

Review

Zetia: Inhibition of Niemann-Pick C1 Like 1 (NPC1L1) to Reduce Intestinal Cholesterol Absorption and Treat Hyperlipidemia

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Zetia (ezetimibe) is a selective cholesterol absorption inhibitor, which potently inhibits the absorption of biliary and dietary cholesterol from the small intestine without affecting the absorption of fat-soluble vitamins, triglycerides or bile acids. Ezetimibe reduces the small intestinal enterocyte uptake and absorption of cholesterol by binding to Niemann-Pick C1 Like 1 (NPC1L1), which keeps cholesterol in the intestinal lumen for excretion. Ezetimibe undergoes glucuronidation to a single metabolite and localizes at the intestinal wall, where it binds with higher affinity for NPC1L1 than ezetimibe to prevent cholesterol absorption. Enterohepatic recirculation of ezetimibe and/or its glucuronide ensures repeated delivery to the intestinal site of action and limited peripheral exposure. Ezetimibe has no effect on the activity of major drug metabolizing enzymes (CYP450), which reduces any potential drug-drug interactions with other medications. Ezetimibe (10 mg/day) was found to inhibit cholesterol absorption by an average of 54% in hypercholesterolemic individuals and by 58% in vegetarians. Ezetimibe alone reduced plasma total and LDL-Cholesterol (18%) levels in patients with primary hypercholesterolemia. When ezetimibe was added to on-going statin treatment, an additional 25% reduction in LDL-C was found in patients with primary hypercholesterolemia and an additional 21% reduction in LDL-C in homozygous familial hypercholesterolemia. Ezetimibe in combination with statins produces additional reductions in plasma cholesterol levels and allows for more patients to achieve their LDL-C goals.

J Atheroscler Thromb, 2007; 14:99-108.

Key words; Ezetimibe, Cholesterol absorption inhibitor, NPC1L1, Cholesterol transporter

Zetia Discovered in Preclinical Models of Hyperlipidemia

Zetia (ezetimibe; SCH 58235; 1-(4-fluorophenyl)-(3R)-[3-(4-fluorophenyl)-(3S)-hydroxypropyl]-(4S)-(4-hydroxyphenyl)-2-azetidinone) was discovered using *in-vivo* models of cholesterol absorption¹. Zetia was identified through the characterization of the active biliary metabolites of its predecessor, SCH 48461, and extensive structure-activity relationship information obtained from a seven day cholesterol-fed hamster model². Ezetimibe dose-dependently inhibited diet-induced hypercholesterolemia in hamsters with an

ED₅₀ of 0.04 mg/kg. In an acute model of intestinal absorption using radiolabeled cholesterol in rats, ezetimibe inhibited the appearance of radiolabeled cholesterol in plasma with an ED₅₀ of 0.0015 mg/kg ninety minutes after dosing, indicating that the onset of activity was rapid³. Ezetimibe has proven to be most potent pre-clinically in cholesterol-fed rhesus monkeys with an ED₅₀=0.0005 mg/kg/day. A single dose of the ezetimibe analog, SCH 48461, administered to cynomolgus monkeys fed a single cholesterol-containing meal caused a significant reduction of cholesterol in chylomicrons and chylomicron remnants during the postprandial phase without affecting triglyceride content⁴.

Ezetimibe selectively inhibits the transport of cholesterol across the intestinal wall. Ezetimibe does not affect intestinal cholesteryl ester hydrolysis and the absorption of fatty acids thus generated. The free cholesterol from this hydrolysis, however, was not absorbed

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Received: April 26, 2007

Accepted for publication: May 16, 2007

in the presence of ezetimibe. Ezetimibe does not affect the absorption of triglyceride, ethinyl estradiol, progesterone, vitamins A and D, and taurocholic acid in rats⁵. Ezetimibe does not affect pancreatic lipase, and therefore shares no properties with orlistat (Xenical). Ezetimibe also does not sequester bile acids or block their absorption and thus differs from cholestyramine (resins). Studies in humans have indicated that ezetimibe does not affect serum fat soluble vitamin status⁶. Ezetimibe potently and selectively inhibits biliary and dietary cholesterol absorption in the intestine.

Metabolism of Zetia

Ezetimibe is rapidly metabolized in the intestine to its phenolic glucuronide by uridine 5-diphosphate (UDP)-glucuronosyl-transferase 1A1, 1A3, and 2B15^{7,8}. Once ezetimibe is glucuronidated, it is excreted in the bile, thereby delivering the drug back to the intestinal site of action³. Cholesterol absorption studies indicated that the glucuronide appeared more potent than ezetimibe itself, because glucuronidated ezetimibe localizes more avidly to the intestine. Autoradiographic analysis demonstrated that drug related material was located in the small intestinal villi, and concentrated at the enterocyte brush border^{3,9}.

In humans, ezetimibe is rapidly absorbed and primarily metabolized in the small intestine and liver to its glucuronide, with little oxidative cytochrome P450 mediated metabolism^{7,8}. Ezetimibe and its glucuronide undergo enterohepatic recycling and have a plasma half-life of approximately 22 hours in humans. Ezetimibe and/or the glucuronide metabolite are excreted in the feces (90%) and urine (10%). Since ezetimibe does not influence the activities of cytochrome P450 enzymes, no significant pharmacokinetic interactions occur with most medications. Pharmacokinetic interaction studies with ezetimibe in humans have found no significant changes in the plasma levels of many medications, including statins (atorvastatin, simvastatin, pravastatin, rosuvastatin, lovastatin, and fluvastatin), fibrates (gemfibrozil and fenofibrate), digoxin, glipizide, warfarin, and oral contraceptives (ethinyl estradiol and levonorgestrel)⁶⁻⁸.

