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Pharmacological aspects of ANGPTL3 and ANGPTL4 inhibitors: New therapeutic approaches for the treatment of atherogenic dyslipidemia

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Abstract

Among the determinants of atherosclerotic cardiovascular disease (ASCVD), genetic and experimental evidence has provided data on a major role of angiopoietin-like proteins 3 and 4 (ANGPTL3 and ANGPTL4) in regulating the activity of lipoprotein lipase (LPL), antagonizing the hydrolysis of triglycerides (TG). Indeed, beyond low-density lipoprotein cholesterol (LDL-C), ASCVD risk is also dependent on a cluster of metabolic abnormalities characterized by elevated fasting and post-prandial levels of TG-rich lipoproteins and their remnants. In a head-to-head comparison between murine models for ANGPTL3 and ANGPTL4, the former was found to be a better pharmacological target for the treatment of hypertriglyceridemia. In humans, loss-of-function mutations of ANGPTL3 are associated with a marked reduction of plasma levels of VLDL, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Carriers of loss-of-function mutations of ANGPTL4 show instead lower TG-rich lipoproteins and a modest but significant increase of HDL. The relevance of ANGPTL3 and ANGPTL4 as new therapeutic targets is proven by the development of monoclonal antibodies or antisense oligonucleotides. Studies in animal models, including non-human primates, have demonstrated that short-term treatment with monoclonal antibodies against ANGPTL3 and ANGPTL4 induces activation of LPL and a marked reduction of plasma TG-rich-lipoproteins, apparently without any major side effects. Inhibition of both targets also partially reduces LDL-C, independent of the LDL receptor. Similar evidence has been observed with the antisense oligonucleotide ANGPTL3-LRX. The genetic studies have paved the way for the development of new ANGPTL3 and 4 antagonists for the treatment of atherogenic dyslipidemias. Conclusive data of phase 2 and 3 clinical trials are still needed in order to define their safety and efficacy profile.

Keywords	angiopoietin-like 3 angiopoietin-like 4 antisense oligonucleotide evinacumab ANGPTL3-LRx
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No data was used for the research described in the article

Dear Editor,

please find enclosed copy of the last version of the review article "Pharmacological aspects of ANGPTL3 and ANGPTL4 inhibitors: new therapeutic approaches for the treatment of atherogenic dyslipidemia" by Massimiliano Ruscica et al. to be considered for publication on Pharmacological Research.

We have answered all the issues have been raised. Moreover, we have added two tables and described the most recent study on ANGPTL3 antisense oligonucleotide (evinacumab) published few days ago on Journal of Lipid Research.

We are most grateful for your help and interest and we wish you a pleasant holiday time.

Massimiliano Ruscica

This is an excellent, well written manuscript addressing a very timely and important subject. Bravo, my ongratulations to the authors.

We really appreciated this comment. Thank you.

Comments:

1. In the paragraph, lines 140-150, please rewrite to represent a summary of the current understanding of the mechanism of TG related risk:

- a. Hepatic hypersecretion of TG enriched VLDL particles, VLDL itself being atherogenic,
- b. Excess accumulation of remnant VLDL particles, especially emphasizing the type III dyslipiemia and role of RLP in general in hyperTG dyslipidemias in general,
- c. Formation of small, dense LDL particles with discordantly high LDL particle concentrations for given LDL-C, Overall non-HDL particle load being the major mediator of risk.
- d. reductions in HDL-C are largely driven by mass action exchange of cholesterol ester and TG between non-HDL and HDL particles via CETP, not a a causal mechanism of risk, shown not to be in the causal pathway by Mendelian

Randomization experiments.

We thank the reviewer for this constructive comment, we agree with. The text has been amended as follows "TG levels are the sum of the TG content in nascent very-low-density lipoproteins (VLDL) and in their remnants in the fasting state, together with TG in chylomicrons and their remnants in the postprandial state. Thus, TGRL remnants, encompassing a mixture of chylomicrons and VLDL particle, can be considered as a surrogate biomarker for plasma levels of both newly secreted TGRL and their remnants (7). As reported in the recent European Guidelines, desirable fasting TG levels are ≤ 150 mg/dL (1.7 mmol/L) and the use of drugs to lower TG should be considered in high-risk patients with TG > 200 mg/dL (2.3 mmol/L) and when TGs cannot be lowered by lifestyle changes (8). As TG levels rise over this range, large VLDL become the major TG-rich species due to either a rise in the hepatic production or a decrement in lipoprotein lipase (LPL) activity. In this scenario, LPL subsequently hydrolyzes VLDL to form smaller and denser lipoprotein particles, believed to be at least as atherogenic as LDL, being enriched in cholesteryl esters (9). Overall, with the exception of very large particles, e.g. chylomicrons, TGRL can enter the arterial wall contributing to the deposition of cholesterol content in the atherosclerotic plaque (10). In individuals with normal TG, for every chylomicron remnant particle there are approximately 10 VLDL particles, that have a short half-life compared to LDL. Considering that for every VLDL particle there are approximately 9 LDL particles, this proportion could explain why a rise in plasma TG will lead to many more LDL particles than VLDL particles (11). This ratio is not exactly maintained in type III hyperlipoproteinemia, a rarer phenotype characterized by abnormal apoB48 remnant particles plus abnormal VLDL apoB100 remnant particles, present at concentrations 30–50 times higher than normal remnant particles (12). The association between TG lowering and reduction of ASCVD risk has been recently reaffirmed in a meta-regression analysis of three classes of therapies (fibrates, niacin, and marine-derived omega-3 fatty acids) in addition to 25 statin trials, showing that TG lowering associates with a lower risk of major vascular events, even after adjustment for LDL-C lowering: Relative Risk (RR) 0.84; 95% CI, 0.75-0.94; per 1-mmol/L (40 mg/dL) TG reduction (13). Although similar conclusions have been reached by genetic studies supporting the causal role of raised TG levels on ASCVD risk (14, 15), it is worth mentioning that, in addition, the clinical benefit of lowering TG and LDL-C levels is proportional to the absolute change in apoB (16)."

2. Lines 266-70, please expand discussion of mechanism of PPAR and interaction with ANGPTL providing greater detail for the reader to better frame the regulatory effects of PPAR at the nuclear level and the peripheral effects of ANGPTL 3/4/8 peripherally.

Thank you. The text now reads as follows "ANGPTL4 expression is also induced by ligands of all peroxisome proliferator-activated receptors (PPAR- α , - δ , and γ) (77), although the fold induction of mRNA in tissues may vary, possibly due to individual variations among animal or in vitro models (78, 79)."

3. Line 288: "...TR?? dependent manner..." is unclear.

We thank the reviewer. The text has been amended and now reads as follows "Thyroid hormones suppress the gene expression of ANGPTL3 but not that of ANGPTL4 via activation of the thyroid hormone receptor β , thus providing a potential mechanism explaining the hypotriglyceridemic properties of thyroid hormone receptor β agonists (74). In patients with clinical and subclinical hypothyroidism, high ANGPTL3 levels have been described, an observation fitting with the negative correlation among ANGPTL3, total tri-iodothyronine and free tri-iodothyronine (81)."

4. In conclusions section, it would be informative to the reader to briefly mention the problems that have arisen with both mAb or RNA interference strategies aimed at apoC3 suppression resulting in thrombocytopenia. Thus far, to my knowledge, this problem has not emerged as a problem with strategies using ANGPTL 3/4 as target of therapy.

Thank you, this part has been added and the text now reads as follows "The ASO was safe, an important aspect, considering the thrombocytopenia found in patients given volanesorsen, an ASO against apoC-III, reducing chylomicron TG by roughly 83% (150)."

Reviewer 2

The manuscript of Ruscica and coll. is a revision regarding the pathophysiology and the emerging therapeutic approaches for the atherogenic dyslipidemia, a condition characterized by an abnormal increase of TG-rich lipoproteins (TRL). The work is interesting, well documented and written, focused on really new aspects of the metabolism of TRL that will be surely an important target for the development of future therapies in the cardiovascular field. I have only few comments, generally aimed to improving the fluidity and the clarity of the message. They are listed here below:

Graphical abstract: the role of LPL should be better represented in the image, with the list of activator/inhibitors of the enzymatic activity. ANGPTL 3 and 4 should be graphically magnified, to stress the concept that they will be the main object of discussion.

