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Emerging oral therapeutic strategies for inhibiting PCSK9

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ABSTRACT

Pharmacological inhibition of Proprotein Convertase Subtilisin/Kexin 9 (PCSK9) have been firmly established to be an effective approach to reduce low-density lipoprotein (LDL) cholesterol levels and cardiovascular events. Subcutaneous administration of monoclonal antibodies (evolocumab and alirocumab) every 2 or 4 weeks determined a 60 % reduction of LDL cholesterol levels, while the GalNac-siRNA anti PCSK9 (inclisiran) provided an effective lipid lowering activity (−50 %) after an initial subcutaneous dose, repeated after 3 months and followed by a maintenance dose every 6 months. Although these two approaches have the potentiality to bring the majority of patients at high and very-high cardiovascular risk to the appropriate LDL cholesterol targets, their cost and subcutaneous administration represent a strong limitation for their large-scale use. These problems could be overcome by the development of small chemical molecules anti PCSK9 as oral therapy for controlling hypercholesterolemia. In the present review, we summarized the pharmacological properties of oral anti PCSK9 molecules that are currently under clinical development (DC371739, CVI-LM001, and AZD0780), including the mimetic peptides enlicitide decanoate (MK-0616) and NNC0385-0434.

1. Introduction

Since the discovery that mutations in PCSK9 cause autosomal dominant hypercholesterolemia [1], this circulating protein has attracted the attention of drug companies as new pharmacological target for controlling hypercholesterolemia and cardiovascular diseases [2]. Considering the extracellular function of PCSK9, it was logical to develop fully human monoclonal antibodies (mAbs) as therapeutic approach for preserving the catabolic pathway of LDL receptor. Indeed, PCSK9 post-translationally regulate the number of cell-surface LDL receptors by binding to its epidermal growth factor-like repeat homology domain A (EGF-A domain). The interaction of PCSK9 with LDL receptor elicits a dual effect (1) acting as a courier, facilitating the exit of LDL receptor from the endoplasmic reticulum, and (2) fostering the degradation of the LDL receptor at the cell surface [3–5]. Thus, by binding to circulating PCSK9, evolocumab and alirocumab inhibit the interaction to the LDL receptor preventing its degradation and allowing the receptor to be recycled back to the hepatic cell surface. This activity increases the efficiency of hepatocytes to capture the apoB-containing lipoproteins, such as LDL and VLDL. Differently, inclisiran is a long-acting, subcutaneously delivered, synthetic siRNA directed against PCSK9 and conjugated to triantennary N-acetylgalactosamine carbohydrates (siRNA-GalNAc). These carbohydrates bind to abundant liver-expressed asialoglycoprotein receptors, leading to inclisiran uptake specifically into hepatocytes. The siRNA molecules engage the natural pathway of RNA interference by binding intracellularly to the RNA-induced silencing complex (RISC), enabling it to cleave mRNA molecules encoding PCSK9. The cleaved mRNA is degraded and thus unavailable for protein translation, which results in decreased levels of PCSK9 protein [6]. Levels of circulating PCSK9 are reduced by almost 80 % in response to inclisiran administration, resulting in a 50 % reduction in LDL cholesterol levels [7].

Although both biological approaches have shown to be very effective and well-tolerated, with no serious side effects, the use of mAbs and siRNA has at least two drawbacks: 1) very high costs; 2) subcutaneous administration. Thus, it is clear there is a need for alternative therapeutic approaches capable of effectively and safely lowering LDL cholesterol either as monotherapy or in combination with statins.

The deep understanding of PCSK9 biology has permitted to identify potential druggable sites for its inhibition by acting at different levels: 1) transcriptional levels; 2) autocatalytic cleavage; 3) protein-protein interaction with LDL receptor; 4) allosteric conformational changes of

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PCSK9; 5) mRNA translation. However, small molecules (500 Da in mass) do not effectively inhibit the interaction between PCSK9 and LDL receptor (EGF-A domain) due to the relatively flat, featureless, and undruggable surface of PCSK9 [8]. Indeed, only one molecule, which is actually a macrocyclic peptide [9], reached the clinical phase as PCSK9 inhibitor by inhibiting the interaction with the LDL receptor, all the others act on the transcription of PCSK9 mRNA or by interacting with PCSK9 itself [10]. In this review we summarized the pharmacological properties and clinical results of new oral PCSK9 inhibitors with peculiar chemical structures (Fig. 1) and mechanisms of action (Fig. 2).

2. DC371739

Tetrahydroprotoberberines are compounds chemically related to the natural berberine which has been shown to stabilize LDL receptor mRNA and inhibit PCSK9 transcription by downregulating the expression of hepatocyte nuclear factor-1α (HNF-1α) [11,12]. However, some limiting factors preclude the efficacy of berberine as oral PCSK9 inhibitor: 1) high oral dose requirement (1.0–1.5 g/day) [13]; 2) poor bioavailability (F <1 %) [14]; 3) potential human ether-a'-go-go related gene (hERG) potassium channel inhibitory activity [15].

