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Lomitapide modifies high-density lipoprotein function in homozygous familial hypercholesterolaemia

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Abstract

Background Lomitapide reduces plasma low-density lipoprotein cholesterol (LDL-C) and is approved for the treatment of homozygous familial hypercholesterolemia (HoFH). This study aims to determine the effect of lomitapide on HDL and cholesterol efflux in a cohort of patients with HoFH.

Patients and methods Analysis included plasma samples from 17 HoFH patients enrolled in the lomitapide phase 3 Aegerion clinical study (NCT00730236). Samples taken at baseline (pre-lomitapide) and weeks 56 and 66 (assumed steady-state on lomitapide) were analyzed for HDL-C levels and cholesterol efflux capacity (CEC) pathways via ABCA1, ABCG1, and SR–BI cholesterol uptake.

Results Treatment with lomitapide is associated with a statistically significant decrease of both LDL-C and apo B when compared to baseline levels, $p < 0.01$. However, the reduction of Lp(a) appears only at a higher dose when compared to baseline (− 27% against values around − 55% for LDL-C and apo B). HDL-C shows a small 4.2% increase between the baseline and the treatment with a high dosage of lomitapide, while apo A–I displays an opposite small 3% decrease. Total efflux and ABCA1 mediated CEC decreased especially at higher dosage of lomitapide, with marked dose-dependent increase of SR–BI cholesterol uptake (+ 21.4% and + 64.3%, respectively, at a low and high dosages of lomitapide). However, ABCG1 did not change consistently.

Conclusions Our report raises the hypothesis that lomitapide promotes lipidation of HDL particles independently of ABCA1 and ABCG1 through a process involving SR–BI pathway. This effect impairs the total efflux process suggesting that lomitapide drives the reverse cholesterol transport through SR–BI receptors in HoFH patients.

Keywords Apo B, Cholesterol efflux capacity, HDL cholesterol, Homozygous familial hypercholesterolaemia, Lomitapide, LDL cholesterol

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Introduction

Homozygous familial hypercholesterolaemia (HoFH) is a rare autosomal dominant lipoprotein disorder caused by mutations in both alleles of the gene coding for the low-density lipoprotein receptor (LDL-R) [1]. Mutations in other genes can cause the HoFH phenotype, but LDLR mutations are the most common causes [2]. The mainstay of therapy for HoFH involves reducing the LDL-C level to prevent progression of atherosclerosis [3]. To address the unmet clinical need in HoFH, several cholesterol lowering therapies have been developed

[4]. These therapies can be broadly classified as either LDL-R-dependent (PCSK9 inhibitors of proprotein convertase subtilisin/kexin type 9, such as evolocumab and alirocumab), statins, ezetimibe, and resin or LDL-R-independent (evinacumab and microsomal triglyceride transfer protein [MTP] inhibitors, such as lomitapide) [5–9]. Subsequent observational studies have confirmed the effectiveness of lomitapide in reducing LDL-C in HoFH in real-world clinical settings [10–14]. Lomitapide blocks MTP which can reduce the assembly of lipoproteins containing apoB in intestine and liver independently of cholesterol efflux [7]. Lomitapide may reduce the levels of HDL derived from the intestine, since MTP-deficiency has been reported to reduce HDL-C secretion from the intestine in mice [15]. Little is known about HDL levels and function particularly in the severe vascular disease in HoFH patients. There is evidence suggesting impaired HDL functionality in patients with FH [16]. Abnormalities of HDL to promote cholesterol efflux have detected in HoFH patients [17, 18]. Recently, we distinctly raised the hypothesis that the anti-atherogenic potential of HDL seems to be unaffected by lomitapide treatment as total CEC did not change consistently in a small cohort of patients receiving the drug [19]. HDL has numerous antiatherosclerotic actions that are not readily reflected by HDL cholesterol levels. A key function of HDL is to promote reverse cholesterol transport from the periphery to the liver in a process called cholesterol efflux. The most studied function of HDL is its ability to remove cholesterol from macrophages, termed cholesterol efflux capacity (CEC) [20]. This is the initial step of RCT and can be measured in vitro with a cell-based assay [21–23]. We hypothesize that, impairment of HDL CEC might be another reason to explain the severe vascular disease in HoFH rather than the obvious explanation of massively increased proatherogenic species, in particular LDL. Herein, we aim to evaluate the effect of lomitapide on HDL cholesterol efflux and uptake through various cell lines expressing different transporters in the reverse cholesterol transport (RCT) pathway. We found that lomitapide-induced changes in apo B-containing lipoproteins are indirectly impacting HDL-related CEC, and these effects on HDL might have a CV benefit for HoFH patients. Results from a study of this type have the potential to inform the exploration of relationships between HDL functionality and HDL remodelling [24].