A modified graphical abstract has been added.

Abstract: the first sentence (L 64-67) should focus on the residual risk in the statins era, i.e on the so-called "atherogenic dyslipidemia" largely determined by a deranged TRL

metabolism. Thank you for this comment we agree with. The text now reads as follows "Among the determinants of atherosclerotic cardiovascular disease (ASCVD), genetic and experimental evidence has provided data on a major role of angiopoietin-like proteins 3 and 4 (ANGPTL3 and ANGPTL4) in regulating the activity of lipoprotein lipase (LPL), antagonizing the hydrolysis of triglycerides (TG). Indeed, beyond low-density lipoprotein cholesterol (LDL-C), ASCVD risk is also

dependent on a cluster of metabolic abnormalities characterized by elevated fasting and post-prandial levels of TG-rich lipoproteins and their remnants.”

L 78-79: the sentence may be shortened (“ANGPTL3 and ANGPTL4 are new therapeutic targets of biological therapies, i.e. monoclonal antibodies or antisense oligonucleotides”).

Thank you. The text has been amended *as per* suggestion.

L 87: Please cut “thus suggesting their potential use in homozygous familial hypercholesterolemia”, a comment that is not useful in Abstract but in Discussion .

Thank you. The text has been amended *as per* suggestion

Introduction: as above indicated, the relevance of the topic is largely dependent on the “residual risk after statins”. The remaining text is indeed fully in line with this consideration. Part 3, L 281: what does it mean “Thyroid hormones ... to suppress ANGPTL3 mRNA in a TR??-dependent manner” In particular TR??- dependent manner? Please specify.

Thank you. The text has been amended accordingly and now reads as follows “Thyroid hormones suppress the gene expression of *ANGPTL3* but not that of *ANGPTL4* via activation of the thyroid hormone receptor β , thus providing a potential mechanism explaining the hypotriglyceridemic properties of thyroid hormone receptor β agonists ([74](#)). In patients with clinical and subclinical hypothyroidism, high *ANGPTL3* levels have been described, an observation fitting with the negative correlation among *ANGPTL3*, total tri-iodothyronine and free tri-iodothyronine ([81](#)).”

In the same phrase the classification (i - ii- iii) does not appear logical; it may be better grouped in “This observation fits well (i) with the negative correlation between ANGPTL3, total tri-iodothyronine (TT3) and free tri-iodothyronine (FT3) (69) and (ii) with the high ANGPTL3 levels.....”

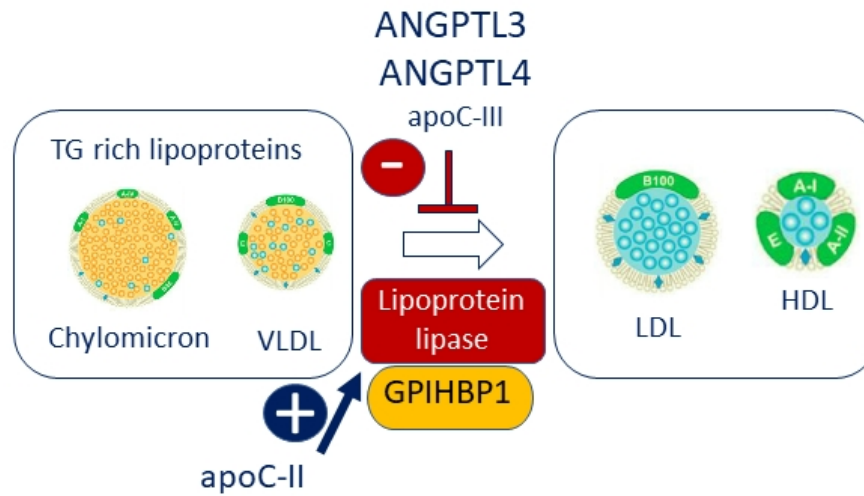
We thank the reviewer for this comment we agree with and the text has been amended accordingly.

Part 4 , L 342: please write ” in extenso” the acronymus BAT and WAT

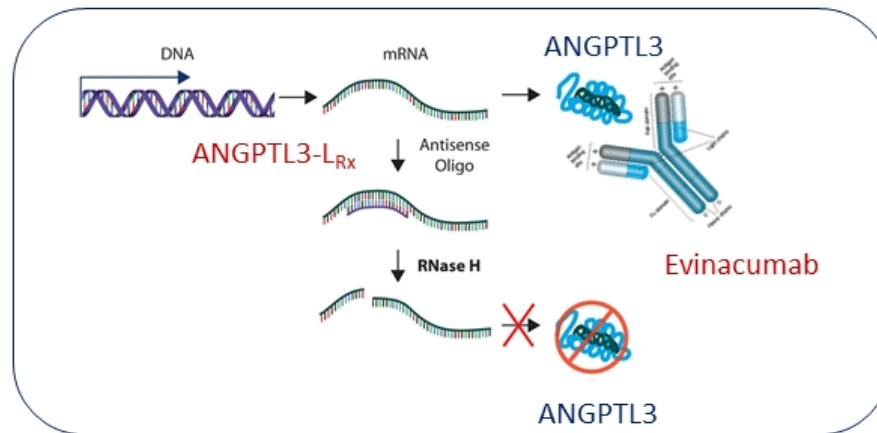
Thank you. BAT and WAT have been reported in extenso throughout the manuscript.

PART 7, L603: please specify that Fh is in the large majority of patients due to the loss of the LDL RECEPTOR..

Thank you. The text has been amended accordingly and now reads as follows “Evinacumab was also tested in nine adults with homozygous familial hypercholesterolemia for LDLR, including two null homozygotes and one compound heterozygote with two null alleles.”



Pharmacological Inhibition of ANGPTL3



**Pharmacological aspects of ANGPTL3 and ANGPTL4 inhibitors:
new therapeutic approaches for the treatment of atherogenic dyslipidemia**

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Abstract

Among the determinants of atherosclerotic cardiovascular disease (ASCVD), genetic and experimental evidence has provided data on a major role of angiopoietin-like proteins 3 and 4 (ANGPTL3 and ANGPTL4) in regulating the activity of lipoprotein lipase (LPL), antagonizing the hydrolysis of triglycerides (TG). Indeed, beyond low-density lipoprotein cholesterol (LDL-C), ASCVD risk is also dependent on a cluster of metabolic abnormalities characterized by elevated fasting and post-prandial levels of TG-rich lipoproteins and their remnants. In a head-to-head comparison between murine models for ANGPTL3 and ANGPTL4, the former was found to be a better pharmacological target for the treatment of hypertriglyceridemia. In humans, loss-of-function mutations of *ANGPTL3* are associated with a marked reduction of plasma levels of VLDL, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Carriers of loss-of-function mutations of *ANGPTL4* show instead lower TG-rich lipoproteins and a modest but significant increase of HDL. The relevance of ANGPTL3 and ANGPTL4 as new therapeutic targets is proven by the development of monoclonal antibodies or antisense oligonucleotides. Studies in animal models, including non-human primates, have demonstrated that short-term treatment with monoclonal antibodies against ANGPTL3 and ANGPTL4 induces activation of LPL and a marked reduction of plasma TG-rich-lipoproteins, apparently without any major side effects. Inhibition of both targets also partially reduces LDL-C, independent of the LDL receptor. Similar evidence has been observed with the antisense oligonucleotide ANGPTL3-L_{RX}. The genetic studies have paved the way for the development of new ANGPTL3 and 4 antagonists for the treatment of atherogenic dyslipidemias. Conclusive data of phase 2 and 3 clinical trials are still needed in order to define their safety and efficacy profile.

