) experienced a higher risk of cardiovascular events (HR: 5.1 [2.2–12.4]; $p < 0.001$) when compared to patients without diabetes mellitus. The total event-rate in those with well controlled T2D (HbA1c ≤ 54 mmol/mol) not was comparable (HR: 1.5 [0.6–4.1; $p = 0.410$]) to those without diabetes mellitus. The MACE did not significantly differ between those with and without T2D (HR: 2.3 [0.6–7.9]; $p = 0.212$). Kaplan–Meier survival curves indicating event-free and MACE-free survival are shown in Fig. 4.

Therapy adjustments, discontinuation, adverse effects

In nine patients the PCSK9 inhibitor agent was altered over time (i.e. change from one agent to the other). Seven patients discontinued PCSK9 inhibitor treatment due to side-effects (mostly because of joint or muscle pain, skin lesions or pruritus, or gastrointestinal symptoms), one because of insufficient response to PCSK9 inhibitor therapy, one did not fulfill the criteria for health insurance coverage of PCSK9 inhibitor treatment (ongoing smoking) and one discontinued by the patient's own request. One patient with T1D reported on elevated insulin requirements and mild ketoacidosis after 4 months of PCSK9 inhibitor therapy leading to cessation of treatment.

Adverse effects of PCSK9 inhibitor therapy were documented in 27 patients. The majority of side effects was reported from patients without concomitant lipid lowering therapy (20 patients; 74%); all of the reported effects were of mild quality. The most frequent side effects were joint or muscle pain, rhinitis, flu-like symptoms, fatigue, skin lesions or pruritus. Side effects associated with the PCSK9 inhibitor therapy and the motivations for therapy adjustments are shown in Table 3.

Discussion

This real-world data analysis evaluated data from patients in a tertiary center who received PCSK9 inhibitors for the treatment of hypercholesterolemia. Within this setting PCSK9 inhibitors were mainly prescribed in secondary prevention for patients with established cardiovascular disease and multiple cardiovascular risk factors. Statin intolerance and side-effects of ezetimibe was reported in the majority of cases (83.1% and 44.7, respectively) and appears to be a main driver for prescription of PCSK9 inhibitor therapy. This frequent intolerance to these first-line agents explains why about half of the patients had no lipid-lowering medication on board at the time of the first prescription of a PCSK9 inhibitor.