Discovery of the Molecular Target of Zetia: Niemann-Pick C1 Like 1

Since the discovery of the cholesterol absorption inhibitor ezetimibe over ten years ago using *in vivo* models at Schering-Plough, there has been an intense effort to determine the molecular target of ezetimibe. The mechanism by which cholesterol moves from the

intestinal lumen into the absorptive enterocytes lining the proximal small intestine has been poorly understood. The identification of ezetimibe as a potent selective inhibitor of intestinal cholesterol uptake and absorption in animals and humans confirmed that the intestinal cholesterol uptake process is mediated by a specific transporter^{2-5,10}. Based on the properties of ezetimibe in animal models of cholesterol uptake, it was predicted that such a transporter would be expressed in jejunal enterocytes, where cholesterol is absorbed, and localized to the brush border membrane. Biochemical and molecular biological techniques were used for many years in an attempt to identify the intestinal transporter ezetimibe inhibited. These studies identified several candidate proteins, like scavenger receptor class B, type I (SR-BI), but when these proteins were deleted in knockout mice they were not found to be involved, because cholesterol absorption was normal and ezetimibe was still active as a cholesterol absorption inhibitor¹¹.

Several years ago, a genomic/bioinformatics approach was used to identify genes involved in intestinal cholesterol uptake¹². It was hypothesized that a cholesterol transporter should possess several critical features. The gene responsible for intestinal absorption of cholesterol must be expressed in the jejunal enterocyte. The protein should be in direct contact with the luminal contents and hence should be expressed on the surface of the enterocyte brush border membrane. In addition, the protein should contain sequence motifs known to interact with sterols¹². A rat intestinal cDNA library was generated and ~16,500 genes were sequenced and annotated by cross-referencing the rat sequences with both mouse and human data. This sequence database was analyzed for all transcripts containing features anticipated in a cholesterol transporter: sequences predictive of transmembrane domains, extracellular signal peptides and N-linked glycosylation sites, and cholesterol interacting motifs such as a sterol sensing domain. From the list of genes identified, only one credible candidate gene emerged from this analysis, the rat homologue of Niemann-Pick C1 like 1 (NPC1L1). NPC1L1 has all of the predicted features of a plasma membrane expressed transporter including a secretion signal, 13 predicted transmembrane domains and extensive N-linked glycosylation sites located within the extracellular loops, and it contains a sterol sensing domain¹².

NPC1L1 was found to be highly expressed in the jejunum and not expressed in other tissues in the mouse. Furthermore, it localized on the surface of the absorptive jejunal enterocytes^{12,13}. Mice deficient in NPC1L1 were generated and their initial characteriza-

tion showed a significant >70% reduction in cholesterol absorption, and the low level of residual cholesterol absorption was insensitive to ezetimibe treatment¹²). These initial results indicated that NPC1L1 resided in the ezetimibe-sensitive pathway responsible for cholesterol absorption. With additional studies, intestinal cholesterol uptake and absorption was found to be significantly reduced in the NPC1L1 null mice¹⁴. Acute cholesterol absorption was reduced by nearly 90% in the NPC1L1 null mice, which is similar to the inhibition of cholesterol absorption found in ezetimibe treated mice, hamsters, and rats²). The uptake of cholesterol into the enterocytes of the jejunum was substantially reduced in the NPC1L1 null mice, indicating that this protein plays an essential role in the uptake of cholesterol from the lumen of the intestine to the brush border membrane of the enterocyte. Ezetimibe-treated mice, hamsters, and rats also demonstrated a reduction the intestinal uptake of cholesterol into jejunal enterocytes. Triglyceride uptake by the intestine and its absorption was not altered in the NPC1L1 null mice. This finding is also similar to that with ezetimibe-treated animals, where fatty acid absorption from triglycerides or cholesteryl esters, or the triglyceride content of postprandial chylomicrons is not altered^{4, 5}). NPC1L1 null mice were completely resistant to diet-induced hypercholesterolemia, similar to an ezetimibe treated wild-type mouse. Overall, the profile of cholesterol absorption in the NPC1L1 null mice closely resembled the profile of an ezetimibe-treated animal¹⁴).

In addition to inhibiting cholesterol absorption, ezetimibe has been shown to reduce plasma phytosterol levels in patients with hypercholesterolemia and in patients with sitosterolemia (see clinical section), which is caused by a mutation in the ATP-binding cassette (ABC) co-transporters, either ABCG5 or ABCG8^{10, 15, 16}). ABCG5 and ABCG8 are expressed on the apical surface of hepatocytes and enterocytes, and their function is required to export phytosterols into the bile and intestinal lumen, respectively. Phytosterols are poorly absorbed compared to cholesterol, and it is not known whether the two sterol classes are taken up by the same mechanism into intestinal enterocytes. Plasma levels of the plant sterols campesterol and sitosterol were found to be nearly undetectable in the NPC1L1 null mice, and reduced by greater than 90% compared to wild type mice¹⁴). Labeled sitosterol absorption and uptake in the intestine of the NPC1L1 null mice was substantially reduced, very similar to the reductions observed in wild type mice treated with ezetimibe. Taken together, these results indicated that NPC1L1 is the intestinal transporter for the uptake of both cholesterol and structurally related phytosterols (**Fig. 1**), and

that ezetimibe acts through the NPC1L1 pathway¹⁴).

It has been demonstrated that ezetimibe treatment inhibits cholesterol absorption, reduces plasma cholesterol and inhibits the development and progression of atherosclerosis in apoE (-/-) mice by >90% when fed western or cholesterol-free diets¹⁷). Consequently, with the discovery of NPC1L1, studies were initiated to determine the effect that mice lacking NPC1L1 would have on the development and progression of atherosclerosis in apoE (-/-) mice. NPC1L1/apoE null (-/-) mice were generated and had a 77% reduction in cholesterol absorption, and when fed chow or western diets, plasma cholesterol levels were reduced, and atherosclerosis inhibited by >90% relative to apoE (-/-) mice. Lack of NPC1L1 in apoE (-/-) mice caused a nearly complete protection from the development of atherosclerosis, under both cholesterol-fed and non-cholesterol-fed conditions, similar to the findings in ezetimibe treated apoE (-/-) mice¹⁸).