These observations promoted the development of other lipid-lowering small molecules with limited side effects, leading to the discovery of DC371739, an indole-containing tetrahydroisoquinoline compound derived from chemical modification of tetrahydroprotoberberines extracted from the Chinese herb *Corydalis ambigua* [16,17].

2.1. Pharmacodynamic properties

Besides the chemical relationship between berberine and DC371739, the two compounds showed a different mechanism of action. Unlike berberine, DC371739 neither increases LDL receptor mRNA nor changes the HNF-1α protein expression in cultured cells [17]. Although DC371739 does not alter HNF-1α expression, it reduces the transcription of its target genes PCSK9 and angiopoietin-like protein 3 (ANGPTL3), two known target genes of HNF-1α [12,17]. DC371739 does not interfere with the dimerization of HNF-1α but directly disrupts its binding to the DNA. The specific site of interaction between DC371739 and HNF-1α has also been identified [17] confirming that the compound interferes with the transcription of PCSK9 and ANGPTL3 through the disruption of the binding of HNF-1α to its HNF-1 response element (HRE) on the promoters of PCSK9 and ANGPTL3 [17]. DC371739 was also shown to reduce the transcription levels of *Pcsk9* and *Angptl3* in hamster liver by 41.3 % and 15.7 %, respectively. Further, DC371739 treatment of high fat diet (HFD)-fed hamsters (10, 30 or 100 mg/kg/d, 21 days) reduced serum total cholesterol levels by 29 %, 35 %, and 39 %, respectively, and the serum LDL cholesterol by 23 %, 31 %, and 35 %, respectively. DC371739 also determined a significant reduction of triglyceride levels by 50 %, 58 %, and 78 %, respectively.

Treatment for 30 days, at the dose of 10 and 30 mg/kg/d, resulted in lower levels of both liver total cholesterol and triglycerides in a dose-dependent manner, with values of −42.91 % and −31.15 %, respectively, after the administration of the highest dose [17].

More importantly, serum cholesterol levels in spontaneous hyperlipidemic rhesus monkeys treated with 3 or 10 mg/kg/d of DC371739 by oral gavage for 28 days were notably lower by 16 % and 28 %, respectively, compared to baseline levels. Likewise, serum LDL cholesterol levels in DC371739-treated monkeys were significantly lower than before treatment (by 14 % and 31 %, respectively) [17].

It is well established that statins augment serum PCSK9 levels by stimulating sterol regulatory element binding protein 2 (SREBP2) transcription, which leads to diminished efficacy on LDL receptor induction [18–20]. Not surprisingly, combined treatment of DC371739 and atorvastatin increased DiI-LDL uptake by 50 % compared to atorvastatin alone. This increase is also associated with greater LDL receptor protein expression by 90 % and lower PCSK9 protein expression by 50 % in HepG2 cells, as compared to atorvastatin alone [17].

2.2. Pharmacokinetics properties

DC371739 displayed a good oral bioavailability in rats (F = 58.3 %) and dogs (F = 19.5 %) with a T_{max} of 6.5 h and 2 h, respectively (Table 1). Notably, DC371739 showed good metabolic stability, as its metabolites were undetectable or at a very low levels in hepatocytes from humans, monkeys, dogs, rats, and mice [17]. Moreover, DC371739 shown lower inhibitory activity on the hERG channel *in vitro* (IC_{50} > 40 mM).