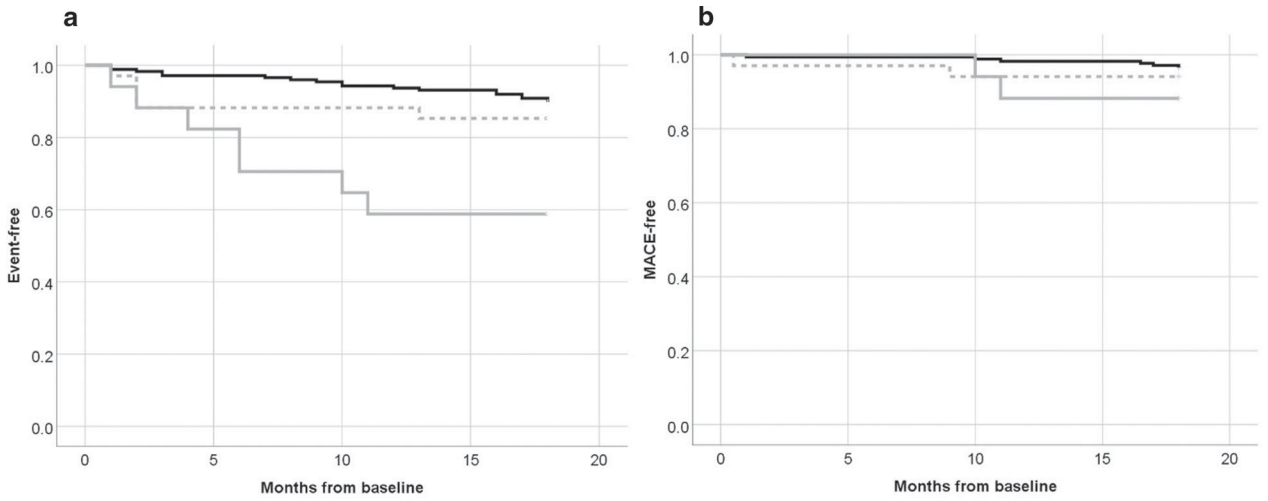


Fig. 4 **a** Event-free and **b** MACE-free survival curves of patients without diabetes mellitus (black line), with T2D and baseline HbA1c ≤ 54 mmol/mol (dashed grey line), and with T2D and baseline HbA1c of > 54 mmol/mol (solid grey line). MACE was defined as composite endpoint including non-fatal myocardial infarction (STEMI or NSTEMI), indication of urgent need for coronary intervention, non-fatal stroke or TIA, and cardiovascular death

Table 3 Side effects of PCSK9 inhibitor therapy reported by patients, cause of therapy adjustments and discontinuation

Adverse effect documented in patients record	
Joint or muscle pain	10
Rhinitis	5
Flu-like symptoms	5
Fatigue	4
Skin lesions or pruritus	4
Gastrointestinal symptoms	2
Insomnia	1
Elevated insulin requirements	1
Cause leading to PCSK9 inhibitor therapy adjustment (switch of PCSK9 inhibitor agent)	
Adverse effect	8
Joint or muscle pain	6
Flu-like symptoms	3
Fatigue	1
Gastrointestinal symptoms	1
Dose reduction (evolocumab 140 mg to alirocumab 75 mg)	1
Cause leading to PCSK9 inhibitor therapy discontinuation	
Adverse effect	7
Joint or muscle pain	3
Skin lesions or pruritus	2
Gastrointestinal symptoms	1
Adverse effects not specified	1
Elevated insulin requirement	1
Non-responder (clearly documented)	1
Reasons of insurance coverage	1
By patient's request	1

Data are number of patients. Patients could have more than one symptom

In our investigation, the significant reduction of LDL-C levels which was comparable with both agents after only a few weeks underlines the effective potential of PCSK9 inhibitor drugs as lipid-lowering therapy. RCTs like the FOURIER and ODYSSEY OUTCOMES trials showed impressive results in lowering LDL-C. As compared to our study, in the FOURIER trial, substantially more patients achieved LDL-C levels < 70 mg/dl and < 40 mg/dl (87% and 67% vs. 56.2% and 19.6% in our study). In both RCTs a reduction to even lower LDL-C levels was achieved (LDL-C of 30 mg/dl after 48 weeks in the FOURIER trial; LDL-C of 48 mg/dl after 12 months in the ODYSSEY OUTCOMES trial vs. LDL-C of 66 mg/dl after 12 months in our study). However, it should be considered that the baseline LDL-C was considerably lower in these RCTs, and that a majority of patients were on concomitant statin therapy (FOURIER: 69.3%; ODYSSEY: 88.8%) with or without ezetimibe (FOURIER: 5.2%; ODYSSEY: 2.9%), which may explain the differences to our data [5, 6]. In routine care, and also as shown by our analysis, there is a high number of patients on PCSK9 inhibitor therapy without concomitant lipid-lowering therapy. In many cases this is due to intolerance against the first-line therapy. This condition thus hinders further intensification of lipid-lowering therapy aside from PCSK9 inhibitor therapy. Within clinical studies, nutrition and adherence to prescribed medication are intensely monitored; this might positively impact LDL-C reductions seen in RCTs and might result in a lesser effect LDL-C reduction by PCSK9 inhibitors in everyday use.

Of note, our population differed from the patients in the ODYSSEY OUTCOMES study, as in this study only patients with recent cardiovascular events (< 12 months) were included [6]. History of cardiovascular diseases was frequent in our patients, but a recent or previous cardiovascular event was not a mandatory criterion to be eligible for analysis.

Our study showed a similar percentage of LDL-C reduction reported in a systematic review on PCSK9 inhibitors (LDL-C reduction of 53.86% at month 6) [13]. Real-world studies also observed inter-individual reduction of LDL-C and postulated that LDL-C goals cannot be reached in all patients [14–16]. This should be considered in the PCSK9 inhibitor treatment in clinical practice. Achievement of treatment goals should be regularly monitored in routine care with special attention to treatment adherence and therapy intensification, if possible.