Methods

Study participants

The current analysis utilised plasma and serum samples from 17 patients enrolled in an open-label, Phase 3, clinical study of lomitapide in patients with HoFH (NCT00730236) [14]. Details of inclusion and exclusion criteria, screening procedures and lomitapide dose escalation protocol have been published previously [14]. The studied Period covering the 56-week data in the Phase 3 was 18 January 2007 to 12 April 2011 (all patients had the week 56 assessment which covers the efficacy and safety phase). The first patient was enrolled on 18 January 2007, with the last patient enrollment occurring on 18 February 2010. All patients provided written informed consent to participate in the Phase 3 trial, which was approved by the Medical Ethics Committees of all participating centers [19]. All eligible patients were informed of the study objectives and overall requirements prior to completing the informed consent form. Patients were also re-consented for the purpose of the current analysis. Before initiation of the study, the informed consent form to be used was submitted for approval to both the sponsor and to the relevant Institutional Review Board or Independent Ethics Committee. Visits 13 and 14 (weeks 56 and 66 of the study) were selected for sampling, because by this time, patients had generally reached stable dose of lomitapide (median dose 40 mg at both visits). During follow-up, lomitapide was not interrupted in any patients.

Blood analysis and measurements

At baseline (prior to the start or immediately after lomitapide treatment), venous blood was drawn by venepuncture after an overnight fast, respectively, from after the LDL apheresis procedure using the DALI® system and were collected into sterile EDTA-containing tubes for isolation of plasma [19, 25]. This was repeated before each dose increase and every 3–4 weeks during the dose titration period [14]. Plasma was immediately separated from blood cells by low-speed centrifugation at 2000 g for 20 min at 4 °C and frozen at − 80 °C in 0.5 mL aliquots until used. A reference standard for plasma/serum was obtained from a pool of six healthy donors and stored in the same manner described above. Plasma samples were directly used for lipids, apolipoproteins and cholesterol efflux analysis without freeze–thaw cycle. Samples from baseline (Visit 2), and weeks 56 and 66 (Visits 13 and Visit 14) were analysed for the current study. All samples from all-timepoints were analysed together in a single run. Lipoprotein profiles were generated using a previously described density-gradient ultracentrifugation method [19, 26]. Lipoproteins were fractioned according to their densities by ultracentrifugation into LDL (1.019–1.063 g/mL), and intermediate- and very-low- density lipoproteins (< 1.019 g/mL) [27]. Cholesterol was measured by an enzymatic method using Selectra E (DDS diagnostic system, Istanbul, Turkey). Plasma lipoprotein (a) (Lp(a) levels were measured using the DiaSys 21 FS immunoturbidimetric assay (DiaSys, Holzheim, Germany) [28].

Apolipoprotein (apo) A–I and apo B levels were measured by immunoturbidimetry on an automatic c311 analyser (Roche Diagnostics) with commercially available polyclonal antibodies. Cellular cholesterol content before and after serum exposure was measured by fluorescence using the Amplex Red Cholesterol Assay Kit (Molecular Probes, Eugene, OR) as previously described [29].