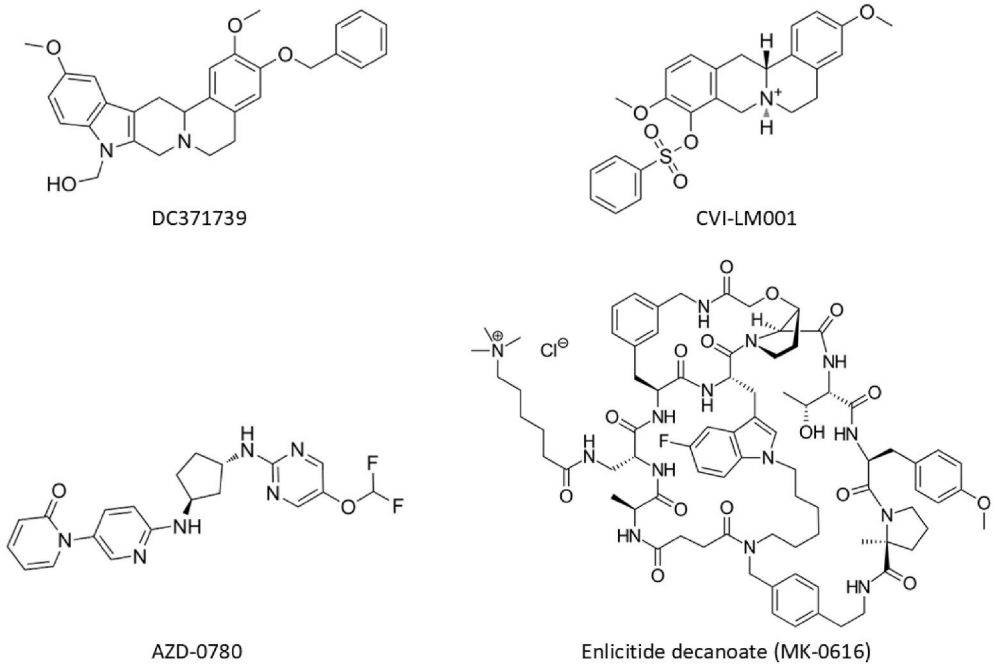

Fig. 1. Chemical structures of oral anti PCSK9 molecules currently under clinical development.

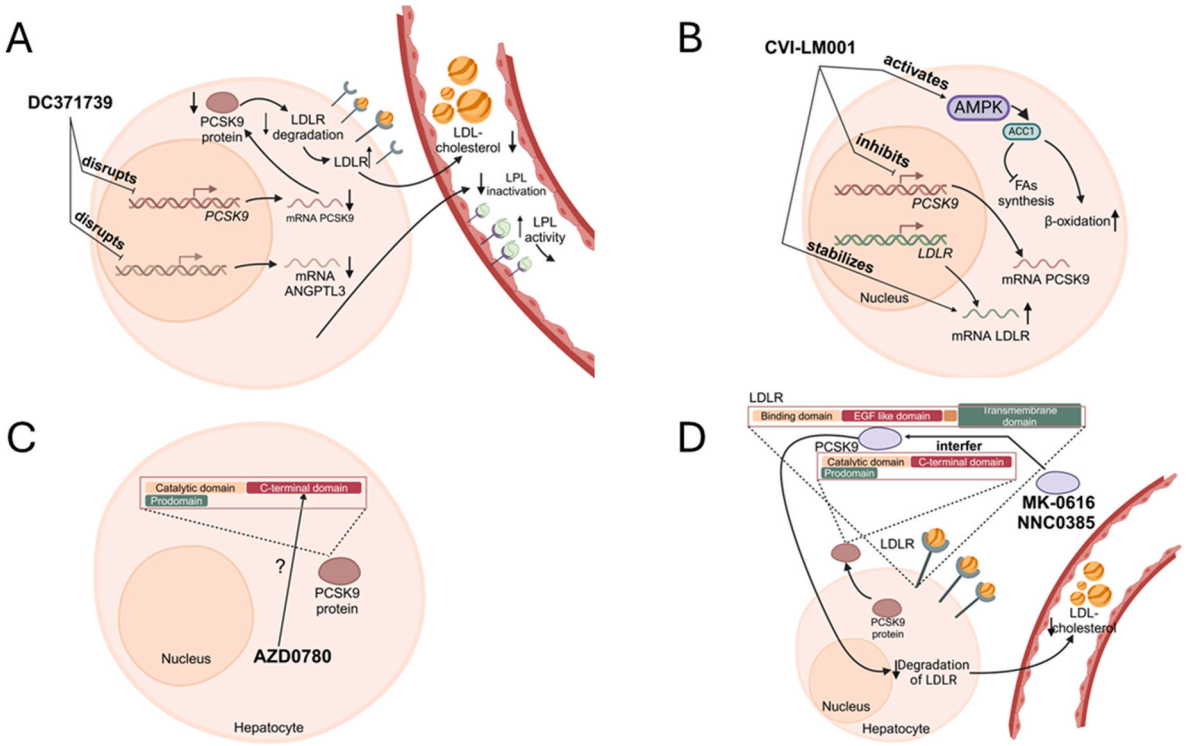

Fig. 2. Mechanisms of action of oral anti PCSK9 molecules. A) DC371739 interferes with the transcription of PCSK9 and ANGPTL3 by inhibiting the binding of HNF-1α to its HNF-1 response element (HRE) on the promoters of PCSK9 and ANGPTL3. B) CVI-LM001 increases LDL receptor levels by both inhibiting PCSK9 transcription and preventing LDL receptor mRNA degradation. CVI-LM001 also activates hepatic adenosine monophosphate-activated protein kinase (AMPK) thus reducing hepatic triglyceride synthesis and boosting fatty acids oxidation. C) AZD0780 directly binds PCSK9 and blocks its function. D) Enlicitide decanoate (MK-0616) is a macrocyclic peptide that interferes with the interaction between PCSK9 and LDL receptor. NNC0385-0434 is a peptide that mimics the EGF-A domain of the human LDL receptor and binds free PCSK9 and subsequently prevents its interaction with the LDL receptor.

Table 1
Pharmacokinetic parameters of DC371739 in rats, dogs[a], and individuals with hypercholesterolemia[b] [17].