In a prespecified analysis of the ODYSSEY OUTCOMES study comparing the effect of alirocumab on patients with diabetes, prediabetes and normoglycemia, no significant differences in LDL-C reduction were found across these subgroups (median LDL-C reduction of 64–65% at month 4) [17]. This finding was confirmed in another sub-cohort of the ODYSSEY collective [18]. In a sub-study of the FOURIER study, no differences in LDL-C reduction were seen when evolocumab was used in patients with and without diabetes (− 57% in patients with and − 60% in patients without diabetes) [7]. In our study, PCSK9 inhibitor therapy markedly lowered LDL-C in both patients with and without diabetes irrespective of glycemic control. Both, the FOURIER and the ODYSSEY OUTCOMES trial found a more pronounced absolute risk reduction of a composite of major cardiovascular events in patients with diabetes mellitus receiving PCSK9 inhibitors compared to those without diabetes [7, 17]. Patients with T2D are at an extraordinary increased cardiovascular risk. Therefore, patient with T2D and hypercholesterinemia especially benefit in particular from an intensified lipid-lowering treatment.

Clinical trials and genetic studies have ascertained a potential link between the use of lipid-lowering therapy and an increased risk for deterioration of glucose control and the development of T2D. In statin users the risk to develop T2D was estimated to be approximately 1:1.000 per year of exposure and patients with additional risk factors to develop T2D (e.g. age, prediabetes, metabolic syndrome) were at an even increased risk [19]. A Chinese study reported on the association between elevated circulating PCSK9 levels and an increased risk for T2D in women with prediabetes [20].

Schmidt et al. investigated the relation of PCSK9 gene variants and glycemic parameters in a mendelian study, suggesting a potential risk for new-onset diabetes in patients on PCSK9 inhibitor treatment. Mimicking the pharmacological effects of PCSK9 inhibition, this study found increased glucose concentrations and an increased risk of T2D in carriers of PCSK9 variants associated with low LDL-C levels. However, these findings represent the life-long effect of these gene variants and may not reflect the pharmacological PCSK9-targeted intervention later in life [10].

No significant changes on patients' Hb1Ac levels were found in our study, in both patients with and without preexisting diabetes. A limitation of our study was that only in a small number of patients all investigated parameters were available at all time-points. Nevertheless, the results are consistent with findings from past studies, which showed no relevant changes in glycemic control during PCSK9 inhibitor therapy [8, 21–24].

In our study we observed adjustments and intensifications of diabetes therapy when required. Of note, due to refund claims by the Austrian health insurance only patients with sufficiently controlled diabetes (HbA1c < 64 mmol/mol (8.0%)) are eligible for PCSK9 inhibitor therapy. Therefore, this study cannot provide data on potential effects of PCSK9 inhibitor therapy on glucose control, LDL-C and other parameters of the lipid panel as well as cardiovascular outcome in patients with insufficiently controlled diabetes mellitus.

While there is a plenty of observational data available demonstrating a reduction of cardiovascular outcomes by therapeutically reduce LDL-C [25, 26], to the best of our knowledge there is no real-world evidence available investigating these potentially beneficial effects when populations with PCSK9 inhibitor therapy were selected.

It has been shown that low PCSK9 levels are associated with the presence of metabolic syndrome and atherosclerosis in patients with coronary heart disease [27] and recently, a study conducted by Peng et al. described an important relationship between elevated circulating PCSK9 levels to be associated with higher cardiovascular morbidity and adverse cardiovascular outcomes specifically in patients with diabetes [28]. The authors suggest to measure PCSK9 levels in patients with diabetes to identify the ones with particularly high cardiovascular risk, indicating that these patients might be the ones who might even profit from lower LDL-targets (e.g. < 40 mg/dl) established by therapeutic PCSK9 inhibition [28, 29].