Keywords: angiopoietin-like 3, angiopoietin-like 4, antisense oligonucleotide, evinacumab, ANGPTL3-L_{RX}

1. Introduction

A series of modifiable and non-modifiable risk factors contributes to the atherosclerotic cardiovascular disease (ASCVD). Among them, the pharmacological control of hypercholesterolemia has represented the most effective therapy in the prevention of CVD. The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, statins, successfully lower low-density lipoprotein cholesterol (LDL-C) and reduce the rate of CV events in many patients. Despite the fact that LDL-C is causal in the development of atherogenesis and ASCVD, new options are required to control high levels of triglyceride-rich lipoproteins (TGRL) (1) or lipoprotein (a)(2). Beyond LDL-C, ASCVD risk is also dependent on a cluster of metabolic abnormalities characterized by elevated fasting and post-prandial levels of TGRL and their remnants (3), low levels of high-density lipoprotein cholesterol (HDL) and elevated small dense LDL (4). Thus, besides therapies directed at reducing only LDL-C levels, pharmacological approaches targeting other lipoproteins, e.g. triglycerides (TG) and/or HDL-C (5), can be considered in order to reduce the CV residual risk (6).

TG levels are the sum of the TG content in nascent very-low-density lipoproteins (VLDL) and in their remnants in the fasting state, together with TG in chylomicrons and their remnants in the postprandial state. Thus, TGRL remnants, encompassing a mixture of chylomicrons and VLDL particle, can be considered as a surrogate biomarker for plasma levels of both newly secreted TGRL and their remnants (7). As reported in the recent European Guidelines, desirable fasting TG levels are ≤ 150 mg/dL (1.7 mmol/L) and the use of drugs to lower TG should be considered in high-risk patients with TG > 200 mg/dL (2.3 mmol/L) and when TGs cannot be lowered by lifestyle changes (8). As TG levels rise over this range, large VLDL become the major TG-rich species due to either a rise in the hepatic production or a decrement in lipoprotein lipase (LPL) activity. In this scenario, LPL subsequently hydrolyzes VLDL to form smaller and denser lipoprotein particles, believed to be at least as atherogenic as LDL, being enriched in cholesteryl esters (9). Overall, with the exception of very large particles, e.g. chylomicrons, TGRL can enter the arterial wall contributing to the deposition of cholesterol content in the atherosclerotic plaque (10). In individuals with normal TG, for every chylomicron remnant particle there are approximately 10 VLDL particles, that have a short half-life compared to LDL. Considering that for every VLDL particle there are approximately 9 LDL particles, this proportion could explain why a rise in plasma TG will lead to many more LDL particles than VLDL particles (11). This ratio is not exactly maintained in type III hyperlipoproteinemia, a rarer phenotype characterized by abnormal apoB48 remnant particles plus abnormal VLDL apoB100 remnant particles, present at concentrations 30–50 times higher than normal remnant particles (12).

The association between TG lowering and reduction of ASCVD risk has been recently reaffirmed in a meta-regression analysis of three classes of therapies (fibrates, niacin, and marine-derived omega-3 fatty acids) in addition to 25 statin trials, showing that TG lowering associates with a lower risk of major vascular events, even after adjustment for LDL-C lowering: Relative Risk (RR) 0.84; 95% CI, 0.75–0.94; per 1-mmol/L (40 mg/dL) TG reduction (13). Although similar conclusions have been reached by genetic studies supporting

the causal role of raised TG levels on ASCVD risk (14, 15), it is worth mentioning that, in addition, the clinical benefit of lowering TG and LDL-C levels is proportional to the absolute change in apoB (16).

Catalyzing the partial hydrolysis of the core TG of circulating chylomicrons and VLDL to non-esterified fatty acids and 2-monoacylglycerol for tissue utilization, LPL represents the ideal target to control hypertriglyceridemia (17). TGRL are too large to cross capillary endothelia and thus, in order to gain access to triacylglycerols in these particles, LPL must be exposed to the luminal surface of the capillary endothelial cells. Once produced and correctly folded in myocytes and adipocytes, LPL is secreted into the subendothelial space and translocated to the lumen of capillaries by the glycosylphosphatidylinositol-anchored HDL binding protein (GPIHBP1) (18, 19), that retains LPL anchored to the endothelium (20-23). Highly charged, membrane bound chains of heparan sulphate-proteoglycans (HSPG) also contribute to anchor LPL on the luminal surface (17, 24), but since this binding is weak, LPL is shuttled to GPIHBP1 (25): the enzymatically active form of LPL is a 1:1 heterodimeric complex with GPIHBP1 (26, 27). Nevertheless, GPIHBP1 stabilizes LPL, thus enhancing its lipase activity (28, 29), an effect confirmed in GPIHBP1 knockout mice and patients with loss-of-function mutations of *GPIHBP1* which associated with hyperchylomicronemia (21, 23). GPIHBP1 is expressed highly in heart, adipose tissue and skeletal muscle, the same tissues that express high levels of LpL. In each of these tissues, GPIHBP1 is located on the luminal face of the capillary endothelial cells and not in the endothelium of brain capillaries (18, 21). This suggests a more important role for HSPG in tethering LPL to atherosclerosis-prone vessels (30). The identification of autoantibodies against GPIHBP1 has explained the reason why patients who have a clinical manifestation of familial chylomicronemia syndrome (about 3%), are negative to the genetic screening of five canonical genes for monogenic chylomicronemia (31, 32).

Rare loss-of-function mutations of genes involved in TG metabolism, such as *LPL* (33, 34), are associated to higher plasma TG levels and CV risk, whereas genetic inactivation of *ApoC3*, *Angptl3* and *Angptl4* genes, which encode for natural inhibitors of LPL, are associated with lower TG levels and a corresponding lower CVD risk (35-37). Importantly, some variants of *LPL* and *LDL receptor (LDLR)* are associated to similar lower coronary heart disease (CHD) risk per unit and to lower levels of apoB-containing lipoproteins (16). This association is additive and proportional to the absolute change in apo-B, indicating that the clinical benefit of TG lowering is similar to the clinical benefit of LDL-C lowering (16). Based on these findings, several novel therapies that potentially reduce TGRL are currently in development (38-40). Although many pharmacological targets can be envisioned to reduce TGRL levels, lipase antagonism may represent a successful example of clinical relevance (41).

In this complex scenario, another main player is represented by the apolipoprotein (apo)C-II. Synthesized in the liver and intestine, it is secreted as a surface component of chylomicrons, VLDL and HDL representing a specific activator/cofactor of LPL (42). The presence of apoC-II is required for a proper enzymatic activity of LPL, whereas three proteins from the angiopoietin-like (ANGPTL) family - ANGPTL3, ANGPTL4 and ANGPTL8 (37, 38, 43-45) as well as apoC-III are its physiological inhibitors (46). Thus, the

present review will discuss the current development of new pharmacological inhibitors of ANGPTL3 and ANGPTL4 as potential treatments for treating hypertriglyceridemia and associated CVD risk.

2. Biology of ANGPTL3, ANGPTL4 and ANGPTL8

Angiopoietins are members of the vascular endothelial growth factor (VEGF) family (47-49). ANGPTL3 is a 70kDa protein mainly expressed and secreted by the liver (43, 50), and to a lesser extent by the kidney (50), from which it is released at higher levels in case of renal damage (51-53). ANGPTL3 shares with its family members a N-terminal and a C-terminal fibrinogen-like domain (FLD), conserving the fibrinogen domain (~40% of sequence identity), except for a signal peptide (16 amino acids) required for its secretion (54, 55) (Figure 1). A linker region between N- and C-terminal domains is necessary for the ANGPTL3 biological activation. After intracellular and extracellular cleavages by furin (PCSK3) and PACE4 (PCSK6), respectively (56), the N-terminal domain is released being able to inhibit the activity of LPL more efficiently than full-length ANGPTL3 (54, 57): ANGPTL3 circulates in the plasma as full-length and truncated forms.