Parameter	Rats[c]	Dogs[d]	Human 20 mg once daily	Human 40 mg once daily
T_{max} (h)	6.5 ± 1.0, p.o.	2.0 ± 0, p.o.	6.5 ± 1.7	5.5 ± 1.1
C_{max} (ng/mL)	130 ± 25.1, p.o.	183 ± 87.7, p.o.	42.4 ± 13.8	102.5 ± 28.5
$AUC_{0-\infty}$ (h*ng/mL)	2110 ± 531, p.o.	1970 ± 770, p.o.	1313 ± 305	2266 ± 391
$AUC_{0-\infty}$ (h*ng/mL)	724 ± 105, i.v.	2013 ± 138, i.v.	/	/
AUC_{0-48} (h*ng/mL)	/	/	923 ± 262	1713 ± 263
$MRT_{0-\infty}$ (h)	9.27 ± 1.96, i.v.	17.17 ± 4.10, i.v.	39.7 ± 6.1	33.3 ± 4.7
$T_{1/2}$ (h)	8.18 ± 1.08, p.o.	12.5 ± 0.95, p.o.	26.2 ± 4.9	22.6 ± 3.9
CL (mL/min/kg)	46.7 ± 6.64, i.v.	138.3 ± 9.83, i.v.	/	/
Clz/F (L/h)	/	/	15.9 ± 3.6	18.2 ± 3.5
Vss (L/kg)	25.9 ± 6.35	8.93 ± 1.19		
Vz/F (L)	/	/	608 ± 188	586 ± 123
F (%)	58.3	19.5		

[a] Average of three animals.
[b] Average of eight individuals with hypercholesterolemia.
[c] 10 mg/kg for p.o. and 2 mg/kg for i.v.
[d] 5 mg/kg for p.o. and 1 mg/kg for i.v. AUC: area under the curve; C_{max}: maximal plasma concentration; CL: clearance; CLz/F: apparent oral clearance; F: bioavailability; MRT: mean residence time; T_{max}: time to reach the maximal plasma concentration after the administration; $T_{1/2}$: half-life time; Vss: steady state volume of distribution; Vz/F: apparent volume of distribution during terminal phase.

DC371739 did not inhibit human CYP isoforms (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4), indicating a low probability of drug-drug interactions. OATP1B1 and OATP1B3 are the major uptake transporters for statins in the liver [21]. Notably, DC371739 is neither a substrate nor an inhibitor (with $IC_{50} > 50$ mM) of either transporter, as revealed by *in vitro* uptake assays [17].

DC371739 had good safety, tolerability, and pharmacokinetic profiles at all preset doses in individuals with hypercholesterolemia. Pharmacokinetic analysis among participants in the study (n = 16) showed that the area under the curve (AUC) of DC371739 increased dose-dependently (Table 1). The half-life ($T_{1/2}$) was determined to be 26.2 h (20 mg/dose) and 22.6 h (40 mg/dose), respectively, and peak times (T_{max}) are 6.5 h (20 mg/dose) and 5.5 h (40 mg/dose), respectively.

2.3. Clinical development

The results of Phase I clinical trial reported that among 20 of the participants with hyperlipidemia, treatment for 28 days with DC371739 at 40 mg per day, significantly reduced both serum total cholesterol and LDL cholesterol levels by 19 %, compared to placebo group [17]. In addition, serum triglyceride and ApoB levels were also significantly lowered by 27 % and 25 %, respectively, while there was a little change in HDL-C levels [17].

DC371739 is currently under clinical development for the treatment of hypercholesterolemia in a Phase Ib/IIa clinical trial (ClinicalTrials.gov, NCT04927221). The study evaluated the safety, tolerability, pharmacokinetics and pharmacodynamics of DC371739 after multiple-dose oral administration, to explore the clinical effective dose, and to initially explore the efficacy and safety in hypercholesterolemic subjects.

A total of 30–50 subjects have been treated with one of the following

doses: 20 mg, 40 mg, 60 mg, 80 mg, and 120 mg per day for 28 days. Each of the dose groups included 10 subjects (8 for DC371739 and 2 for placebo). The study has been completed in 2021-12-21 and the results have not been fully disclosed.

Taken together DC371739 appears to be an interesting molecule with a unique mechanism of action that may classified it as a dual PCSK9/ANGPTL3 inhibitor. Although some doubts regarding the specificity of action of the molecule remain, DC371739 has shown to be very well tolerated. No dose-limiting toxicities have been observed in the Phase I trial, at doses up to 40 mg once daily for 28 days. The maximum tolerated dose was not reached. Considering the efficacy of the monoclonal antibody anti ANGPTL3, evinacumab, in patents with Homozygous familial hypercholesterolemia [22,23], DC371739 could be very effective also in patients with mutations on the LDL receptor, condition where anti PCSK9 therapies lose their lipid lowering action.