Cholesterol efflux capacity

HDL–CEC assays were performed using apolipoprotein B (apo B)-depleted plasma obtained with the polyethylene glycol (PEG) precipitation method as previously reported [22, 30]. A cell-based efflux system employed 3[H]-cholesterol-labelled J774 murine macrophages and Chinese hamster ovary (CHO), to quantify cholesterol efflux pathways (ABCA1, and ABCG1), respectively, as described previously [31–33]. J774 macrophages, in the absence or presence of cAMP were used for evaluation of aqueous diffusion and total (ABCA1 + ABCG1 + SR–BI + passive diffusion) CEC [32]. The specific contribution of ABCA1 to cholesterol efflux in cAMP stimulated J774 cells was calculated as the difference between total and diffusional-related effluxes [33]. In all assays, J774 and CHO cells were labelled with 0.5 ml/well of 2 µCi/mL 3[H]-cholesterol (Perkin Elmer, Milan, Italy) for 24 h in the presence of 1% FBS [31]. Briefly, ABCG1 expressed in CHO cells was induced by incubating cells for 18–20 h in DMEM with 1 mg/ml fatty acid-free bovine serum albumin and 10 nM mifepristone [34, 35]. After an equilibration period in 0.2% bovine serum albumin (BSA) in medium, cells were exposed to 20% apo B depleted patient serum or plasma for 4 or 6 h. Cholesterol efflux capacity was calculated as cholesterol efflux percent = 3[H] cpm medium/(3[H] cpm medium + 3[H] cpm cells) × 100%. Each patient sample was run in triplicate. The coefficient of inter-variability was 5.83%. To correct for inter-assay variation across plates the pooled serum standard was included on each plate and values were normalized to this control pool (patient efflux/control pool efflux) [36].

Cholesterol uptake through SR–BI receptor in Fu5AH cells

The measurement of HDL influx in Fu5AH rat hepatoma cells was performed as previously described [37]. Briefly, purified HDL were labeled by exchanging 3[H]-cholesterol (20–40 µCi/mg HDL protein) from the glass wall of a test tube onto which the 3[H]-cholesterol had been dried under N2. After incubating the HDL with the 3[H]-cholesterol overnight at 4 °C, the particles were sterilized by filtration through a 0.45 µm filter. The radiolabeled HDL were diluted in DMEM and incubated with the unlabeled Fu5AH cells for 6 h. The Fu5AH cell lipids were then extracted with isopropyl alcohol as previously described [37]. The total of 3[H]-label present in the lipid extract from cells (% cpm) was quantified by liquid scintillation and plotted as cell-associated radioactivity as described for HDL cholesterol influx by Yancey et al. [37]. The specific contribution of SR–BI was assessed via a 2 h pre-treatment of Fu5AH cells with 10 µM blocker of lipid transport-1 (BLT-1) to inhibit any transport of cholesterol from HDL to the cells via SR–BI [38, 39].

Statistical analysis

Descriptive analyses were conducted on samples obtained at baseline and during lomitapide treatment (weeks 56 and 66 of the Phase 3 study). These data are presented as percentage changes from baseline. The effect of lomitapide on various dependent variables [LDL, HDL, total efflux, ABCA1, ABCG1, SRBI, apo B, apo A–I, and Lp(a)] was tested. Due to the small sample size, statistical analysis was limited to comparison of the measures of plasma levels before and after treatment with lomitapide (regardless of dosage). After treatment, the average values of blood parameters at weeks 56 and 66 were used (Fig. 1A, B). In this way, we could utilize a simple test for paired data, namely, the sign test, which is a non-parametric test for the equality of medians, specifically designed for data that do not adhere to normality and for which the distribution of differences is not symmetric. More precisely, the null hypothesis here tested is that the median of the paired differences is zero. The Bonferroni correction was then applied to correct for multiple testing, and the results are presented in Table 3. Data were analyzed using STATA14 and Microsoft Excel.