The *in vivo* studies established that NPC1L1 is central to cholesterol and phytosterol uptake into enterocytes and is in a pathway sensitive to ezetimibe. However, the specific molecular role for NPC1L1 in the action of ezetimibe remained unclear until a binding assay for NPC1L1 and ezetimibe was established¹⁹). Recombinant rat and human NPC1L1 were expressed in HEK 293 cells. Binding of a BODIPY-labeled fluorescent ezetimibe glucuronide analog to NPC1L1 expressing cells demonstrated a specific, single site, saturable binding profile. Binding of the fluorescent ezetimibe clearly showed cell surface membrane binding to the NPC1L1-expressing cells and was completely abolished in the presence of excess unlabeled ezetimibe glucuronide. Similar binding studies using [3H]-ezetimibe glucuronide also revealed specific binding to membrane preparations from cells expressing NPC1L1. These results demonstrated that ezetimibe binds specifically to NPC1L1 and clearly indicated that NPC1L1 is the direct molecular target of ezetimibe¹⁹).

To obtain more evidence that NPC1L1 is the direct binding target of ezetimibe, binding affinities of ezetimibe glucuronide and several key structural analogs were determined using recombinant rat and human NPC1L1 in HEK - 293 cell membranes and compared to those for native rat and rhesus monkey intestinal enterocyte brush border membranes¹⁹). A series of ezetimibe analogs was selected with subtle structural diversity, but with binding affinities to native brush border membranes that covered a range of 1,000-fold. The K_i values for the series of ezetimibe analogs for recombinant rat NPC1L1 and native rat brush border membranes were virtually identical, which showed that NPC1L1 is the molecular target of ezetimibe *in vivo*.

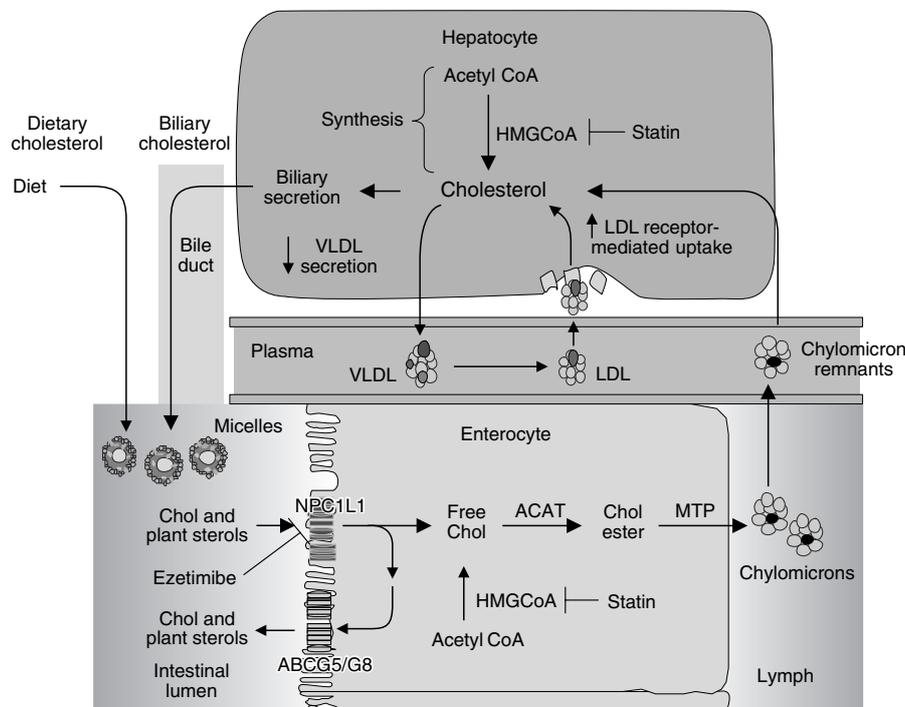


Fig. 1. NPC1L1, Ezetimibe and Cholesterol Metabolism

Intestinal cholesterol absorption begins with the micellar solubilization of both dietary and biliary cholesterol in the lumen of the small intestine. In general, approximately 50% of intestinal cholesterol is absorbed, in which two thirds is derived from the bile and the other one third is derived from the diet. The cholesterol is then transferred from the micelles to the surface of the brush border membrane of the enterocyte, where NPC1L1 plays a critical role for the uptake of cholesterol and phytosterols, and is the target of the cholesterol absorption inhibitor Zetia (ezetimibe). Cholesterol enters the cytoplasmic compartment and moves to the endoplasmic reticulum where it may be esterified by ACAT2 to form cholesteryl ester. Free cholesterol and cholesteryl esters are packaged into chylomicrons, which are then secreted into the mesenteric lymph. Once in the circulation, chylomicrons and their remnants are rapidly cleared by the liver. The consequences of cholesterol absorption inhibition by ezetimibe include decreased cholesterol delivery to the liver, reduced hepatocyte cholesterol stores, decreased VLDL production, increased LDL clearance and, subsequently, decreased LDL cholesterol levels. Inhibition of cholesterol synthesis by statins complements the activity of inhibiting cholesterol absorption by ezetimibe, resulting in enhanced reductions in LDL cholesterol levels.

These studies were extended to include binding to mouse, hamster, rabbit, and canine NPC1L1, which correlated to the *in vivo* activity of ezetimibe²⁰. Binding studies with multiple species NPC1L1 orthologs also demonstrated that the binding affinity for the glucuronide metabolite was 2-10 fold higher than ezetimibe. The final, conclusive evidence proving the hypothesis that NPC1L1 is the target of ezetimibe was provided by studies with tissues from NPC1L1 null mice. Enterocyte brush border membranes prepared from NPC1L1 null mice showed no detectable specific binding affinity of [³H]-ezetimibe glucuronide, whereas membranes from wild-type mice showed a high level of specific binding¹⁹.