3. CVI-LM001

CVI-LM001 is a fluorobenzenesulfonate derivative of corydaline acting by suppressing the expression of PCSK9 gene and by stabilizing LDL mRNA. Thus, CVI-LM001 increases the LDL receptor levels by both inhibiting PCSK9 transcription and preventing LDL receptor mRNA degradation. Moreover, similar to berberine [11,24], CVI-LM001 activates hepatic adenosine monophosphate-activated protein kinase (AMPK), a master regulator of cellular energy, which turns on pathways that reduce hepatic triglyceride synthesis and boost fatty acids oxidation [25].

3.1. Pharmacodynamic properties

The hypocholesterolemic effect of CVI-LM001 has been investigated in hyperlipidemic hamsters. CVI-LM001 was administered at 40, 80 and 160 mg/kg OD for 4 weeks. A dose-dependently increased of liver LDL receptor protein levels was observed with a maximal effect of 3.5-fold with 160 mg/kg OD [25]. Circulating PCSK9 levels was reduced by more than 90 % compared to control group at the highest dose, this was accompanied by significant reductions in serum LDL cholesterol (−42.6 % vs control group). In this animal model, CVI-LM001 significantly reduced mRNA levels of PCSK9 and induced AMPK phosphorylation, confirming its molecular mechanism *in vivo* [25]. Finally, CVI-LM001 treatment was associated with a reduction in hepatic total cholesterol and triglycerides levels.

3.2. Pharmacokinetics properties

The pharmacokinetic properties of CVI-LM001 were investigated in double-blind, randomized single ascending-dose (100–800 mg per day) and multiple ascending-dose studies. Subjects (Chinese males and females, 18–45 years) received 100, 200, or 300 mg of CVI-LM001 or placebo, once daily for 10 days. CVI-LM001 undergoes hepatic metabolism forming two bioactive metabolite M1 (more potent) and M2 (less potent). The total exposure (AUC_{last}) increased with the dose, reaching a plateau at 400 mg per day. The molecule is rapidly absorbed with a T_{max} of 1.5 ± 0.2 h and a very long half-life time of elimination ($T_{1/2}$ = 39 ± 2 h) (Table 2). The C_{max} also increased with the dose, reaching a maximum of 440 ng/mL after administration of 600 mg of CVI-LM001 once daily (Table 2). Treatment for 10 days determined a significant reduction of PCSK9 plasma levels by 35 %.

3.3. Clinical development

CVI-LM001 is advanced to Phase II stage to treat hypercholesterolemic patients. In a double-blind, randomized Phase Ia study conducted in healthy volunteers with normal lipid levels, compared to baseline, it was observed a 36.4 % ($p < 0.001$) reduction in serum PCSK9 levels after 10 days of oral treatment with CVI-LM001 (300 mg, OD) [26].

Table 2
Summary of CVI-LM001 pharmacokinetic parameters in healthy subjects.

Parameter	100 mg once daily	200 mg once daily	400 mg once daily	600 mg once daily	800 mg once daily
T_{max} (h)	1.25	1.6	1.3	1.65	1.4
C_{max} (ng/mL)	NA	NA	NA	440	NA
$T_{1/2}$ (h)	37	38	35	41	45
AUC_{last} (h*ng/mL)	900	2050	3100	3150	3300

NA: not available. AUC: area under the curve; C_{max}: maximal plasma concentration; T_{max}: time to reach the maximal plasma concentration after the administration; $T_{1/2}$: half-life time.

Moreover, in a proof of mechanism Phase Ib study treatment with CVI-LM001 (300 mg OD) for 28 days significantly reduced serum LDL cholesterol (−26.3 %), total cholesterol (−20.1 %), ApoB (−17.4 %) and PCSK9 (−39.2 %) in subjects with elevated LDL-C, compared with placebo cohort [26]. CVI-LM001 had a benign safety profile and was well tolerated in 105 treated healthy volunteers and 33 treated hyperlipidemic subjects [26].

4. AZD0780

AZD0780 is an oral, small molecule PCSK9 inhibitor that is being developed as a first-in-class therapy for patients with dyslipidemia which cannot be controlled by statins alone. Few information is available on this molecule that, however, exhibited bind affinity for PCSK9 with a Kd value lower than 200 nM.

4.1. Clinical development

The Phase I trial included participants receiving AZD0780 30 mg or 60 mg daily versus placebo (n = 15 for each arm) for four weeks. An additional arm including 35 participants with hypercholesterolemia (LDL cholesterol >100 mg/dL to 190 mg/dL) received rosuvastatin 20 mg for three weeks, followed by AZD0780 30 mg or placebo for four weeks [27]. The trial assessed safety, tolerability, pharmacokinetics, and pharmacodynamics of AZD0780 in lowering LDL cholesterol in plasma when administered as monotherapy and in combination with rosuvastatin. AZD0780 demonstrated a statistically significant reduction of 52 % in LDL cholesterol levels on top of rosuvastatin treatment, with 78 % total reduction from baseline, in treatment-naive participants with hypercholesterolemia [27]. Preliminary data showed similar pharmacokinetic profile either under fasting or fed condition. No serious adverse events were reported and AZD0780 was well tolerated. AZD0780 progressed into Phase II trials in patients with dyslipidemia in 2024.