Within our population, 42 cardiovascular events of any kind during an 18 months observation, of those 15 events met the criteria to be characterized as a major adverse cardiovascular outcome event (MACE). Noteworthy, we have found significantly more all cause events in patients with T2D at baseline and intriguingly, despite a higher LDL reduction than the non-diabetic population those patients with T2D with HbA1c > 54 mmol/mol

experienced a fivefold higher risk of any cardiovascular event compared to those without diabetes. This finding emphasizes diabetes to be a further potent cardiovascular risk factor, specifically when insufficiently controlled which needs to be accordingly paid attention.

In our analysis we observed a reduction in triglyceride levels of about 20% throughout the observational period. This finding has to be interpreted with caution as patients do not mandatorily need to be in a fasting state for blood sampling in our clinic. Still, this result is in-line with previous research indicating a 12–30% reduction of triglycerides due to PCSK9 inhibitor therapy [30, 31]. In our population, this finding might be attributed to various factors: first, due to the national reimbursement criteria, all patients must receive professional nutritional advice prior to initiation of PCSK9 inhibitor therapy. Secondly, previous data suggests that intra- and extracellular PCSK9 might act in a complementary fashion to regulate triglyceride levels, on the one hand, intracellularly, by the ability of PCSK9 to modulate apolipoprotein B (APOB) secretion and on the other hand, extracellularly, by triggering LDL-receptor mediated catabolism [32]. Further investigations, focusing on the physiologic mechanisms which might explain the positive correlation of PCSK9 inhibitor therapy on triglyceride levels are in progress.

A further well-known cardiovascular risk factor is lipoprotein(a) [Lp(a)]. At present there are no approved medical agents to reduce Lp(a) available. Lp(a) currently can be lowered only by means of lipid apheresis and to some extent by dietary interventions [12]. In our study Lp(a) data over time are only available to a very limited extent. This is caused by current guidelines that suggest only a single Lp(a) determination to determine whether lipid apheresis might be necessary [12]. In secondary analyses of the FOURIER and the ODYSSEY OUTCOMES trials, alirocumab and evolocumab demonstrated only a small reduction (− 20%) of Lp(a) [33, 34], which is similar to the results seen in our study. A novel and not yet approved medical approach (antisense-oligonucleotide therapy) might lead to clinically relevant Lp(a) reduction in the future [35].

In our analysis, adverse effects of alirocumab or evolocumab were rare. Since the documented symptoms stated by the patients were mainly unspecific (e.g. joint or muscle pain, rhinitis), it remains speculative whether they were associated with PCSK9 inhibitor therapy. Most of the adverse events leading to discontinuation of PCSK9 inhibitor therapy occurred in patients without concomitant lipid-lowering therapy. Thus, the potential side effect can probably truly be attributed to the PCSK9 inhibitor therapy. The adverse effects described in our study are similar to those found in other studies [5, 6, 36, 37]. Overall, PCSK9 inhibitors appear were tolerated, although long-term data on adverse health effects are not available yet.

The strength of this retrospective study is the large sample size of 237 patients. Many real-world studies available have investigated only one of the two agents and were mainly sponsor-initiated studies [38]. Furthermore, real-world evidence is substantially lacking investigating the efficacy of PCSK9 inhibitor therapy on LDL-C reduction in patients with diabetes. In our study we describe for the first time, that patients with insufficiently controlled T2D might have a superior LDL-C reduction by PCSK9 inhibitor therapy compared to patients without diabetes mellitus. This finding needs to be addressed in prospective randomized controlled clinical trials. Additionally, special attention should be given to patients with. Also, insufficiently controlled T2D as glycemic control seems to have a relevant strong impact on cardiovascular events despite achieving LDL-C treatment goals. This finding might be attributed to the fact that in our population the proportion of patients on sodium glucose co-transporters 2 (SGLT2) inhibitors [39–41] and GLP1-RA [42, 43] was rather low at baseline. Both agents have demonstrated beneficial cardiovascular outcome in patients with T2D. The broad uptake of these medications into routine care only occurred in more recent years following guideline recommendations [44]. In the beginning these agents were mainly prescribed by diabetologists and endocrinologists whereas a large proportion of our patients were seen by a GP for diabetes care until the first presentation at our center. Potential beneficial cardiovascular effects might be expected in patients with diabetes receiving both PCSK9 inhibitor therapy as lipid-lowering agent and SGLT2 inhibitors and/or GLP1-RA for diabetes management.