The human ANGPTL4 glycoprotein (~45-65 KDa) contains an N-terminal coiled-coil structure and a C-terminal FLD (58) (Figure 1). ANGPTL4 shares with ANGPTL3 31% of the amino acid sequence identity as well as modular structure. ANGPTL4, discovered independently during a search of additional angiopoietin-related proteins (59), is induced by fasting (60) and during preadipocyte differentiation (61). ANGPTL4 is produced in many cells and tissues, including adipose tissue, liver, intestine and muscle (61). Before secretion ANGPTL4 forms dimers and tetramers, whereas after secretion it undergoes cleavage at a canonical proprotein convertase cleavage site, *i.e.*, 161-Arg-Arg-Lys-Arg-164 (62). After cleavage, the N-terminal fragment, which remains oligomerized, is allowed to bind transiently to LPL. This process converts LPL from catalytically active dimers to inactive monomers, thus reducing the LPL activity (63).

Differently from the above described members of the same family, ANGPTL8 (also known as betatrophin) lacks the FLD leading to a protein of 22 kDa, *i.e.* less than half the size of ANGPTL3 and ANGPTL4 (45, 64) (Figure 1). The comparison between the ANGPTL8 sequence and the N-terminal domains of ANGPTL3 and ANGPTL4 highlighted the presence of a Specific Epitope1 (SE1) region, also mapped on ANGPTL3 and ANGPTL4, as necessary and sufficient to inhibit LPL activity (65-67). ANGPTL8 is a feeding-induced hepatokine, highly expressed in liver, white adipose tissue and brown adipose tissue (45, 68, 69).

3. Transcriptional regulation of ANGPTL3 and ANGPTL4

ANGPTL3 is transcriptionally regulated by Liver X receptors (LXRs) and Hepatocyte Nuclear Factor 1 α (HNF1 α) (70). This evidence derived from mice fed to a high-cholesterol diet and treated with a synthetic LXR-selective agonist (T0901317); this raises the hepatic gene expression of *Angptl3* (70). The analysis of the transcriptional response elements of mouse and human ANGPTL3 genes identified an LXR binding site, required for the regulation by LXR transcription factors (70, 71). LXR also drives the expression of LPL (72)

and ANGPTL8 (73), thus suggesting the presence of a common regulatory pathway. In contrast, HNF1 α indirectly inhibits the expression of ANGPTL3, i.e. through the activation of the thyroid hormone receptor (74). A repressive stimulus on the transcriptional activity of ANGPTL3 is mediated by insulin and leptin, thus supporting the hypothesis of a possible link between diabetic condition and dyslipidemia (75, 76). In adipose tissue and liver, *ANGPTL4* expression is regulated by feeding, fasting, with a strong induction under this latter condition, suggesting a pivotal role in fat metabolism (60). *ANGPTL4* expression is also induced by ligands of all peroxisome proliferator-activated receptors (PPAR- α , - δ , and γ) (77), although the fold induction of mRNA in tissues may vary, possibly due to individual variations among animal or *in vitro* models (78, 79).

A head-to-head comparison between ANGPTL3 and ANGPTL4 clearly pointed out that, although both inhibit LPL activity and raise plasma TG levels, they are regulated by different nuclear receptors (78). In particular, ANGPTL4 expression is induced in many tissues, including heart and skeletal muscle, whereas LPL hydrolyzes circulating lipoproteins for energy expenditure (78). Consistently, ANGPTL4 overexpression in the heart reduces the use of lipoprotein-derived free fatty-acids (FFA) in cardiac tissue, a mechanism mediated by a repression of the LPL activity (80).

Thyroid hormones suppress the gene expression of *ANGPTL3* but not that of *ANGPTL4* via activation of the thyroid hormone receptor β , thus providing a potential mechanism explaining the hypotriglyceridemic properties of thyroid hormone receptor β agonists (74). In patients with clinical and subclinical hypothyroidism, high ANGPTL3 levels have been described, an observation fitting with the negative correlation among ANGPTL3, total tri-iodothyronine and free tri-iodothyronine (81).

4. Role of ANGPTL3 and ANGPTL4 on lipid metabolism

Both ANGPTL3 and ANGPTL4 are involved in the regulation of breakdown and lipid storage. ANGPTL3 decreases VLDL-TG clearance by different mechanisms: by inhibiting LPL activity (82) and by a direct activation of lipolysis in adipocytes (83), a process resulting in FFA and glycerol release into the circulation (83). LPL is involved in lipid-related pathological conditions, including atherosclerosis (84), diabetes and obesity, Alzheimer's disease and cachexia (17). Beyond LPL, evidence coming from experimental models suggested an inhibitory effect of ANGPTL3 on endothelial lipase (EL) (85). This enzyme is expressed by endothelial cells and acts in the plasma similar to LPL (85, 86).

Since the N-terminal domain of ANGPTL3 interacts directly with LPL (65) and EL (85), it is not surprising that the presence of this domain is required for the inhibitory activity (54). This feature is completely abolished in the absence of the heparan sulphate-proteoglycans (HSPG) that anchor EL to endothelial cells (85). However, the molecular mechanism underlying the inhibition of LPL by ANGPTL3 is still unclear. It has been hypothesized that ANGPTL3 may induce LPL cleavage by the way of proprotein convertases PACE4 and furin, an effect specific for LPL but not for EL (87). Overall, the mechanism of ANGPTL3 is thus to foster TG to adipose tissue for storage during feeding through the tissue specific expression of the

modulator ANGPTL8 (64). Regarding ANGPTL8, its inhibitory activity on LPL is observed only in the presence of ANGPTL3 (88-90). Beyond LPL, ANGPTL3 inhibits EL, a crucial enzyme regulating plasma HDL levels (91). In humans, ANGPTL3 concentration positively correlated with HDL-C (85), a finding dependent on the inhibition of EL activity.

All-in-all, it is possible to envision that ANGPTL3, 4 and 8 regulate TG metabolism by inhibiting LPL in different tissues and under different nutritional status. During fasting an induction of ANGPTL4 and a suppression of ANGPTL8 (92-94) were observed, a condition affecting ANGPTL3 activity. The suppression of ANGPTL8 during fasting state is mediated by the increased levels of glucocorticoids and their binding to negative glucocorticoid responsive elements in the promoter region (94). Downregulation of ANGPTL8 leads to a higher activity of LPL in the skeletal muscle and heart (95), thus promoting TG hydrolysis and increased circulating FFA concentrations. Conversely, increased ANGPTL4 protein reduces LPL activity in the adipose tissue (96), promoting lipolysis in the adipocytes (97). This site-specific modulation of LPL activity leads the circulating TG toward peripheral tissues for utilization. Conversely, feeding leads to the upregulation of ANGPTL8 and the downregulation of ANGPTL4, directing plasma TG to adipose tissues for storage. Apart from fasting, during physical exercise, ANGPTL4 is induced in non-exercising skeletal muscle, likely serving to divert plasma TG to exercising muscle to be used as energy source (98). In addition, during cold exposure, ANGPTL4 is suppressed in brown adipose tissue and induced in white adipose tissue, thus ensuring an adequate energetic provision of TG to brown fat cells (96, 99). Overall, ANGPTL4 is to be considered as part of a shuttling mechanism directing fatty acids derived from circulating TGRL to brown adipose tissue during sustained cold exposure (96).

5. Phenotype of *Angptl3* and *Angptl4* knock-out and transgenic mice

The first genetic evidence of the role of ANGPTL3 on lipid metabolism was made by Koishi *et al* who identified an insertion mutation of the *Angptl3* gene associated with a hypolipidemic phenotype in obese KK mice (100). Levels of TG, total cholesterol and free fatty acids (FFA) in these mice were lower than those of wild type mice. Overexpression of *Angptl3* in murine models, e.g. by injection of adenovirus or human ANGPTL3 or administration of recombinant ANGPTL3, led to significant increases of TG, total cholesterol and FFA levels within one day post injection, reaching a peak after about four days (100). Generation of *Angptl3* knock-out mice corroborated its role in lipid metabolism (101). The absence of ANGPTL3 reduces TG, total cholesterol and FFA concentrations with a concomitant rise in LPL activity, i.e. +1.57 fold compared to wild type mice (101). In addition, the *Angptl3*-null mice fed a high fat diet had lower adipose tissue weight despite no differences in adipocyte size (101). The same experimental model showed a significant fall in the uptake of circulating VLDL-TG into white adipose tissue, rather than into skeletal muscle, brown adipose tissue and heart (102). The effect on the adipose tissue has been further corroborated by observations that in the absence of ANGPTL3 or ANGPTL8 fat mass is reduced after a +1°C increase in temperature in the fed (not fasted)

condition, without any change in physical activity or food intake (103). In addition, short-term cooling was shown to increase plasma ANGPTL4, ANGPTL3 and ANGPTL8 levels in young, healthy, lean men (104). These ANGPTLs are thought to act in concert to facilitate TG partitioning among tissues in response to cold (104).