These results demonstrate that ezetimibe binds to native intestinal membranes and cells expressing recombinant NPC1L1 with comparable affinity, and does not bind to membranes from NPC1L1 null mice, indicating a specific binding interaction between NPC1L1 and ezetimibe. Together with the findings that mice deficient in NPC1L1 are defective in intestinal cholesterol and phytosterol uptake, and are no longer responsive to ezetimibe, definitively established NPC1L1 as the direct molecular target of ezetimibe (**Fig. 1**).

The finding that NPC1L1 is the molecular target of ezetimibe has begun to be applied to human cholesterol absorption and clinical studies with ezetimibe alone or in combination with statins²¹⁻²⁴. These

clinical studies have identified polymorphisms in the NPC1L1 gene and reported that these polymorphisms alter cholesterol absorption and the magnitude that ezetimibe reduced LDL-cholesterol levels.

Efficacy of Zetia (Ezetimibe) in Humans

The effect of ezetimibe (10 mg/day) on human cholesterol absorption was investigated in mildly hypercholesterolemic individuals and vegetarians^{10, 25}. Fractional cholesterol absorption rate was measured by the continuous dual-isotope feeding method using deuterium-labeled cholesterol and sitostanol. In mildly hypercholesterolemic individuals fractional cholesterol absorption rates averaged 49.8% on placebo and 22.7% on ezetimibe, indicating an average reduction of 54%. LDL and total cholesterol levels following ezetimibe treatment were reduced 20.4% and 15.1%, respectively, whereas campesterol and sitosterol were decreased by 48% and 41%, respectively¹⁰. The reduction of plasma concentrations of the non-cholesterol sterols, sitosterol and campesterol, which are not endogenously synthesized, indicated a direct effect on the absorption of these sterols by ezetimibe inhibiting NPC1L1. In vegetarians fractional cholesterol absorption rates averaged 48.2% on placebo and 20.2% on ezetimibe, indicating an average reduction of 58%. Although these individuals were consuming less than 30 mg of dietary cholesterol, LDL cholesterol levels following ezetimibe treatment were still reduced 17.3%²⁵. These results indicate that Zetia significantly reduces LDL-C by inhibiting the reabsorption of biliary cholesterol in individuals consuming very little dietary cholesterol, and Zetia's LDL-C lowering activity is not diet dependent.

Ezetimibe Monotherapy

The efficacy and safety of ezetimibe in patients with primary hypercholesterolemia was evaluated in two multicenter, double-blind studies^{26, 27}. Following dietary stabilization, a 2- to 12-week washout period, and a 4-week placebo lead-in period, patients were randomized to ezetimibe 10 mg or placebo once daily for 12 weeks. In a pooled analysis of the 1,719 patients from both studies²⁸, ezetimibe significantly reduced mean LDL-C by 18.2% (vs a 0.9% increase with placebo, $p < 0.01$) and resulted in a statistically significant increase in HDL-C, and statistically significant reductions in triglycerides and apolipoprotein B. The response to ezetimibe was consistent across all subgroups analyzed. Ezetimibe was well tolerated, with a safety profile similar to placebo.

Ezetimibe in Combination with Statins

Ezetimibe inhibits the absorption of biliary and dietary cholesterol and statins inhibit cholesterol synthesis, these mechanisms of action should be complementary in reducing plasma LDL-cholesterol levels (Fig. 1). The LDL-C lowering efficacy of inhibiting both cholesterol absorption and synthesis has been evaluated in several large clinical trials. These trials have evaluated the efficacy of adding ezetimibe to ongoing statin therapy as well as the efficacy of coadministration of ezetimibe and a statin.

In the ezetimibe add-on to statin for effectiveness (EASE) trial²⁹, the benefit of adding ezetimibe to ongoing statin therapy was evaluated for patients who had not achieved National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III LDL-C goals after 6 weeks of statin therapy. A total of 3,030 patients were randomized to receive either statin plus ezetimibe ($n = 2,020$) or statin plus placebo ($n = 1,010$) for 6 weeks. Addition of ezetimibe to ongoing statin therapy resulted in a mean decrease in LDL-C of 25.8% compared to 2.7% with placebo ($p < 0.001$). Addition of ezetimibe was similarly beneficial in patients with CHD or a CHD equivalent, patients with =2 risk factors for CHD, and patients with <2 risk factors ($p < 0.001$). Further, addition of ezetimibe resulted in 71% of patients attaining their LDL-C goal compared with 21% for placebo. Target LDL-C achievement was also evident across patient subgroups ($p < 0.001$). Addition of ezetimibe was also more efficacious in lowering other lipid parameters, including triglycerides, non-HDL-C, and apo B ($p < 0.05$). The two treatment regimens had similar safety and tolerability profiles²⁹.

The addition of ezetimibe to 40 mg of atorvastatin or simvastatin was evaluated in patients with homozygous familial hypercholesterolemia (HoFH)⁶. Patients with HoFH on 40 mg of statin had their statin dose doubled, were given ezetimibe (10 mg) or both for 12 weeks. Compared to increasing the statin dose to 80 mg, the addition of ezetimibe to 40 or 80 mg of statin resulted in an additional 14% LDL-cholesterol reduction (-20.7% vs -6.7%) and adding ezetimibe to 80 mg statin an additional 21% LDL-cholesterol reduction occurred (27.5% vs 7%). LDL-cholesterol lowering through the inhibition of cholesterol absorption by ezetimibe does not require the expression of LDL receptors, and offers an additional treatment option for patients with HoFH.