Results

Baseline characteristics

The baseline characteristics of the study subjects are presented in Table 1. All patients ($n = 17$) had history of CVD. Patients 8–17 ($n = 10$) were undergoing apheresis (lipoprotein–apheresis or plasmapheresis) to reduce their exposure to proatherogenic variables as HDL and apoA-I (Fig. 1). One patient was receiving lipoprotein apheresis at the time of obtaining the baseline blood sample (Visit 2), but apheresis was stopped before weeks 56 and 66 on lomitapide.

Atherogenic variables [LDL-C, apo B, and Lp(a)]

According to the descriptive results shown in Table 2, the levels of LDL-C, apo B, and Lp(a) all decrease in a lomitapide-dose-dependent manner. However, the reduction of Lp(a) appears slower and substantial only at a higher dose when compared to baseline (−27% compared to values around −55% for LDL-C and apo B). According to the sign test (Table 3), the treatment with lomitapide is

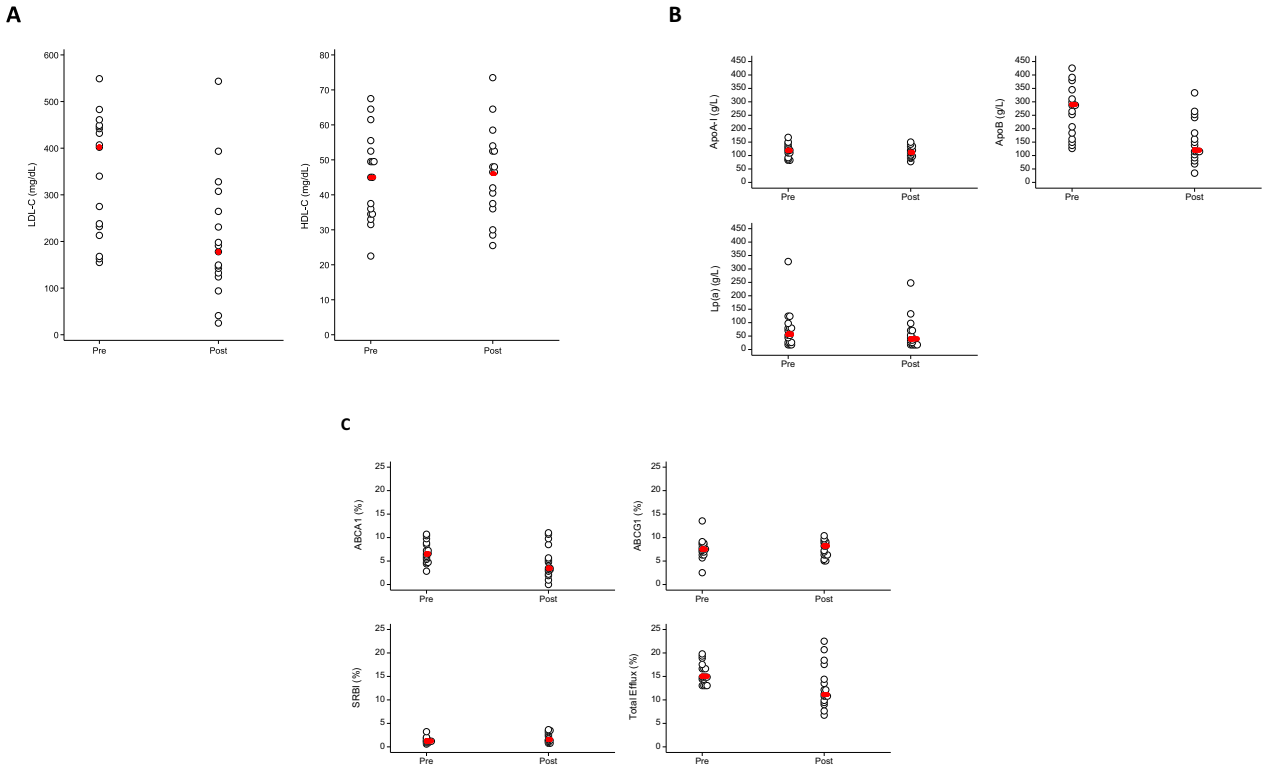

Fig. 1 Dot plots of the parameters investigated before (pre) and after (post) treatment with lomitapide. Median values in red

associated with a statistically significant decrease of both LDL-C and apo B—but not for Lp(a)—when compared to baseline levels after correction for multiple testing (Table 3 and Fig. 1A, B).