5. Enlicitide decanoate (MK-0616)

Enlicitide decanoate is a macrocyclic peptide that efficiently interferes with the interaction between PCSK9 and the LDL receptor [28]. Based on structural data enlicitide decanoate was demonstrated to interact with the LDL receptor binding domain of PCSK9 and inhibit the protein-protein interaction with an IC_{50} value of 2.5 ± 0.1 nM [29]. Modeling enlicitide decanoate from related crystal structures suggests that this molecule interacts with a flat surface on the PCSK9 catalytic domain, interrupting the interaction with the EGF-A domain of LDLR [29].

5.1. Pharmacodynamic properties

A randomized, double-blind, placebo controlled, single-ascending-dose clinical study in 60 healthy male participants was assessed to evaluate the efficacy of orally dosed enlicitide decanoate [29]. All doses (10 mg, 35 mg, 100 mg, 200 mg, 300 mg) of enlicitide decanoate,

reduced free plasma PCSK9 by more than 93 % from baseline [29]. With enlicitide decanoate doses 35 mg and higher, formulated with permeation enhancers (sodium caprate), free plasma PCSK9 was reduced by >80 % from baseline and maintained for >24 h.

The effect of multiple administrations of enlicitide decanoate was investigated at the dose of 10 mg and 20 mg once daily for 14 days. At both doses, plasma levels of free PCSK9 were reduced by more than 90 % across the 14 days. The pharmacodynamic effect of enlicitide decanoate was lower when a 10 mg dose was given 30 min post-meal. The maximum reduction of free PCSK9 was observed at time points corresponding to the T_{max} of MK-0616 on day 1 and day 14. Differently from free form, the plasma levels of total PCSK9 were increased 24 h after the 14th dose of 20 mg MK-0616 (+122 ± 45 % from baseline).

The administration of enlicitide decanoate, at both doses, determined a continuous gradual reduction of plasma LDL cholesterol during the 14-day dosing period. After 14 days of treatment, the percent reductions from baseline of LDL cholesterol were 58.2 and 60.5 % for 10 and 20 mg daily doses, respectively. This effect was strongly reduced when MK-0616 was given 30 min after a standard meal (−11.6 %).

Taken together, enlicitide decanoate demonstrated an effective inhibitory activity on PCSK9, which was associated to a strong lipid lowering effect. As expected, considering the LDL particles turnover, the maximal effect of enlicitide decanoate was observed between 7 and 14 days at the dose of 10 and 20 mg.

5.2. Pharmacokinetics properties

Oral administration of enlicitide decanoate is formulated with permeation enhancers. When fasted participants were dosed orally with 10–300 mg of enlicitide decanoate formulated with the permeation enhancers Labrasol, the increase in $AUC_{0-\infty}$ was less than dose proportional. The increase in C_{max} was generally dose proportional (Table 3).

The $T_{1/2}$ ranged from 35 h at the lowest dose to 130 h at the highest dose (Table 3). The apparent total plasma clearance of drug and the apparent volume of distribution during the terminal phase increased with increasing dose of enlicitide decanoate (Table 3). The observed T_{max} ranged from 1.50 to 2.02 h (Table 3). The C_{max} of a 200 mg dose of enlicitide decanoate in the absence of 1800 mg Labrasol was >5-fold lower compared with the same dose with the permeation enhancers PE. At a 100 mg dose of enlicitide decanoate, Labrasol and sodium caprate formulations exhibited similar exposures of drug. The ratios (fed/fasted) of $AUC_{0-\infty}$ and C_{max} for a 40 mg dose given 30 min after a high-fat breakfast (55.6 g fat, 55 g carbohydrate, 31.1 g protein) compared with the same dose given under fasted conditions (an overnight fast of at least 8 h duration, and no food for 4 h post-dose) were 0.33 and 0.25, respectively. The effect of lower-fat breakfast (8 g fat, 65 g carbohydrate, 13 g protein) was less pronounced.

After multiple-administration of enlicitide decanoate the increase of AUC_{0-24} and C_{max} for doses at 10 and 20 mg were less than dose proportional (Table 3). On day 14, the T_{max} for all fasted doses was less than 2 h and the $T_{1/2}$ was between 178 and 244 h (Table 3). According to the elimination half-life time, the steady state was achieved by the end of the first week of treatment. A low-fat breakfast given 30 min prior to a 10 mg enlicitide decanoate dose reduced the AUC_{0-24} and C_{max} of enlicitide decanoate by 40–55 % and delayed the T_{max} by approximately 6 h, compared with the same dose given under fasting conditions (Table 3).