The efficacy of co-administration of ezetimibe (10 mg/day) with statins has been evaluated in four parallel 12-week phase III studies, with simvastatin³⁰ and atorvastatin³¹ at 10-80 mg/day, and lovastatin³² and

pravastatin³³) at 10-40 mg/day. LDL-C reductions with the combination of ezetimibe with statins ranged from -44% to -57% for simvastatin (10-80 mg), -50% to -60% for atorvastatin (10-80 mg), -34% to -41% for pravastatin (10-40 mg), and -33% to -45% for lovastatin (10-40 mg). With all statins, the combination of ezetimibe with the lowest statin dose (10 mg) resulted in similar reductions in LDL-cholesterol as the highest statin alone dose (80 mg for atorvastatin and simvastatin, 40 mg for pravastatin and lovastatin). These trials demonstrate a consistent, significant additional reduction in LDL-C with coadministration of ezetimibe plus statin vs statin alone regardless of the statin or dose. In addition, C-reactive protein (CRP), an inflammatory marker which may be a risk factor for atherosclerosis, was found to be reduced nearly two fold greater in the ezetimibe plus simvastatin pooled groups compared to the simvastatin alone pooled groups (-33.3% vs -14.3%; $p < 0.01$)³⁴.

Considerable LDL-C reductions with the coadministration of ezetimibe and simvastatin compared with simvastatin were also reported by Goldberg *et al.*³⁵. Ezetimibe plus simvastatin reduced LDL-C by 46.2% to 60.8% over the 10- to 80-mg dose range, compared with 31.3% to 45.6% with 10 to 80 mg simvastatin alone. Moreover, treatment with ezetimibe plus simvastatin allowed 82.4% of patients to achieve their LDL-C goal of <100 mg/dL compared to 42.9% of patients treated with simvastatin alone ($p < 0.001$).

The development of a fixed-dose combination of ezetimibe and simvastatin (Vytorin[®]) provided the additive efficacy of both agents in the convenience of a single tablet in the United States and many other countries. In a study by Bays *et al.*³⁶, the efficacy of the ezetimibe/simvastatin tablet was consistent with coadministration studies, with LDL-C reductions of 44.8% to 60.2% over the 10 to 80 mg dose range. Ezetimibe/simvastatin was also associated with significantly greater reductions in CRP and remnant-like particle-cholesterol than simvastatin alone ($p < 0.001$). More patients receiving ezetimibe/simvastatin versus simvastatin achieved LDL-C levels <100 mg/dL (78.6% vs 45.9%; $p < 0.001$). Ezetimibe/simvastatin had a safety profile similar to simvastatin monotherapy. There were no significant differences between ezetimibe/simvastatin and simvastatin in the incidence of consecutive liver transaminase levels = 3 times the upper limit of normal or creatine kinase levels = 10 times ULN.

Ezetimibe/Simvastatin vs Atorvastatin

Ezetimibe/Simvastatin has been directly compared to atorvastatin in both titration and fixed-dose studies.

In the titration study³⁷, 788 patients were randomized 1:1:1 to 3 treatment groups. Each group was force-titrated over four 6-week treatment periods: patients in group 1 were initially treated with atorvastatin 10 mg, which was titrated to 20, 40, and 80 mg; patients in group 2 were treated with ezetimibe/simvastatin 10/10 mg titrated to 10/20, 10/40, and 10/80 mg; patients in group 3 were treated with ezetimibe/simvastatin 10/20 mg titrated to 10/40 mg for 2 treatment periods and 10/80 mg. At the end of treatment period 1, the mean decrease of LDL-C was significantly ($p = 0.001$) greater for co-administration of 10/10 mg and 10/20 mg of ezetimibe/simvastatin than for 10 mg of atorvastatin. At the end of treatment period 4 and after comparing maximum doses, ezetimibe/simvastatin was superior to atorvastatin 80 mg in the percent LDL-C reduction (-59.4% vs -52.5%, $p < 0.001$) and HDL-C increase (12.3% vs 6.5%; $p < 0.001$). All treatments were well tolerated³⁷.

In the VYtorin versus Atorvastatin (VYVA) trial³⁸, ezetimibe/simvastatin was compared to atorvastatin at various fixed-doses. A total of 1,902 patients not at their NCEP ATP III LDL-C goal were randomized to atorvastatin (10, 20, 40, or 80 mg) or to ezetimibe/simvastatin (10/10, 10/20, 10/40, or 10/80 mg). At each milligram-equivalent statin dose, significantly greater LDL-C reductions were achieved with ezetimibe/simvastatin (47%-59%) than with atorvastatin (36%-53%). Ezetimibe/simvastatin 10/40 and 10/80 mg also provided significantly greater increases in HDL-C vs atorvastatin 40 and 80 mg, respectively. Triglyceride reductions were similar for all comparisons. Among patients with coronary heart disease (CHD) or CHD risk equivalents, a significantly greater percentage of patients treated with ezetimibe/simvastatin attained the ATP III LDL-C goals of <100 mg/dL and <70 mg/dL than patients treated with atorvastatin. CRP reductions were comparable between treatment groups. A significantly greater number of patients treated with atorvastatin had consecutive elevations in alanine aminotransferase and/or aspartate aminotransferase than did ezetimibe/simvastatin patients (1.2% vs 0.1%; $p = 0.006$). Only one patient in the study (treated with atorvastatin) had CK elevation (= 10 X ULN).

Ezetimibe/simvastatin and atorvastatin have also been compared in patients with type 2 diabetes mellitus and hypercholesterolemia³⁹, a patient group who are at high-risk for CHD events. In a double-blind, multicenter study, 1,229 patients were randomized to the usual starting doses (ezetimibe/simvastatin, 10/20 mg/d, vs atorvastatin, 10 or 20 mg/d) or next highest doses (ezetimibe/simvastatin, 10/40 mg/d, vs atorvastatin, 40 mg/d). Significantly greater mean LDL-C reduc-

tions were seen with ezetimibe/simvastatin 10/20 mg (-53.6%) vs atorvastatin 10 mg (-38.3%, $p < 0.001$) or atorvastatin 20 mg (-44.6%; $p < 0.001$) and for ezetimibe/simvastatin 10/40 mg vs atorvastatin 40 mg (-57.6% vs -50.9%; $p < 0.001$). At all dose comparisons, the percentage of patients who attained an LDL-C goal of < 70 mg/dL was significantly greater with ezetimibe/simvastatin vs atorvastatin ($p < 0.001$). Significantly greater improvements in total cholesterol, HDL-C, and non-HDL-C were also reported with ezetimibe/simvastatin vs atorvastatin ($p = 0.001$). Adverse events, including repeated elevation of hepatic transaminases or CK levels, were similar for both treatments³⁹.