By the retrospective nature of the study several limitations have to be addressed. The major limitation is the varying availability of data, especially laboratory data. This is due to the fact that in routine clinical care, no stringent protocol as in a clinical trial is applied. Many of our patients performed the laboratory analysis with their GP who might only chose a limited laboratory panel than recommended in our patient letters. Additionally, follow-up visits in many cases are performed via telemedical methods (i.e. video call, telephone call) which has a negative impact on availability of laboratory measurements or further baseline characteristics as BMI or blood pressure.

There was a relatively large number of patients (11.4%; n = 27) who were lost to follow-up (LTF). This might result in some selection bias and thus can impact the results. One can assume that more compliant and adherent patients thus are represented by our data. Also, no telephone follow-up was performed due to the nature of the study. So, there might even have been a fatal event in

one of patients LTF, as only fatalities within the Styrian hospital cluster (KAGES) and adjacent hospitals would have been noticed.

Additionally, concurrent lipid-lowering medication was ascertained only at baseline and potential adjustments were not considered in the analysis. Further, adherence to lifestyle recommendations were not systematically documented. As all patients who are prescribed with and reimbursed for PCSK9 inhibitor therapy obtain professional nutritional advice, the effect of this training should be evenly distributed among the investigated population.

Another limitation is the lack of continuous documentation of further cardiovascular risk factors such as body weight, body mass index or blood pressure. This is due to the fact that the enrolled patients presented in a specialized clinic for lipid metabolism disorders which focuses their recommendations on metabolic disease (lipids and diabetes); other cardiovascular risk factors are adjusted elsewhere. Are not taken and the patient is not always available on-site.

Conclusions

In conclusion, significant reductions in LDL-C and a high percentage of patients achieving the recommended treatment targets were observed in a real-world population over the course of 18 months. Interestingly, patients with T2D, and specifically those with insufficiently controlled T2D showed a superior response to PCSK9 inhibitor therapy. Still, they experienced a substantial number of cardiovascular events. Thus, efforts also must be made to achieve better glycemic control. Even when receiving PCSK9 inhibitor therapy, currently the most potent lipid-lowering agent, some patients did not meet LDL-C treatment goals as recommended in current guidelines. Special attention to these patients is required in the future to achieve these goals to avoid subsequent adverse cardiovascular outcomes.

Abbreviations

ASCVD: Atherosclerotic cardiovascular disease; EAS: European Atherosclerosis Society; ESC: European Society of Cardiology; FH: Familial hypercholesterolemia; GLP1: Glucagon- like peptide 1; HbA1c: Hemoglobin A1c; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; Lp(a): Lipoprotein (a); LTF: Lost to follow up; PCSK9: Proprotein convertase subtilisin/kexin type 9; RCTs: Randomized controlled trials; SGLT2: Sodium-glucose co-transporter 2; T1D: Diabetes mellitus type 1; T2D: Diabetes mellitus type 2; TIA: Transient ischemic attack; TG: Triglycerides.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12933-021-01283-w.

Additional file 1: Table S1. Cardiovascular disease at baseline (by medical condition). Data are number of patients (% from subgroup). **Table S2.** LDL-C levels over time of the overall cohort and by PCSK9 inhibitor agent (A) and according to diabetes status (B). Data are median (IQR). *P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time-point. **Table S3.** Triglyceride levels over time of the overall cohort and by PCSK9 inhibitor (A) and according to diabetes status (B). Data are median (IQR). *P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time-point. **Table S4.** HDL cholesterol levels over time of the overall cohort and by PCSK9 inhibitor agent (A) and diabetes status (B). Data are median (IQR). *P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time-point. **Table S5.** Lipoprotein(a) levels and follow-up measurement of the overall cohort, by PCSK9 inhibitor agent and by diabetes status. Data are median (IQR). *P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time. Only patients with follow-up data for Lp(a) were included in the analysis. † First performed follow-up measurement after initiation of PCSK9 inhibitor therapy was included in the analysis. **Table S6.** HbA1c levels over time of the overall cohort, without and with diabetes mellitus at baseline. Data are median (IQR). * P-values were calculated by using the Wilcoxon signed-rank test and refers to the comparisons of laboratory data between baseline and defined time-point.