Thus, ANGPTL3 or ANGPTL8 are essential for an efficient storage of dietary TG and deletion of these genes increases energy use and feeding-induced thermogenesis (103). In fed mice, the pharmacological inactivation of ANGPTL3 by the use of antibodies leads to a reduced hepatic secretion and plasma levels of TG (105). Besides TG, ANGPTL3 inactivation reduced LDL-C and apoB levels, despite no changes in hepatic apoB secretion, thus suggesting an increased clearance of apoB-containing lipoproteins (105). Similar lipid-modifying effects have been described, in a model of dyslipidemic Cynomolgus monkeys, after the administration of an antibody against ANGPTL3 (106).

Considering the ability of ANGPTL4 to inhibit LPL, knock-out mice showed a decrement of plasma TG and a faster initial weight gain when these were fed with a HFD (107, 108). Unexpectedly, the growth of *Angptl4*-null mice reached a plateau after 12 weeks of age, after that an opposite effect was seen, *i.e.* weight loss associated to an anorexic state. This condition led to a premature death between weeks 15 and 25 due to the development of severe fibrinopurulent peritonitis with ascites (107). In the same model, an intestinal fibrosis was observed, with a compressed liver and a hyperplastic spleen. Moreover, mesenteric lymph nodes underwent dramatic expansion and contained numerous lipid-laden macrophages (107).

The regulation of LPL by ANGPTL4 is essential for the protection from the proinflammatory effects induced by saturated fatty acids, leading to lipid accumulation into macrophages within mesenteric lymph nodes. This effect is associated to foam cell formation and a massive inflammatory response characterized by severe mesenteric lymphadenitis. These data suggest that the homozygous carrier status of the E40K mutation in *Angptl4*, associated to higher LPL activity and lower plasma TG (62, 109), may be more sensitive to the proinflammatory effects of dietary saturated fat. Even more intriguingly, recent evidence highlighted a relevant anti-inflammatory action of ANGPTL4, exerted by modulating macrophage polarization by mesenchymal stem cells during cardiac repair (110).

Finally, relative to glucose metabolism findings are not conclusive. In transgenic mice overexpressing *Angptl4* it has been reported (i) no impairment on glucose levels, (ii) a rise in blood glucose levels (44, 111), and (iii) an improvement or an impairment in glucose tolerance (112-114). In contrast, the functional studies on *Angptl4*-null mice demonstrated an improvement in insulin sensitivity and glucose homeostasis (115), suggesting that the inhibition of ANGPTL4 may reduce the risk of type 2 diabetes.

6. Preclinical development of ANGPTL3 and ANGPTL4 inhibitors

The identification of individuals carrying rare loss-of-function variants in *Angptl3* has confirmed the relevant role of this protein in the metabolism of TGRL, also considering that these subjects have lower TG levels, LDL-C and HDL-C (43, 116-119). This lipid profile is defined familial combined hypolipidemia and it seems not

associated with peculiar pathological manifestations (120). Similar findings in the lipid profile were described for variants of ANGPTL4, although these subjects had reduced TG but elevated HDL-C (78).

Interestingly, the use of a humanized mouse model deficient in the *Ldlr* highlighted that silencing of ANGPTL3 led to a modest reduction on LDL-C, thus suggesting that the LDL-C lowering was linked to *Ldlr* (92). This observation has been supported in *Ldlr*^{+/-} mice injected with silencing *Angptl3* and PCSK9. Reduction of total cholesterol (TC) and LDL-C levels were larger than those achieved from silencing *Angptl3* or *PCSK9* alone (121). Currently, an open question is how inactivation of ANGPTL3 leads to the reduction in LDL-C levels. Some hypotheses have been raised: (i) changes in VLDL apoB production rate or LDL apoB fractional catabolic rate (86), (ii) reduced apoB secretion and enhanced uptake of apoB-containing lipoproteins (93), and (iii) increased LDL clearance due to a rise in the inactive form of PCSK9 (94). On the other hand, the raising effect of ANGPTL3 on HDL seems to be the direct consequence of the inhibitory phospholipase activity on EL (122).

A role of ANGPTL3 and ANGPTL4 has also been documented with respect to the HDL promoting-cholesterol efflux. A direct correlation has been in part observed between plasma levels of these proteins and the HDL cholesterol efflux capacity in humans (123-125). Overall, the plasma concentrations of HDL of the studied subjects mainly accounted for the variation observed in HDL cholesterol efflux capacity.

Genetic variants leading to reduction in the levels of ANGPTL3 and ANGPTL4 are associated with a reduced CHD risk, with odds ratios ranging between 0.61 and 0.66 (11, 12, 14). In particular, null variants of ANGPTL3 lead to a unique form of familial hypobetalipoproteinemia, characterized by lower levels of all lipoproteins (116) and enhanced insulin sensitivity without an increased prevalence of fatty liver disease (116).

The explanation for a reduced CVD risk may be related to a life-long exposure to low levels of LDL-C. However, the extent of risk reduction in ANGPTL3 loss-of-function variants is estimated to be larger than the one predicted by the LDL lowering effect, suggesting that the stimulation of LPL-promoted lipolysis might translate into additional cardiovascular protection (126). These genetic observations strongly support the utility of developing new ANGPTL3 and 4 inhibitors to reduce TG and the incidence of CVD. A summary of the pre-clinical evidence relative to ANGPTL3 or ANGPTL4 inhibitors is shown in Table 1A.

Evinacumab (REGN1500) is a fully human monoclonal antibody directed to ANGPTL3 (Box1)(127). By using surface plasmon resonance, Gusarova et al. measured the relative affinity of evinacumab to human, monkey, rat and mouse ANGPTL3 (106) and showed that the drug binds ANGPTL3 with comparable affinities in all four species ($K_d=0.26\div1.28$ nM). In addition, evinacumab reverted the inhibition of LPL mediated by ANGPTL3 with IC_{50} values of $2.9\div9.6$ nM (106). In an experimental model of hypercholesterolemic mice, the injection of 25 mg/kg once weekly of evinacumab for 8 weeks dramatically reduced TG (-53%), total cholesterol (-35%) and LDL-C (-45%) (106). These findings were then reproduced in nonhuman primates (*Cynomolgus* monkeys) treated with single doses of evinacumab (106). The 3 mg/kg dose reduced by 48% plasma TG levels, with a doubling of effect with 10 mg/kg (-89%). The lipid lowering effect was maintained

for 33 days after the single injection (106). Very recently, in APOE*3-Leiden.CETP mice, triple treatment with atorvastatin + alirocumab + evinacumab was superior to atorvastatin in reducing plasma total cholesterol (-68%), non-HDL-C (-84%), and TG levels (-67%). Relative to atheroma formation and composition, the effect of triple combo blocked progression and led to a stronger regression of atherosclerotic lesion size, resulting in a further -56% macrophage content compared with control, in parallel with a rise in α -smooth muscle cells and collagen content (128).