Ezetimibe/Simvastatin vs Rosuvastatin

Ezetimibe/simvastatin has also been compared with rosuvastatin⁴⁰. In a double-blind, multicenter study, 2,959 hypercholesterolemic patients were randomized to ezetimibe/simvastatin or rosuvastatin, respectively, at the usual starting (10/20 or 10 mg), the next highest (10/40 or 20 mg), and maximum doses (10/80 or 40 mg). Patients treated with ezetimibe/simvastatin had significantly greater reductions in LDL-C vs rosuvastatin at the starting (52% vs 46%), next highest (55% vs 52%) and maximum (61% vs 57%) doses ($p = 0.001$ for all comparisons). Ezetimibe/simvastatin also provided significantly greater rates of attaining LDL-C levels of < 70 mg/dL vs rosuvastatin at all dose comparisons and in a comparison of pooled doses. Ezetimibe/simvastatin produced significantly greater reductions in total cholesterol ($p < 0.001$), non-HDL ($p < 0.001$), and apo B ($p < 0.05$). Increases in HDL-C and decreases in hsCRP were similar between treatment groups. Safety profiles for both treatments were comparable, although a significantly greater percentage of patients treated with rosuvastatin vs ezetimibe/simvastatin had proteinuria, respectively, at 10 mg versus 10/20 mg ($p = 0.004$) and 40 mg versus 10/80 mg ($p < 0.001$)⁴⁰.

Ezetimibe Plus Rosuvastatin vs Rosuvastatin Alone

The achievement of large LDL-C reductions with ezetimibe plus statin therapy has also been demonstrated in the Examination of Potential Lipid-modifying effects Of Rosuvastatin alone (EXPLORER) trial⁴¹. In this open-label, multicenter trial, 469 patients with hypercholesterolemia and CHD (clinical or sub-clinical) or CHD risk $> 20\%$ were randomized to the maximum dose of rosuvastatin (40 mg) or to ezetimibe

plus rosuvastatin 40 mg. After 6 weeks, the combination of ezetimibe and rosuvastatin provided an LDL-C reduction of 70%, compared to a 57% LDL-C reduction with rosuvastatin alone ($p < 0.001$). Addition of ezetimibe to rosuvastatin was also associated with significantly ($p < 0.001$) greater reductions in non-HDL-C (-65% vs -52%), apo B (-56% vs -45%), and triglycerides (-35% vs -25%). The reduction in hsCRP was also significantly greater with ezetimibe plus rosuvastatin (46% vs 29% with rosuvastatin, $p < 0.001$). A significantly greater percentage of patients receiving rosuvastatin plus ezetimibe vs rosuvastatin alone attained their NCEP ATP III LDL-C goal (< 100 mg/dL, 94.0% vs 79.1%, $p < 0.001$) and the optional LDL-C goal of < 70 mg/dL (79.6% vs 35.0%, $p < 0.001$). Both treatments were generally well tolerated. These findings further support the significant incremental benefits of ezetimibe on multiple lipid parameters and LDL-C goal attainment when combined with statins and demonstrate that substantial improvements in the atherogenic lipid profile can be attained with ezetimibe-statin combination therapy.

Ezetimibe and Ezetimibe/Simvastatin in Combination with Fenofibrate

The effects of combining ezetimibe with fenofibrate on atherogenic lipid profile have also been evaluated^{42, 43}. In a multicenter, double-blind, randomized trial, 625 patients with mixed hyperlipidemia (LDL-C 130-220 mg/dL, TG 150-500 mg/dL), were randomized in a 3:3:3:1 ratio to one of 4 treatments for 12 weeks: ezetimibe 10 mg, fenofibrate 160 mg, fenofibrate 160 mg plus ezetimibe 10 mg, and placebo⁴². After completing the 12-week study, 576 patients continued into a double-blind, 48-week extension phase comparing fenofibrate ($n = 236$) to fenofibrate plus ezetimibe ($n = 340$)⁴³. Patients in the fenofibrate plus ezetimibe and fenofibrate groups continued on their respective previous treatment, and patients in the ezetimibe and placebo groups were switched to fenofibrate plus ezetimibe and fenofibrate, respectively. The fenofibrate plus ezetimibe produced significantly greater reductions in LDL-C compared with fenofibrate (-22% vs -9%, respectively; $p < 0.001$). There were also significantly greater improvements in non-HDL-C (-32% vs -19%), apo B (-25% vs -16%) triglycerides (-46% vs -42%), HDL-C (+21% vs +18%), with fenofibrate plus ezetimibe compared with fenofibrate. Changes in apolipoprotein A-I and hsCRP were comparable between groups. The combination of fenofibrate plus ezetimibe was well tolerated during the extension study, with similar rates of consecutive ALT/AST elevations

=3 X ULN between the fenofibrate plus ezetimibe (1.2%) and fenofibrate (1.7%) groups. No cases of creatine phosphokinase elevations =10 times ULN or myopathy were observed in either group. Long-term, 48-week co-administration of fenofibrate plus ezetimibe was well tolerated and more efficacious than fenofibrate in patients with mixed hyperlipidemia. These findings demonstrate that the combination of ezetimibe plus fenofibrate improves several atherogenic lipid parameters in patients with mixed hyperlipidemia.