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Authors' contributions

All of the authors have sufficiently contributed to this work. FA, JKM and LTF drafted the manuscript, TP and LTF performed the data collection. DAH, LK and TP approved the latest version to be published. All authors read and approved the final manuscript.

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Availability of data and materials

Data was extracted from medical records and saved on a separate password-protected file with anonymized data.

Declarations

Ethics approval and consent to participate

This study was a retrospective data analysis approved by the Medical University of Graz, Austria (EK Number 32-018 ex 19/20). By the nature of the study, it was not necessary to obtain an individual patient consent an individual basis per Austria regulations.

Consent for publication

This study was a retrospective data analysis approved by the Medical University of Graz, Austria (EK number 32-018 ex 19/20). By the nature of the study, it was not necessary to obtain an individual patient consent on an individual basis per Austria regulations. Consent for publication is covered within application and approval of the local ethics committee of the Medical University of Graz.

Competing interests

FA received speaker honoraria from Eli Lilly, Merck Sharp & Dome, Boehringer Ingelheim, Astra Zeneca. JKM is a member in the advisory board of Becton–Dickinson, Boehringer Ingelheim, Eli Lilly, Medtronic, Prediktor SA and Sanofi, and received speaker honoraria from Abbott Diabetes Care, Astra Zeneca, Eli Lilly, Dexcom, Medtronic, Novo Nordisk, Roche Diabetes Care, Sanofi, Servier, and Takeda.