Anti-atherogenic variables (HDL-C, apo A–I)

Unlike atherogenic variables, HDL-C and apo A–I levels do not appear to change after treatment with lomitapide at any dosage (Table 2). HDL-C shows a small 4.2% increase between the baseline and the treatment with a high dosage of lomitapide, whereas apo A–I displays a slight small 3% decrease. The sign test confirms that none of the anti-atherogenic variables showed any statistically significant difference between before and after treatment with lomitapide (Table 3 and Fig. 1A, B)

Cholesterol efflux capacity and cholesterol uptake

From a descriptive point of view, overall CEC showed only a minor reduction after treatment with lomitapide and only at higher dosages (Table 2). Such a small reduction was not proven to be statistically significant according to the sign-test results on medians. As for the effects of the treatment with lomitapide on total CEC pathways (ABCA1, ABCG1, and SR–BI receptors), the descriptive analysis points to a decrease of ABCA1 mediated CEC levels, particularly at higher dosage of lomitapide (-48.5% with respect to baseline), a marked dose-dependent increase of SR–BI (+21.4% and +64.3%, respectively, at a low and high dosages of lomitapide), and a substantial stability of ABCG1 efflux, which decreases slightly at low dosage (−15.6%) and then increases again at higher dosage of lomitapide (+13.0%), always in comparison with baseline. However, neither the total efflux nor the ABCA1, ABCG1, and SR–BI-cholesterol uptake pathways show any statistically significant difference before and after treatment with lomitapide according to the sign test after correction for multiple testing (Table 3 and Fig. 1C).

Discussion

Lomitapide is a MTP inhibitor that works independently of the LDL receptor, resulting in the reduction of LDL-C levels in patients with HoFH. The results presented in this research reaffirm that lomitapide treatment affects lipoprotein profile [LDL-C, apo B, and Lp(a)] and HDL functionality in patients with HoFH (Table 2). Our data confirm no significant differences on HDL-C levels between the lomitapide treatments in patients, as well as insignificant difference at weeks 56–66 (Table 2). Nevertheless, we observed that levels of apo A–I remained unchanged across the lomitapide dose categories. Previous studies showed that lomitapide treatment is

Table 1 Patients' baseline characteristics (before receiving lomitapide)

Patient No	Age	Gender	LDL-C mg/dL	HDL-C mg/dL	apoA–I mg/dL	apoB mg/dL	Lp(a) mg/dL	Total Efflux %cpm	ABCA1%cpm	ABCG1%cpm	SR–BI %cpm
1	18	M	483	50	127	390	83.7	16.7	5.3	8.5	1.0
2	55	M	275	23	85	264	56.8	13.2	4.4	9.0	1.2
3	18	F	163	45	120	132	14.3	15.0	2.7	7.5	1.2
4	19	F	446	67	136	293	43.1	14.4	4.6	7.1	3.3
5	33	M	461	35	92	374	16.0	14.9	6.7	7.1	1.4
6	21	F	232	36	111	187	26.0	12.9	5.7	7.8	1.0
7	41	F	549	61	110	429	323.6	17.7	10.4	8.4	0.9
8†	19	F	168	38	130	146	29.7	13.1	6.1	7.6	1.2
9	22	M	238	32	87	208	76.8	14.8	6.2	9.0	1.6
10	45	M	340	55	143	250	121.2	16.7	6.5	7.5	2.0
11	23	M	432	45	117	315	67.5	15.0	7.1	13.7	1.8
12	26	M	213	53	147	159	76.0	12.9	4.9	9.0	2.0
13	55	F	155	50	151	139	94.0	16.7	8.9	6.4	1.2
14	22	F	402	33	82	290	53.7	14.5	7.3	7.3	1.3
15	36	M	407	64	168	295	28.3	19.0	8.4	5.6	0.9
16	22	M	442	49	119	345	119.9	20.0	9.6	6.3	1.3
17	22	F	449	34	83	293	15.6	19.5	10.6	2.6	0.6
Mean	29.2		344.4	45.3	118.1	265.2	73.3	15.7	6.8	7.7	1.4