The oral bioavailability of enlicitide decanoate formulated with permeation enhancers was estimated to be approximately 2 %, based upon the plasma exposure achieved at a given oral dose. Enlicitide decanoate is a macrocyclic peptide and due to its chemical characteristics is not cell permeable. Indeed, its oral administration required the coformulation with a permeation enhancer like the medium-chain fatty acid sodium caprate. Caprate modulates the opening of tight junctions enabling paracellular absorption of molecules that are otherwise impermeable. Nevertheless, the very low bioavailability of enlicitide decanoate does not seem to impact its pharmacological activity, even taking into consideration the long terminal half-life time of elimination.

Table 3
Pharmacokinetic parameter values of enlicitide decanoate in healthy participants after single doses of 10, 35, 100, 200, and 300 mg.

Parameter	Enlicitide decanoate single dose				
	10 mg	35 mg	100 mg	200 mg	300 mg
$AUC_{0-\infty}$ (h*nmol/L)	259	540	1080	1260	2260
AUC_{0-24} (h*nmol/L)	93.0	165	309	339	778
AUC_{0-last} (h*nmol/L)	235	501	1020	1170	2000
C_{max} (nmol/L)	5.21	17.8	46.2	45.3	149
T_{max} (h)	1.50	1.50	1.50	2.02	1.50
$T_{1/2}$ (h)	35.14	42.86	81.52	95.47	129.95
CL/F (L/h)	24.98	42.96	59.83	103.17	84.64
Vz/F (L)	1266.52	2656.39	7037.04	14209.96	15868.31

AUC: area under the curve; C_{max}: maximal plasma concentration; CL/F: apparent oral clearance; T_{max}: time to reach the maximal plasma concentration after the administration; $T_{1/2}$: half-life time; Vz/F: apparent volume of distribution during terminal phase.

5.3. Clinical development

Enlicitide decanoate is currently in Phase III of clinical development with results of Phase IIb study already published. The Phase IIb, randomized, double-blind, placebo-controlled, multicenter trial aimed to evaluate the efficacy and safety of enlicitide decanoate in participants with hypercholesterolemia [30]. Participants were assigned randomly to enlicitide decanoate at the dose of 6, 12, 18, or 30 mg once daily or matching placebo [30]. At 8 week follow-up, enlicitide decanoate reduced, in a dose dependent manner, LDL cholesterol by −41.2 %, −55.7 %, −59.1 %, and −60.9 % after oral administration of 6 mg, 12 mg, 18 mg, and 30 mg, respectively. Near complete efficacy was reached by week 2 and the effect was persistent during the 8-week treatment period.

Among the exploratory endpoints, enlicitide decanoate demonstrated to reduce the Lp(a) plasma levels at week 8 by −12.2 % (6 mg), −21.3 % (12 mg), −21.7 % (18 mg), and −23.7 % (30 mg) [30]. This effect is not unexpected since also mAbs anti PCSK9 reduce Lp(a) by 20–25 % [31]. Importantly, during the 16-week study, including the 8-week treatment period and the 8-week safety follow-up period, the proportion of participants with adverse events was similar in all arms. There were no adverse events that increased in incidence in a dose-dependent manner and there were no overall trends in adverse events across treatment groups [30].

Phase III study CORALreef Outcomes is a randomized, placebo-controlled study investigating the efficacy and safety of enlicitide decanoate in participants with high cardiovascular risk (ClinicalTrials.gov ID NCT06008756). The primary objective is to evaluate the efficacy of enlicitide decanoate, compared with placebo, in increasing the time to the first occurrence of major adverse cardiovascular events (MACE) including coronary heart disease (CHD) death, ischemic stroke, myocardial infarction (MI), acute limb ischemia or major amputation, or urgent arterial revascularization.

6. NNC0385-0434

NNC0385-0434 is a peptide that mimics the EGF-A domain of the human LDL receptor. NNC0385-0434 competitively binds free PCSK9 and subsequently prevents PCSK9 binding to the LDL receptor. This peptide is administered orally with the absorption enhancement excipient and fatty acid derivative, sodium N-[8-(2-hydroxybenzoyl)amino] caprylate (SNAC).

6.1. Clinical development

The Phase II clinical trial was designed to study the efficacy and

safety of NNC0385-0434 [32]. This was a randomized, double-blind, placebo-controlled, parallel-group trial with an open-label active control arm. The subjects recruited were patients with hypercholesterolemia, aged 40 years or older with established atherosclerotic cardiovascular disease (defined as coronary heart disease, cerebrovascular disease, or peripheral artery disease, or any combination of these). People older than 50 years with moderate chronic kidney disease (eGFR between 30 mL/min/1·73 m^2 and 59 mL/min/1·73 m^2), type 2 diabetes, or both moderate kidney disease and type 2 diabetes, were also included in the study as at high risk of atherosclerotic cardiovascular disease.