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References

1. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017;38(32):2459–72.
2. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet. 2005;366(9493):1267–78.
3. Kim MK, Han K, Joung HN, Baek KH, Song KH, Kwon HS. Cholesterol levels and development of cardiovascular disease in Koreans with type 2 diabetes mellitus and without pre-existing cardiovascular disease. Cardiovasc Diabetol. 2019;18(1):139.
4. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk: the task force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). Eur Heart J. 2019;41(1):111–88.
5. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med. 2017;376(18):1713–22.
6. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, et al. Alirocumab and cardiovascular outcomes after acute coronary syndrome. N Engl J Med. 2018;379(22):2097–107.
7. Sabatine MS, Leiter LA, Wiviott SD, Giugliano RP, Deedwania P, De Ferrari GM, et al. Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the FOURIER randomised controlled trial. Lancet Diabetes Endocrinol. 2017;5(12):941–50.
8. Leiter LA, Zamorano JL, Bujas-Bobanovic M, Louie MJ, Lecorps G, Cannon CP, et al. Lipid-lowering efficacy and safety of alirocumab in patients with or without diabetes: a sub-analysis of ODYSSEY COMBO II. Diabetes Obes Metab. 2017;19(7):989–96.
9. Ray K. Statin diabetogenicity: guidance for clinicians. Cardiovasc Diabetol. 2013;12(Suppl 1):S3.
10. Schmidt AF, Swerdlow DI, Holmes MV, Patel RS, Fairhurst-Hunter Z, Lyall DM, et al. PCSK9 genetic variants and risk of type 2 diabetes: a mendelian randomisation study. Lancet Diabetes Endocrinol. 2017;5(2):97–105.
11. Monami M, Sesti G, Mannucci E. PCSK9 inhibitor therapy: a systematic review and meta-analysis of metabolic and cardiovascular outcomes in patients with diabetes. Diabetes Obes Metab. 2019;21(4):903–8.
12. Nordestgaard BG, Chapman MJ, Ray K, Boren J, Andreotti F, Watts GF, et al. Lipoprotein(a) as a cardiovascular risk factor: current status. Eur Heart J. 2010;31(23):2844–53.
13. Schmidt AF, Pearce LS, Wilkins JT, Overington JP, Hingorani AD, Casas JP. PCSK9 monoclonal antibodies for the primary and secondary prevention of cardiovascular disease. Cochrane Database Syst Rev. 2017;4(4):Cd011748.
14. Zafrir B, Jubran A. Lipid-lowering therapy with PCSK9-inhibitors in the real-world setting: two-year experience of a regional lipid clinic. Cardiovasc Ther. 2018;36(5):e12439.
15. Stoekenbroek RM, Hartgers ML, Rutte R, de Wijer DD, Stroes ES, Hovingh GK. PCSK9 inhibitors in clinical practice: delivering on the promise? Atherosclerosis. 2018;270:205–10.
16. Saborowski M, Dölle M, Manns MP, Leitolf H, Zender S. Lipid-lowering therapy with PCSK9-inhibitors in the management of cardiovascular high-risk patients: effectiveness, therapy adherence and safety in a real world cohort. Cardiol J. 2018;25(1):32–41.
17. Ray KK, Colhoun HM, Szarek M, Baccara-Dinet M, Bhatt DL, Bittner VA, et al. Effects of alirocumab on cardiovascular and metabolic outcomes after acute coronary syndrome in patients with or without diabetes: a prespecified analysis of the ODYSSEY OUTCOMES randomised controlled trial. Lancet Diabetes Endocrinol. 2019;7(8):618–28.
18. Ray KK, Leiter LA, Muller-Wieland D, Cariou B, Colhoun HM, Henry RR, et al. Alirocumab vs usual lipid-lowering care as add-on to statin therapy in individuals with type 2 diabetes and mixed dyslipidaemia: the ODYSSEY DM-DYSLIPIDEMIA randomized trial. Diabetes Obes Metab. 2018;20(6):1479–89.
19. Mach F, Ray KK, Wiklund O, Corsini A, Catapano AL, Bruckert E, et al. Adverse effects of statin therapy: perception vs. the evidence—focus on glucose homeostasis, cognitive, renal and hepatic function, haemorrhagic stroke and cataract. Eur Heart J. 2018;39(27):2526–39.
20. Shi J, Zhang W, Niu Y, Lin N, Li X, Zhang H, et al. Association of circulating proprotein convertase subtilisin/kexin type 9 levels and the risk of incident type 2 diabetes in subjects with prediabetes: a population-based cohort study. Cardiovasc Diabetol. 2020;19(1):209.
21. Leiter L, Tinahones F, Karalis D, Bujas-Bobanovic M, Letierce A, Mandel J, et al. Alirocumab safety in people with and without diabetes mellitus: pooled data from 14 ODYSSEY trials. Diabet Med. 2018;35(12):1742–51.
22. Ray KK, Leiter LA, Müller-Wieland D, Cariou B, Colhoun HM, Henry RR, et al. Alirocumab vs usual lipid-lowering care as add-on to statin therapy in individuals with type 2 diabetes and mixed dyslipidaemia: the ODYSSEY DM-DYSLIPIDEMIA randomized trial. Diabetes Obes Metab. 2018;20(6):1479–89.