Descriptive statistics of all the parameters investigated, with means and SDs. Apo A–I, apolipoprotein A1; apo B, apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; SR–BI, Scavenger receptor BI

* The first 7 patients received apheresis, but all values shown in the table are from blood samples drawn pre-apheresis

† Patient was receiving apheresis at the time of obtaining the baseline blood sample (visit 2) but apheresis was stopped before on-lomitapide samplings (visits 13 and 14)

Table 2 Concentrations of atherogenic variables [LDL-C, apo B and Lp(a)] and antiatherogenic variables (HDL-C, apo A–I) by lomitapide dose category (mean values and SDs)

	Lomitapide dosage category		
	No (baseline)	**5–20 mg/dL**	**40–60 mg/dL**
LDL-C, mg/dL	344.4 ± 129.0	270.9 ± 140.9	159.5 ± 105.6
HDL-C, mg/dL	45.3 ± 12.4	44.6 ± 14.3	47.1 ± 12.1
Apo A–I, mg/dL	118.1 ± 26.2	112.1 ± 17.7	114.8 ± 22.8
Apo B, mg/dL	265.2 ± 91.8	196.0 ± 87.0	121.1 ± 62.6
Lp(a), mg/dL	73.3 ± 73.1	66.9 ± 80.3	52.1 ± 39.5
Total Efflux, %cpm	15.7 ± 2.3	14.4 ± 5.3	11.8 ± 3.8
ABCA1, %cpm	6.8 ± 2.2	6.3 ± 3.5	3.9 ± 3.1
ABCG1, %cpm	7.7 ± 2.2	6.5 ± 1.4	8.5 ± 1.5
SR–B1, %cpm	1.4 ± 0.6	1.7 ± 0.9	2.3 ± 1.1
N	17	7	10

N = number of samples analyzed

* Samples from patients receiving no lomitapide were drawn at the Baseline visit (week 2), and samples from patients receiving lomitapide were drawn at visits 13 and 14 (weeks 56 and 66). In the patients who were receiving apheresis, all values shown are from blood samples drawn pre-apheresis

Table 3 Sign test for the equality of medians before and after treatment with lomitapide. *P* values

Variables	***p* value**
LDL	**0.0003**
HDL	0.9993
Apo A–I	0.2101
Apo B	**0.0003**
Lp(a)	0.0489
Total Efflux	0.0124
ABCA1	0.0127
ABCG1	0.6291
SR–B1	0.0490

Non-parametric tests on median differences before and after treatment with lomitapide

* In bold, statistically significant differences before and after treatment. Statistical significance was based on a probability threshold of $\alpha/n = 0.0056$ after correction for multiple testing

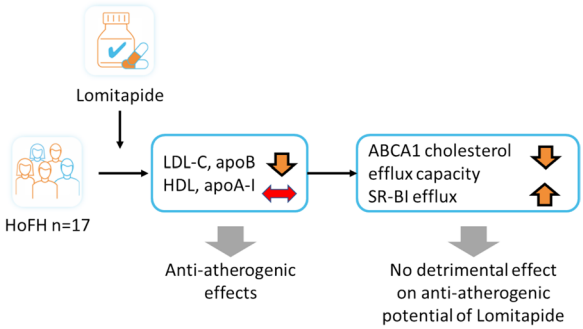

Fig. 2 Graphical abstract. The effect of treatment with lomitapide on high-density lipoprotein (HDL) functionality of serum from patients with homozygous familial hypercholesterolaemia (HoFH)