In addition to evinacumab, antisense oligonucleotides (ASOs) targeting ANGPTL3 messenger RNA are under clinical evaluation (Figure 2). ANGPTL3_{Rx} is a second-generation 2'-O-methoxyethyl (2'-MOE) chimeric antisense oligonucleotide targeted to ANGPTL3 mRNA consisting of the nucleotide sequence 5'-GGACATTGCCAGTAATCGCA-3'. ANGPTL3-L_{Rx} is a second-generation ASO drug targeting ANGPTL3 mRNA with the same sequence and sugar modifications as ANGPTL3_{Rx} but the addition of a covalent linkage with a triantennary N-acetyl galactosamine (GalNAc) cluster, conferring high affinity for the hepatocyte-specific asialoglycoprotein receptor (ASGPR)(129) (Box2). The GalNAc cluster enhances delivery of ANGPTL3-L_{Rx} to hepatocytes over other cell types and consequently increases drug potency for targets expressed by these cells (130).

Mouse *Angptl3* ASO has been tested in different animal models, including the *Ldlr*^{-/-} and *Apoc3*^{-/-} mice, and mice expressing human apoC-III, either fed with chow diet or Western diet (131). Administration of the murine *Angptl3* ASOs led to a drop in *Angptl3* mRNA expression between 69 and 91% corresponding to a decrement in protein levels of 50-90% in each of these mouse models. Relative to the lipid profile, TG, LDL-C and HDL-C were all reduced, i.e. between 35-85%, 7-64% and 3-23%, respectively (131). These findings show that the TG and LDL-C lowering driven by the silencing of ANGPTL3 is independent of the LDLR pathway and occurs in the absence or presence of an excess of apolipoprotein C-III which exhibits inhibitory LPL activity (46).

Experiments on the *Ldlr*^{-/-} model allowed to verify that upon a Western-diet administration, the *Angptl3* ASO (50 mg/kg) halved the progression of *en face* atherosclerosis compared to the group receiving control ASO: 5.4% vs 11.4%. In these animals, administration of the GalNAc modified ASO resulted in a 20 fold more potent suppression of ANGPTL3 with an ED₅₀ value equal to 10.4 mg (131).

Importantly, ANGPTL3-L_{Rx} also reduces hepatic TG secretion, suggesting that a drug targeting ANGPTL3 would ameliorate hepatic steatosis, frequently associated to hypertriglyceridemia and insulin resistance. This effect was observed in diet-induced obese mice and ANGPTL3 deficient humans (131).

The monoclonal antibody anti-ANGPTL4 was developed by immunizing *Angptl4*^{-/-} mice with the recombinant mouse protein for preclinical studies (132). The mAb 14D12 is directed to amino acids Gln29-His53 of the specific epitope 1 (SE1) (65). Hybridomas have been generated by the fusion of splenocytes which were isolated from an immunoresponsive mouse with NS1 myeloma cells. From this fusion, the hybridoma was identified to express IgGs that specifically inhibit LPL enzymatic activity. C57BL/6J mice

591 treated with mAb anti-ANGPTL4 had lower fasting TG when maintained on chow and HFD (-50% and -59%,
592 respectively) compared to vehicle-treated mice (132). Interestingly, mAb anti-ANGPTL4 reduces TG also in
593 *Ldlr*^{-/-}, *ApoE*^{-/-} and db/db mice (132). Importantly, the inhibition of ANGPTL4 reduces instead total cholesterol
594 in C57BL/6J mice. This effect was partially recapitulated in *Ldlr*^{-/-} and db/db mice, but not in *ApoE*^{-/-} mice
595 (132). Interestingly, inhibition of ANGPTL4 showed a rapid drop in serum TG after an i.v. lipid challenge,
596 indicating an increase of TG clearance. The inhibition of ANGPTL4 appears to decrease VLDL production,
597 although this data was not confirmed by overexpressing ANGPTL4 with adenoviral (133) or transgene in
598 adipose tissue and skeletal muscle (114).
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600 Finally, gene editing through the CRISPR-Cas9 technology represents a new approach able to induce
601 an ANGPTL3 permanent LOF mutation. A proof-of-concept study reported that injection of base editor 3
602 *Angptl3* into 5-week-old male mice resulted in a 49%, 31%, and 19% fall in the levels of ANGPTL3, TG and
603 total cholesterol, respectively. When editing was carried on hyperlipidemic *Ldlr*^{-/-} mice the reduction in TG
604 and total cholesterol were 56% and 51%, respectively (134). However, clinical application of the gene editing
605 approach as a pharmacological tool for preventing CVD remains questionable due to potential off-target
606 mutagenesis, unknown toxicity or immunogenicity (135).
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608 7. Clinical development of ANGPTL3 inhibitors

609 In a phase 1, first-in-human, clinical trial, safety and efficacy of evinacumab were tested after s.c. or i.v.
610 injections in subjects with raised TG (150 ≤ TG ≤ 450 mg/dL) and/or LDL-C levels (≥ 100 mg/dL). The ascending
611 single-dose were fixed to 75 mg, 150 mg or 250 mg for s.c. administration and to 5 mg/kg, 10 mg/kg or 20
612 mg/kg for the i.v. ones. Evinacumab was well tolerated and the most frequent emergent adverse events were
613 headache (11.3%) and increase in ALT/AST2 enzymes, i.e. 2 treated subjects experiencing with >3X ULN.
614 Compared to placebo, evinacumab reduced TG in a range between 1% (the lowest dose) to 75% (the highest
615 dose) and LDL-C between 3.4% to 25.5% (136) (Table 2).
616

617 The results of two additional phase 1 trials with evinacumab have been recently reported (137).
618 Subjects with TG levels between 150 and 450 mg/dL were randomized to two different treatment protocol,
619 a single ascending dose study and a multiple ascending dose study. In the single ascending dose study, TG
620 reduction was dose-dependent and rapid, with maximum drops at day 3. Dose-dependent reductions in TG
621 were observed in both studies, with maximum reductions of 76.9% at day 3 with 10 mg/kg i.v. in the single
622 ascending dose and of 83.1% at day 2 with 20 mg/kg i.v. Q4W in the multiple ascending dose study. Significant
623 reductions were also observed in non-HDL-C, apoB, total cholesterol, HDL-C and apoA-I levels in most
624 evinacumab treatment groups compared to placebo (137). Interestingly, evinacumab treatment in both
625 studies did not result in significant changes in Lp(a) levels (137) (Table 2).
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627 Evinacumab was also tested in nine adults with homozygous familial hypercholesterolemia for LDLR,
628 including two null homozygotes and one compound heterozygote with two null alleles. Patients, already
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taking aggressive lipid-lowering therapy, received evinacumab 250 mg s.c. at baseline and 15 mg/kg i.v. at week 2. After 4 weeks of treatment, evinacumab decreased LDL-C by a mean of $49 \pm 23\%$ (range, 25 to 90), with an absolute decrease from baseline of 157 ± 90 mg per deciliter (range, 71 to 323) (39). An approximately significant 48% reduction of apoB, non HDL-C and TG was also observed (39) (Table 2). Treatment was well tolerated, all nine patients reporting the occurrence of at least one adverse event, but no event led to treatment discontinuation. Thus, evinacumab was shown to efficiently reduce LDL-C and TG in homozygous patients already under intensive lipid lowering therapies. This evidence is in line with the fact that the lipid lowering effect of ANGPTL3 inhibitors are obtained in a LDLR-independent manner.

A phase 1 trial, in healthy volunteers aged 18 to 65 years, tested the pharmacokinetics, safety, tolerability and pharmacodynamics of single and multiple ascending doses of ANGPTL3-L_{Rx} (131). Pharmacokinetic analysis of ANGPTL3-L_{Rx} shows a linear and dose-dependent increase of maximum plasma concentrations (C_{max}) within 10 and 60 mg doses, after a rapid distribution phase. As the ANGPTL3-L_{Rx} concentrations decreases, ANGPTL3 protein concentrations return toward baseline values. The calculated half-life ($t_{1/2}$) was approximately 3-5 weeks (131). ANGPTL3-L_{Rx} administered in a multiple-dose design was effective at day 43 to lower TG (from -33.2% to -63.1%), LDL-C (from -1.3% to -32.9%), VLDL-C (from -27.9% to -60%), non-HDL-C (from -10% to -36.6%), apoB (from -3.4% to -25.7%) and apoC-III (from -18.9% to -58.8%) compared to placebo group. At day 43, ANGPTL3 levels were reduced from baseline by 46.6% (10 mg), 72.5% (20 mg), 81.3% (40 mg) and 84.5% (60 mg). No clinical signs of prothrombotic effects, bleeding episodes, significant decreases in platelet counts and of liver or renal function damages were found (131) (Table2).