23. Blom DJ, Koren MJ, Roth E, Monsalvo ML, Djedjos CS, Nelson P, et al. Evaluation of the efficacy, safety and glycaemic effects of evolocumab (AMG 145) in hypercholesterolaemic patients stratified by glycaemic status and metabolic syndrome. Diabetes Obes Metab. 2017;19(1):98–107.
24. Rosenson RS, Daviglus ML, Handelsman Y, Pozzilli P, Bays H, Monsalvo ML, et al. Efficacy and safety of evolocumab in individuals with type 2 diabetes mellitus: primary results of the randomised controlled BANTING study. Diabetologia. 2019;62(6):948–58.
25. Schubert J, Lindahl B, Melhus H, Renlund H, Leosdottir M, Yari A, et al. Low-density lipoprotein cholesterol reduction and statin intensity in myocardial infarction patients and major adverse outcomes: a Swedish nationwide cohort study. Eur Heart J. 2021;42(3):243–52.
26. Chamberlain AM, Gong Y, Shaw KM, Bian J, Song WL, Linton MF, et al. PCSK9 inhibitor use in the real world: data from the national patient-centered research network. J Am Heart Assoc. 2019;8(9):e011246.
27. Caselli C, Del Turco S, Ragusa R, Lorenzoni V, De Graaf M, Basta G, et al. Association of PCSK9 plasma levels with metabolic patterns and coronary atherosclerosis in patients with stable angina. Cardiovasc Diabetol. 2019;18(1):144.
28. Peng J, Zhu CG, Li JJ. The predictive utility of circulating PCSK9 levels on diabetes mellitus. Cardiovasc Diabetol. 2021;20(1):45.
29. Peng J, Liu MM, Jin JL, Cao YX, Guo YL, Wu NQ, et al. Association of circulating PCSK9 concentration with cardiovascular metabolic markers and outcomes in stable coronary artery disease patients with or without diabetes: a prospective, observational cohort study. Cardiovasc Diabetol. 2020;19(1):167.
30. Raal FJ, Stein EA, Dufour R, Turner T, Civeira F, Burgess L, et al. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. Lancet. 2015;385(9965):331–40.
31. Robinson JG, Nedergaard BS, Rogers WJ, Fialkow J, Neutel JM, Ramstad D, et al. Effect of evolocumab or ezetimibe added to moderate- or high-intensity statin therapy on LDL-C lowering in patients with hypercholesterolemia: the LAPLACE-2 randomized clinical trial. JAMA. 2014;311(18):1870–82.
32. Dijk W, Le May C, Cariou B. Beyond LDL: what role for PCSK9 in triglyceride-rich lipoprotein metabolism? Trends Endocrinol Metab. 2018;29(6):420–34.
33. Raal FJ, Hovingh GK, Blom D, Santos RD, Harada-Shiba M, Bruckert E, et al. Long-term treatment with evolocumab added to conventional drug therapy, with or without apheresis, in patients with homozygous familial hypercholesterolaemia: an interim subset analysis of the open-label TAUSSIG study. Lancet Diabetes Endocrinol. 2017;5(4):280–90.
34. Kastelein JJ, Ginsberg HN, Langslet G, Hovingh GK, Ceska R, Dufour R, et al. ODYSSEY FH I and FH II: 78 week results with alirocumab treatment in 735 patients with heterozygous familial hypercholesterolaemia. Eur Heart J. 2015;36(43):2996–3003.
35. Tsimikas S, Karwatowska-Prokopczuk E, Gouni-Berthold I, Tardif JC, Baum SJ, Steinhagen-Thiessen E, et al. Lipoprotein(a) reduction in persons with cardiovascular disease. N Engl J Med. 2020;382(3):244–55.
36. Blom DJ, Hala T, Bolognese M, Lillestol MJ, Toth PD, Burgess L, et al. A 52-week placebo-controlled trial of evolocumab in hyperlipidemia. N Engl J Med. 2014;370(19):1809–19.
37. Kereiakes DJ, Robinson JG, Cannon CP, Lorenzato C, Pordy R, Chaudhari U, et al. Efficacy and safety of the proprotein convertase subtilisin/kexin

type 9 inhibitor alirocumab among high cardiovascular risk patients on maximally tolerated statin therapy: the ODYSSEY COMBO I study. Am Heart J. 2015;169(6):906-15.e13.
38. Leitner DR, Toplak H, Kedenko L, Steinmaurer T, Graff V, Metzner T, et al. Efficacy and tolerability of alirocumab in Austrian clinical practice—results of the non-interventional PEARL-AT study. Curr Med Res Opin. 2020;36(9):1419–25.
39. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015;373(22):2117–28.
40. Wiviott SD, Raz I, Sabatine MS. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. Reply N Engl J Med. 2019;380(19):1881–2.
41. Neal B, Perkovic V, Matthews DR. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med. 2017;377(21):2099.
42. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2016;375(4):311–22.
43. Gerstein HC, Colhoun HM, Dagenais GR, Diaz R, Lakshmanan M, Pais P, et al. Dulaglutide and cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind, randomised placebo-controlled trial. Lancet. 2019;394(10193):121–30.
44. Buse JB, Wexler DJ, Tsapas A, Rossing P, Mingrone G, Mathieu C, et al. 2019 update to: Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia. 2020;63(2):221–8.

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