associated with a moderate decrease of both HDL-C and apo A–I levels during the titration period of the drug suggesting a possible compensatory mechanism [14, 19, 40, 41]. In this study, lomitapide was unable to activate the rate limiting step of RCT mediated by ABCA1 (Fig. 2). Conversely, the total cholesterol efflux continued to decrease constituently at high lomitapide doses (Table 2, Fig. 1C). In that, higher total efflux capacity was shown to include a significant increase in ABCA1-mediated efflux [21]. This indicates that ABCA1 efflux influences total cholesterol efflux, which in turn correlates with the abundance of a specific pool of HDL particles in plasma [21]. Patients with coronary heart disease have higher than normal levels of preβ-1 HDL particle concentration with decreased functionality and lower than normal levels of large-HDL particle concentration, which have enhanced functionality [42]. Thus, HDL CEC might be influenced by both the concentration and the functionality of these specific HDL particles in plasma. Fortunately, lomitapide treatment may favor an increase of HDL-C pool in plasma, possibly associated with SR–BI pathway activation in hepatocytes. This process can facilitate selective HDL lipid uptake and clearance from plasma, thereby driving the RCT process (Fig. 2) [43]. Hepatic SR–BI is important in inhibiting atherosclerosis and reducing foam cell formation by regulating cholesterol transport [44]. In peripheral cells SR–B1 also promotes cholesterol outflow to mature HDL particles, but its role in the RCT pathway is particularly significant in the liver, where it mediates the selective uptake of cholesteryl esters from HDL particles [43]. In our previous small study of four patients with HoFH (two men and two women), the effect of lomitapide on HDL functionality was supported by a shift to larger HDL pool, mainly HDL2 cholesterol particles that promote SR–BI-mediated cholesterol influx [19]. In addition, during regular heparin-based apheresis treatment, a significant increase of HDL and HDL3 cholesterol levels was reported [45]. This effect was not observed in our previous cohort, where we did not see increase in HDL3, which may interfere with lomitapide treatment [19]. However, such observed changes in CEC might also be indirectly associated with a certain effect of lomitapide on plasma HDL particles quality. When combined with the reduction of atherogenic variables (e.g., LDL-C), this suggests a net benefit of lomitapide [19]. In contrast to another study by Cedó et al., involving a group of patients with FH, 7 male and 8 female adults, and 6 male and 7 female adolescents. It was shown

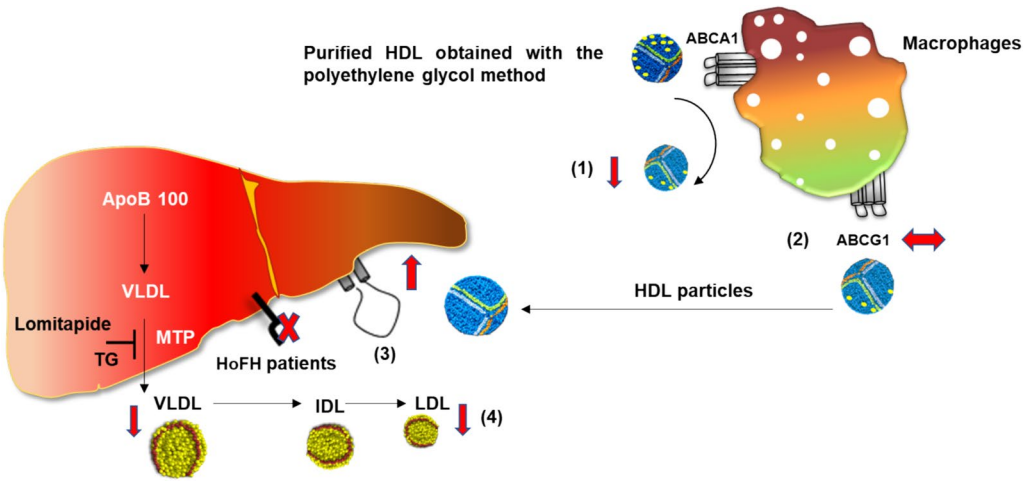

Fig. 3 Schematic representation lomitapide of effect on reverse cholesterol transport in HoFH patients. (1) Total efflux and ABCA1 mediated CEC decreased especially after the introduction of high dose lomitapide (2) ABCG1 mediated CEC was not changed (3). Cholesterol uptake through SR–BI is markedly increased by lomitapide (4). Plasma HDL-C levels increased by 4.2% after the introduction of high doses of lomitapide, while plasma apoA–I levels decreased by 3% under the same conditions