8. Conclusions

The magnitude of contribution of TG to CVD risk is evident both from long-term prospective studies (138) and genetic analyses (16). TGRL may penetrate the arterial wall by interacting with the positive charged residues on apoB and the negative charged groups on the arterial wall proteoglycans. This process allows TGRL to be retained within the sub-endothelial space and to undergo an oxidative modification which favors the development of atherosclerotic plaques and ASCVD (139). The lipolysis of TGRL rich in cholesterol and apoE releases oxidized FFA and lysolecithin which induce endothelial cell inflammation and coagulation (140). Recently, we have listed ANGPTL3 as an early predictor of peripheral artery disease, influencing the endothelial cell adhesion and stimulating the proliferation of haematopoietic stem cells, both processes exacerbating atherosclerosis (141).

Among pharmacological targets envisioned to reduce TG levels, activators of PPARs (*i.e.* fibrates being mild PPAR α agonists) (10) have shown possible benefit in patients with primary hypertriglyceridemia, mixed hyperlipidemia and type 2 diabetes with raised TG and low HDL-C (6, 142-144). The efficacy of controlling the hypertriglyceridemia was also recently confirmed by the use of icosapent ethyl in patients with elevated TG levels despite the use of statins (145). The REDUCE-IT (Reduction of Cardiovascular Events with Icosapent

Ethyl-Intervention) trial demonstrated that after a median follow-up of 4.9 years, the primary endpoint, *i.e.* a composite of CV death, non-fatal MI, non-fatal stroke, coronary revascularization, or unstable angina, was reduced by 25% in the icosapent ethyl group vs placebo. Furthermore, icosapent ethyl was also superior to placebo to reduce total events, namely the occurrence of first and all recurrent major CV events by 30%. Specifically, first events fell by 25%, second ones by 32%, third ones by 31% and fourth ones or more by 48% (146). Of note, lomitapide, a first-in-class microsomal triglyceride transfer protein (MTP) inhibitor (147), has been shown to significantly control TG levels; it prevented pancreatitis in a patient with an inactivating mutation on the LPL, although with a potential long-term cost of hepatotoxicity (148). Despite these pharmacological opportunities, very effective and safe drugs for reducing TG levels are still missing. Within this scenario and along with the inhibition of apoC-III protein (149), the evidence of the role of ANGPTL3 and ANGPTL4 for controlling LPL activity and TG levels indicates a promising pharmacological target, although the adverse phenotype observed in *Angptl4*^{-/-} mice (107, 112, 113), highlights ANGPTL3 as a better target. Although we are still at phase 1 of clinical development, the use of monoclonal antibodies and/or ASO directed to ANGPTL3 have shown very effective in lowering TG and LDL-C and increasing HDL-C (Figure 2). The ASO was safe, an important aspect, considering the thrombocytopenia found in patients given volanesorsen, an ASO against apoC-III, reducing chylomicron TG by roughly 83% (150). Thus, these therapies can be considered as a future valid implementation of the current use of PPAR-α agonists in the management of this very frequent clinical condition, namely hypertriglyceridemia.

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Figure legends

Figure 1. Schematic representation of ANGPTL3, ANGPTL4 and ANGPTL8 protein structures. ANGPTL3 (Angiopoietin-like protein 3) is composed by an N-terminal domain involved in the inhibition of lipoprotein lipase (LPL) and endothelial lipase and by a C-terminal fibrinogen-like domain. ANGPTL4 shares with ANGPTL3 both the coiled-coil domain and the fibrinogen-like domain (FLD). ANGPTL8 is paralog of the N-terminal region of ANGPTL3 and it is required for ANGPTL3 activation. All isoforms contain a Specific Epitope1 (SE1) required to inhibit LPL activity. LR stands for linker region. Modified from Lupo et al. (49)

Figure 2. Biology and pharmacological inhibition of ANGPTL3 and ANGPTL4. ANGPTL3 (Angiopoietin-like protein 3) is secreted by hepatocytes and inhibits lipoprotein lipase (LPL) in peripheral tissues, such as skeletal muscle and adipose tissues. Differently, ANGPTL4 is induced by fasting or physical exercise; it is released by the liver, the skeletal muscle and the white adipose tissue. ANGPTL3 and ANGPTL4 inhibit LPL and endothelial lipase (EL), thus increasing the levels of TG-rich-lipoproteins, FFA and glycerol. Phase I clinical trials with monoclonal antibodies (evinacumab) and antisense oligonucleotide (ASO) against anti-ANGPTL3 are ongoing, while an antibody anti-ANGPTL4, 14D12, is still in preclinical development. ER: endoplasmic reticulum; FFA, free fatty acids; HDL, high-density lipoprotein; TG, triglycerides; TGRL, triglyceride-rich lipoproteins.

Table 1. Effect of ANGPTL3 or ANGPTL4 inhibition on lipid profile in pre-clinical studies.

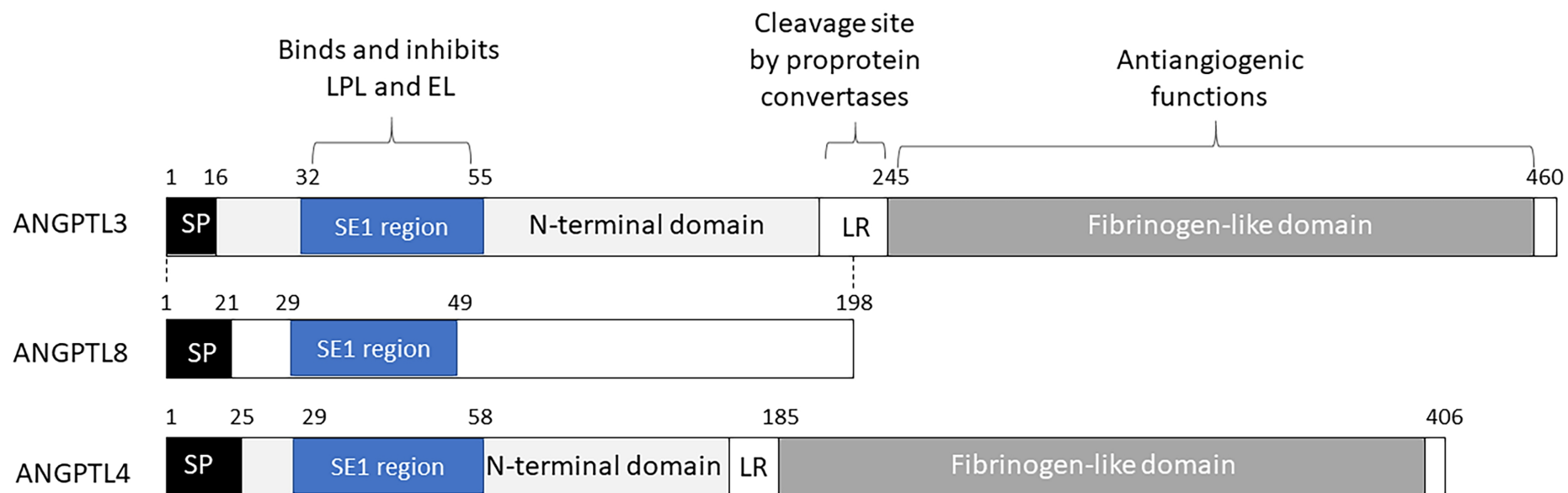
Treatment	Model	Efficacy
Evinacumab	Wild type and LDLr ^{-/-} mice (105)	TG -40%; TC -33%; reduced apoB, increased clearance of apoB-containing lipoproteins
	Wild type mice fed High fat diet (106)	TG -53%; TC -35%; LDL-C -45%
	Spontaneous hypertriglyceridemic Cynomolgus monkey (106)	TG -48% (3mg/Kg) /-89% (10mg/Kg); non-HDL-C -44%; LDL-C: no changes
	ApoE3-Leiden CETP mice fed Western diet (128)	In combination with alirocumab and atorvastatin: TG -67%; TC -68%; non-HDL-C -84% compared to atorvastatin; stronger regression of atherosclerosis lesion size, improved plaque composition compared to control
Angptl3 ASO	(i) LDLr ^{-/-} and apoC-III ^{-/-} mice fed chow and high fat diets (ii) mice expressing human apoC-III (13)	TG -35/-85%; LDL-C -7/-64%; delayed <i>en face</i> atherosclerosis progression in LDLr ^{-/-} mice Fed Western-diet
Angptl3 CRISPR-Cas9 base editing	Wild type mice (134)	TG -31%, TC -19%
	Hyperlipidemic LDL ^{-/-} mice (134)	TG -56%, TC -51%
anti-Angptl4 mAb 14D12	Wild type mice fed chow and high fat diets (132)	TG:-50/-59%; TC ≈-30%; rised TG clearance; reduced VLDL production
	apoE ^{-/-} , LDLr ^{-/-} and db/db mice (132)	TG ≈ -55%; TC ≈ -25% in LDLr ^{-/-} and db/db mice, no changes in apoE ^{-/-} mice