These results are further complemented by the results of combining ezetimibe/simvastatin with fenofibrate. In a multicenter, randomized, double-blind, placebo-controlled trial⁴⁴, eligible patients ($n=611$) with mixed hyperlipidemia (LDL-C 130-220 mg/dL, TG 150-500 mg/L) were randomized in a 3:3:3:1 ratio to one of 4 treatment arms for 12 weeks: ezetimibe/simvastatin 10/20 mg plus fenofibrate 160 mg, ezetimibe/simvastatin 10/20 mg, fenofibrate 160 mg, or placebo. Ezetimibe/simvastatin plus fenofibrate provided significantly ($p<0.05$) greater reductions in LDL-C (-45.8%) vs fenofibrate (-15.7%) or placebo (-3.5%), but not vs ezetimibe/simvastatin (-47.1%). HDL-C and apolipoprotein A-I levels were significantly increased with ezetimibe/simvastatin plus fenofibrate (18.7% and 11.1%, respectively) vs ezetimibe/simvastatin (9.3% and 6.6%) or placebo (1.1% and 1.6%), but not vs fenofibrate (18.2% and 10.8%). However, reductions in triglyceride, non-HDL-C, and apo B levels were significantly greater with ezetimibe/simvastatin plus fenofibrate versus all other treatments. Ezetimibe/simvastatin plus fenofibrate had a safety profile similar to ezetimibe/simvastatin and fenofibrate. These findings demonstrate that the combination of ezetimibe/simvastatin plus fenofibrate improves several atherogenic lipid parameters in patients with mixed hyperlipidemia.

Ongoing Surrogate and Clinical Outcome Trials with Ezetimibe/Simvastatin

Four large randomized, double-blind, clinical trials have been initiated to evaluate the impact on clinical outcome of greater reduction of LDL-C with ezetimibe/simvastatin. Overall, greater than 22,000 patients will be randomized in these trials. The 2-year Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE) study⁴⁵, to be conducted mostly in Europe, will evaluate the effects of combined ezetimibe and simvastatin 80 mg vs simvastatin 80 mg alone in reversing atherosclerotic thickening in the carotid artery wall in 720 patients with heterozygous FH. The primary end point is mean change from baseline to 2 years in carotid in-

tima-media thickness (IMT), using composite measures from the right and left far wall common carotid artery, carotid bulb, and internal carotid artery. The Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) study⁴⁶ is the largest randomized trial to date in patients with asymptomatic, degenerative aortic stenosis. This 4-year study in >1,800 patients will evaluate the mortality and morbidity reduction with ezetimibe/simvastatin 10/40 mg vs placebo. Serial transthoracic doppler-echocardiography is also being performed to assess aortic valve hemodynamics. The Study of Heart and Renal Protection (SHARP) will evaluate the effect of ezetimibe/simvastatin 10/20 mg vs placebo on major vascular events in approximately 9,500 patients with chronic kidney disease (CKD). This large-scale trial will assess the effects of LDL-C reduction on the risk of major vascular events (cardiac death, myocardial infarction, stroke, or revascularization procedure) and also on the rate of loss of renal function in patients with various degrees of renal impairment, including end-stage renal disease. The study will follow patients for at least 4 years. The Improved Reduction of Outcomes: VYTORIN Efficacy International Trial (IMPROVE-IT) study will evaluate the benefit of ezetimibe/simvastatin 10/40 vs simvastatin 40 mg in over 10,000 patients with acute coronary syndromes. The primary endpoint is the composite of cardiovascular death, myocardial infarction, stroke, hospital admission for ACS and revascularization >30 days. Follow-up is a minimum of 2.5 years. Importantly, not only will this trial assess the incremental effects of ezetimibe on cardiovascular morbidity and mortality, but the median LDL-C achieved will test the "even lower is better" hypothesis. The median LDL-C on ezetimibe/simvastatin is expected to be 52 mg/dL compared to 68 mg/dL for the simvastatin alone arm.

Clinical Summary

Ezetimibe has been shown to significantly reduce LDL-C and improve other lipid parameters as monotherapy or when added to other lipid lowering therapies. Ezetimibe provided incremental LDL-C reductions when added to statin therapy, regardless of the statin or dose. Statin/ezetimibe therapy also provides greater reductions in non-HDL-C, apolipoprotein B, and CRP vs statin therapy alone. Ezetimibe also demonstrates significant improvements in the overall atherogenic lipid profile when added to fenofibrate, in both the absence and presence of concurrent statin therapy. Large randomized trials evaluating the effects of ezetimibe/statin therapy vs statin therapy alone on surrogate and clinical endpoints in different popula-

tions at risk for CHD will determine if the beneficial effects of ezetimibe on LDL-C and other lipid parameters will translate into superior clinical outcomes.