that high concentrations of LDL-C in these patients is linked to dysfunctional HDL-C, which is characterised by altered remodeling and a reduction in the capacity to promote cholesterol removal from macrophages [46]. In the current study, and in contrast to previous data [14, 19, 40], HDL-C levels either remained stable with increasing lomitapide dosages, i.e., 40–60 mg/dL or showed a slight increase at a higher dose of lomitapide (Table 2). The reduction in apo B-containing lipoproteins with lomitapide may influence the capacity of plasma to affect CEC from macrophages warranting further investigations. The anti-atherogenic benefits of lowering apo B-containing lipoproteins may reverse the negative impact of reduced cholesterol efflux observed in vitro after lomitapide treatment. However, according to Yin et al., this process was questioned and may reflect a feedback mechanism in response to the reduction of in vivo flux of cholesterol into the artery wall from LDL and VLDL [8]. At present, data suggest that lomitapide reduces cardiovascular events in HoFH patients [47]. This may be achieved by adequately controlling LDL-C levels, which may enable healthcare teams and patients to consider ceasing or reducing the frequency of apheresis, as previously shown [10, 14, 48, 49]. Together our results indicate that the use of lomitapide could potentially mitigate the elevated cardiovascular risk among HoFH patients regardless ABCA1 CEC pathway (Fig. 2).

Limitations

This study has limitations. We only studied a small number of participants ($n=17$) welcoming further investigation to support our findings. We also, highlight that there may be a different interpretation of lomitapide dose dependency. This holds true only when examining the grouping of low doses versus high doses, but when looked at in terms of actual dose this is not accurate, for example, 40 mg is sometimes more efficient than 60 mgs. It should also be considered that age-associated reduction in expression of ABC transporters impair the efflux capacity of macrophages and result in increased cholesterol accumulation [50]. Furthermore, we were unable to compare patients with and without apheresis against HDL CEC due to small sample size of our cohort. We believe that possible shifts in size of HDL particles might occur as it was shown in our previous study [19]. A more detailed examination of changes in HDL composition and size to correlate with changes in CEC needs to be performed.

Conclusion

Lomitapide reduces ABCA1 mediated CEC in plasma samples of HoFH patients in vitro. This may seem clinically unexpected given the documented inverse relationship between ABCA1 mediated HDL CEC and incident cardiovascular events [22, 30]. Lomitapide affects HDL CEC functionality, which may involve changes by reducing the size of the HDL-C and LDL-C pools to prevent progression of atherosclerosis. However, the impact of lomitapide on HDL maturation and metabolism deserves further study. Our report raises the hypothesis that lomitapide promotes HDL-C particle uptake by SR–BI independently of ABCA1 and ABCG1. Changes in the different components of CEC do not impair total efflux, suggesting that lomitapide drives the final part of RCT

through the SR–BI receptor in HoFH patients thereby delivering cholesterol to the liver and regulating cholesterol transport (Fig. 3). Whether such observed changes are good or bad or neutral remains to be determined.

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Author contribution
Anouar Hafiane: E.F. A.B. and C.D. Annalisa Ronca: A.B. and C.D. Matteo Incerti: E.F. Alessandra Rossi: A.B. and C.D. Matteo Manfredini: E.F. Elda Favari: A.B. and C.D. All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All the investigation of the current study was performed under the approval of the ethic committee of University of Parma as well as the Declaration of Helsinki. Patients' recruitment has been done by the Phase 3, Aegerion clinical study of lomitapide in patients with HoFH (NCT00730236).

Consent for publication
All the authors approved the final manuscript and the submission to this journal.

Competing interest
All authors have no conflicts to disclose that are relevant to this publication.

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