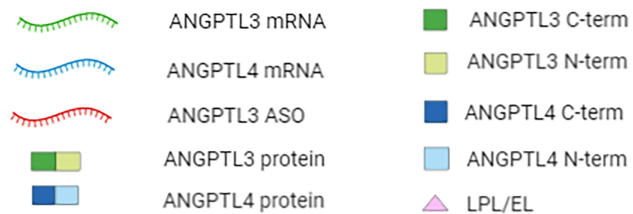
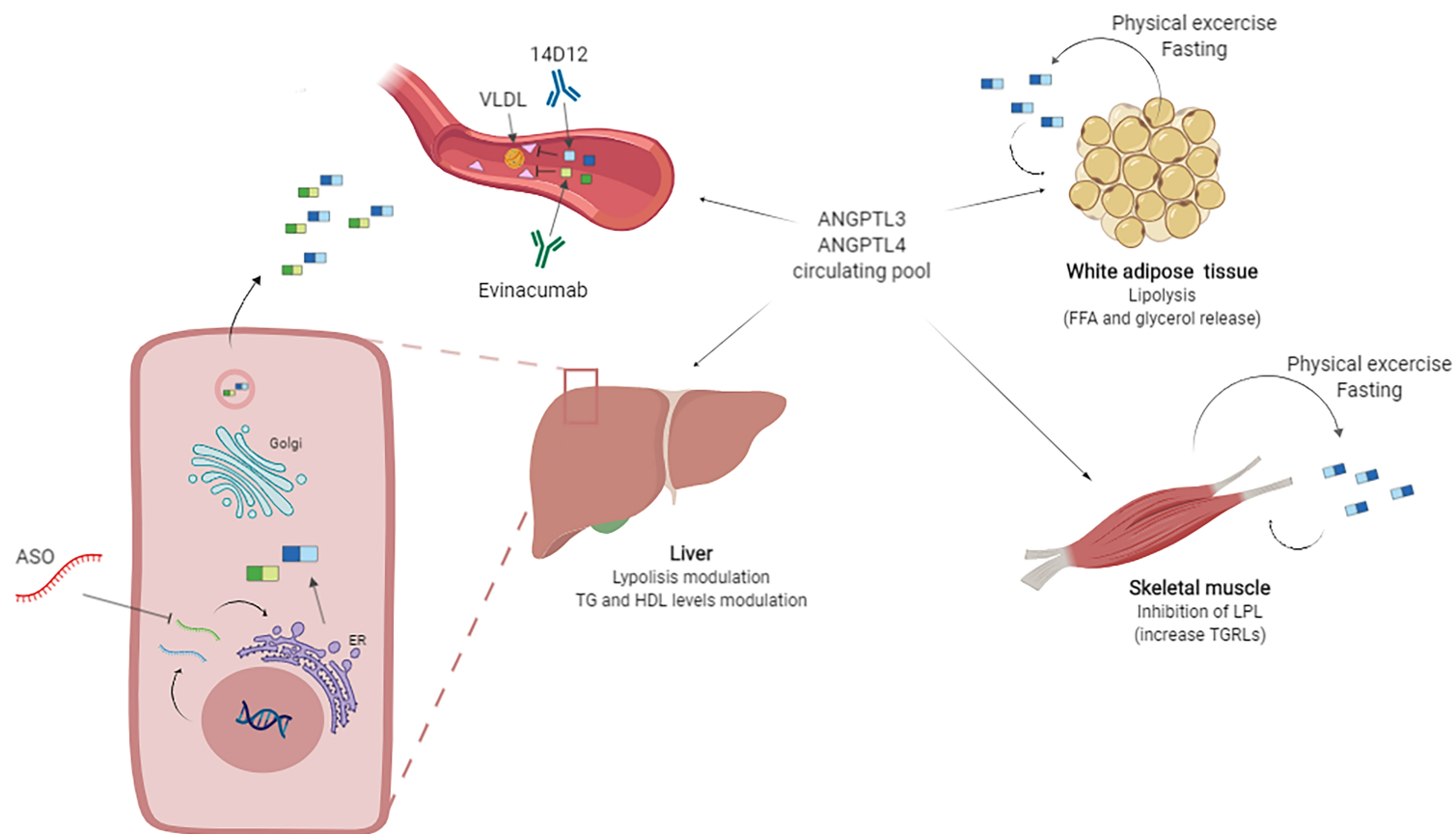
Angptl3, angiopoietin like 3; apoB, apolipoprotein B; apoE, apolipoprotein E; ASO, Antisense Oligonucleotide; CETP, cholesteryl transfer protein; HDL, high density lipoproteins; LDL, low density lipoproteins; TC, total cholesterol; TG, triglycerides; VLDL, very low-density lipoproteins

Table 2. Effect of ANGPTL3 inhibition on lipid profile in clinical studies.

Treatment	Dose	Efficacy
Evinacumab in subjects with 150≤ TG ≤450 mg/dL (136)	Ascending single-dose 75, 150, 250 mg for s.c. or 5, 10, 20 mg/Kg for i.v.	TG -1% (lowest dose) to -75% (highest dose) LDL-C -3.4% (lowest dose) to -25.5% (highest dose)
	Ascending single-dose 75, 150, 250 mg for s.c. or 5, 10, 20 mg/Kg for i.v.	TG maximum reduction of 76.9% with 10 mg/kg i.v.
Evinacumab in subjects with 150≤ TG ≤450 mg/dL (137)	Multiple ascending dose 150/300/450 mg once weekly, 300/450 mg every 2 weeks for s.c. or 20 mg/kg once every 4 weeks i.v.	TG maximum reduction of 83.1% with 20 mg/kg i.v. once every 4 weeks
Evinacumab in adults with homozygous familial hypercholesterolemia (39)	250 mg s.c. at baseline and 15 mg/kg i.v. at week 2	LDL-C -49% (range, 25 to 90) TG -47% (interquartile range, 38 to 57)
ANGPTL3-L _{Rx} in healthy volunteers aged 18-65 years with TG>150 mg/dL (131)	Multiple-ascending dose 10, 20, 40 or 60 mg per week for 6 weeks	TG from -33.2% to -63.1%, LDL-C from -1.3% to -32.9% VLDL-C from -27.9% to -60%

ANGPTL3, angiopoietin like 3; i.v., intravenous; LDL, low density lipoproteins; TC, total cholesterol; TG, triglycerides; s.c., subcutaneous; VLDL, very low-density lipoproteins





Relative to the manuscript “Pharmacological aspects of ANGPTL3 and AGPTL4 inhibitors: new therapeutic approaches for the treatment of atherogenic dyslipidemia” the Authors declare no conflict of interests.