The LDL cholesterol levels were at least 70 mg/dL in patients receiving maximum tolerated statins and stable lipid-lowering therapy. The study randomly allocated participants (3:1) to receive once dailiy either NNC0385-0434 (15 mg, 40 mg, or 100 mg), placebo or open-label evolocumab (140 mg) every 2 weeks administered subcutaneously [32].

Compared with the placebo group, LDL cholesterol concentrations decreased from baseline to week 12 by 32.0 % with 15 mg dose of NNC0385-0434, 44.9 % with 40 mg dose, and 61.8 % in response to 100 mg dose. Interestingly, evolocumab reduced by 59.6 % LDL cholesterol levels, thus similar to the effect observed with 100 mg daily of NNC0385-0434. LDL cholesterol decreased significantly in all active treatment groups at 2 weeks, with a maximum effect between weeks 6 and 12, and then returned to baseline at the end of treatment, 7 weeks and 4 days after the last dose. As with other PCSK9 inhibitors, also NNC0385-0434 significantly lower Lp(a) concentrations by 36 % at 100 mg dose [32]. Overall, participants tolerated NNC0385-0434 well with similar numbers of patients that experienced adverse events across study groups. The most frequent treatment-related adverse event was related to gastrointestinal disorders. Most of these side effects were mild or moderate in severity and their frequencies did not appear to be dose dependent. Although these clinical results were considered positive, on Nov 2, 2022, the sponsor announced the decision not to develop NNC0385-0434 further due to portfolio considerations.

7. Conclusions

Since the discovery of PCSK9 as new key player in cholesterol homeostasis, the development of small molecules with inhibitory activity to this target has been a highly challenging task due to the flat and featureless nature of the interface between PCSK9 and the EGF-A domain of the LDL receptor. For this reason, monoclonal antibodies and siRNA were considered the easiest and most effective approach for the development of new lipid lowering class of drugs. However, these formulations require regular subcutaneous injections. The patient compliance could, therefore, be increased with the use of oral anti PCSK9.

Here we have described the most effective small molecules or macrocyclic peptides currently under clinical investigation with promising results and potentially to become the next class of anti PCSK9 therapy (Table 4). DC371739 and CVI-LM001 act as transcriptional inhibitors of PCSK9, while enlicitide decanoate (MK-0616), NNC0385-0434 and AZD0780 inhibit the interaction between PCSK9 and the LDL receptor. All these molecules have shown good pharmacokinetic properties with once daily administration, thus suitable for combination therapy with statins, bempedoic acid and ezetimibe. In terms of efficacy, enlicitide decanoate showed the strongest lipid lowering property exceeding the 50 % reduction of LDL cholesterol, target request for high and very-high risk cardiovascular patients [33]. In addition, this molecule showed a significant reduction of Lp(a) [30], as observed with mAbs anti PCSK9 [31], further confirming a metabolic link between PCSK9 and Lp(a). The effect of the others oral PCSK9 inhibitors on Lp(a) is still unknown, however it is reasonable to predict a significant reduction of this proatherogenic particle in response to PCSK9 inhibition. Small molecules like CVI-LM001 and DC371739 appears to be less effective than enlicitide decanoate in reducing LDL cholesterol levels, although the inhibitory effect of DC371739 on ANGPTL3 represents an interesting additional property that deserves further investigation. Finally, the effect of PCSK9 inhibition by oral molecules on the high-sensitivity Creactive protein (hs-CRP) levels should be evaluated in the future. Considering that mAbs anti PCSK9 do not show a significant effect on chronic inflammation [34], we do not expect to see any significant effect of small oral anti PCSK9 molecule on hs-CRP.

Table 4
Summary of pharmacological properties and clinical development of new oral anti PCSK9 molecules.

PCSK9 inhibitor	Mechanism of action	Posology	LDL cholesterol lowering	Clinical studies
DC371739	Reduces the transcription of PCSK9 and ANGPTL3	20 mg, 40 mg, 60 mg, 80 mg, or 120 mg OD	19 %	Phase IIa/ IIb
CVI-LM001	Reduces the transcription of PCSK9 Activates AMPK	300 mg OD	26.3 %	Phase II
AZD0780	Binds PCSK9	30 mg or 60 mg OD	52 %	Phase II
Enlicitide decanoate (MK-0616)	Inhibits PCSK9 – LDL receptor interaction	6, 12, 18, or 30 mg OD	60.9 %	Phase III
NNC0385-0434	Inhibits PCSK9 – LDL receptor interaction	15 mg, 40 mg, or 100 mg OD	59.6 %	Stopped in Phase IIb

In conclusion, oral PCSK9 inhibitors seems to represent the future of hypocholesterolemic therapy after almost 40 years of clinical approval of statins, which use could be strongly reconsidered in the near future, if the safety and efficacy profile of this new class of drugs will be confirmed in Phase III clinical trial.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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