References

- 1) van Heek M, France C, Compton DS, Mcleod RL, Yumibe NP, Alton KB, Sybertz EJ, and Davis HR: In-vivo mechanism-based discovery of a potent cholesterol absorption inhibitor (SCH58235) through the identification of the active metabolites of SCH48461. *J Pharmacol Exp Ther*, 1997; 283:157-163
- 2) Clader J: The discovery of ezetimibe: A view from outside the receptor. *J Med Chem*, 2004; 47:1-9
- 3) van Heek M, France C, Compton D, Hoos L, Alton K, Sybertz E, and Davis HR: Comparison of the activity and disposition of the novel cholesterol absorption inhibitor, SCH58235, and its glucuronide, SCH60663. *Br J Pharmacology*, 2000; 129:1748-1754
- 4) van Heek M, Compton DS, and Davis HR: The cholesterol absorption inhibitor, ezetimibe, decreases diet-induced hypercholesterolemia in monkeys. *European J Pharm*, 2001; 415:79-84
- 5) van Heek M, Farley C, Compton DS, Hoos L, and Davis HR: Ezetimibe selectively inhibits intestinal cholesterol absorption in rodents in the presence and absence of exocrine pancreatic function. *British J Pharm*, 2001; 134:409-417
- 6) Ballantyne CM: Ezetimibe: efficacy and safety in clinical trials. *Eur Heart J*, 2002; 4(Suppl J):J9-J18
- 7) Patrick JE, Kosoglou T, Stauber KL, Alton KB, Maxwell SE, Zhu Y, Statkevich P, Iannucci R, Chowdhury S, Afrime M, and Cayen MN: Disposition of the selective cholesterol absorption inhibitor ezetimibe in healthy male subjects. *Drug Metab Dispos*, 2003; 30:430-437
- 8) Kosoglou T, Statkevich P, Johnson-Levonas OA, Paolini JF, Bergman AJ, and Alton KB: Ezetimibe: a review of its metabolism, pharmacokinetics and drug interactions. *Clin Pharmacokinet*, 2005; 44:467-494
- 9) Davis HR: Ezetimibe: First in a new class of cholesterol absorption inhibitors. *International Congress Series, Atherosclerosis XIII. Proceedings of the 13th International Atherosclerosis Symposium*, Edited by Matsuzawa Y, Kita T, Nagai R, and Teramoto T, Volume 1262, pp 243-246, Elsevier BV, Amsterdam, 2004
- 10) Sudhop T, Lutjohann D, Kodal A, Tribble D, Shah S, Perovozskaya I, and von Bergmann K: Inhibition of cholesterol absorption by ezetimibe in humans. *Circulation*, 2002; 106:1943-1948
- 11) Altmann SW, Davis HR, Yao X, Laverty M, Compton DS, Zhu L, Crona JH, Caplen MA, Hoos LM, Tetzloff G, Priestley T, Burnett DA, Strader CD, and Graziano MP: The identification of intestinal Scavenger Receptor Class B, Type I (SR-BI) by expression cloning and its role in cholesterol absorption. *Biochimica et Biophysica Acta*, 2002; 1580:77-93
- 12) Altmann SW, Davis HR, Zhu L, Yao X, Hoos LM, Tetzloff G, Iyer SN, Maguire M, Golovko A, Zeng M, Wang L, Murgolo N, and Graziano MP: Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science*, 2004; 303:1201-1204
- 13) Iyer SN, Yao X, Crona JH, Hoos LM, Tetzloff G, Davis HR, Graziano MP, and Altmann SW: Characterization of the putative and recombinant rat sterol transporter Niemann-Pick C1 Like 1 (NPC1L1) protein. *Biochimica et Biophysica Acta*, 2005; 1722:282-292
- 14) Davis HR, Zhu L, Hoos LM, Tetzloff G, Maguire M, Liu J, Yao X, Iyer S, Lam M, Lund EG, Detmers PA, Graziano MP, and Altmann SW: Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole body cholesterol homeostasis. *J Biol Chem*, 2004; 279:33486-33592
- 15) Salen G, von Bergmann K, Lutjohann D, Kwitervich P, Kane J, Patel SB, Musliner T, Stein P, Musser B, and Multicenter Sitosterolemia Study Group: Ezetimibe effectively reduces plasma plant sterols in patients with sitosterolemia. *Circulation*, 2004; 109:966-971
- 16) Salen G, Starc T, Sisk CM, and Patel SB: Intestinal cholesterol absorption inhibitor ezetimibe added to cholestyramine for sitosterolemia and xanthomatosis. *Gastroenterology*, 2006; 130:1853-1857
- 17) Davis HR, Compton D, Hoos L, and Tetzloff G: Ezetimibe, a potent cholesterol absorption inhibitor, inhibits the development of atherosclerosis in apoE knockout mice. *Arterioscler Thromb Vasc Biol*, 2001; 21:2032-2038
- 18) Davis HR, Hoos LM, Tetzloff G, Maguire M, Zhu L, Graziano MP, and Altmann SW: Deficiency of Niemann-Pick C1 Like 1 (NPC1L1) prevents atherosclerosis in apoE -/- mice. *Arterioscler Thromb Vasc Biol*, 2007; 27:841-849
- 19) Garcia-Calvo M, Lisnock J, Bull HG, Hawes BE, Burnett DA, Braun MP, Crona JH, Davis HR, Dean DC, Detmers PA, Graziano MP, Hughes M, MacIntyre DE, Ogasawa A, O'Neill KA, Iyer SN, Shevell DE, Smith MM, Tang YS, Ujjainwalla F, Altmann SW, Chapman KT, and Thornberry NA: The target of ezetimibe is Niemann-Pick C1 Like 1 (NPC1L1). *Proc Natl Acad Sci USA*, 2005; 102:8132-8137
- 20) Hawes BE, O'Neill KA, Yao X, Crona JH, Davis HR, Graziano MP, and Altmann SW: In vivo responsiveness to ezetimibe correlates with NPC1L1 binding affinity: Comparison of multiple species NPC1L1 orthologs. *Mol Pharmacol*, 2007; 71:19-29
- 21) Hegele RA, Guy J, Ban MR, and Wang J: NPC1L1 haplotype is associated with inter-individual variation in plasma low-density lipoprotein response to ezetimibe. *Lipids Health Dis*, 2005; 4:16-20
- 22) Simon JS, Karnoub MC, Devlin DJ, Arreaza MG, Qiu P, Monks SA, Severino ME, Deutsch P, Palmisano J, Sachs AB, Bayne ML, Plump AS, and Schadt EE: Sequence variation in NPC1L1 and association with improved LDL-cholesterol lowering in response to ezetimibe treatment. *Genomics*, 2005; 86:648-656
- 23) Cohen JC, Pertsemlidis A, Fahmi S, Esmail S, Vega GL, Grundy SM, and Hobbs HH: Multiple rare variants in NPC1L1 associated with reduced sterol absorption and plasma low-density lipoprotein levels. *Proc Natl Acad Sci USA*, 2006; 7:103:1810-1825
- 24) Huff MW, Pollex RL, and Hegele RA: NPC1L1: Evolution

- from pharmacological target to physiological sterol transporter. *Arterioscler Thromb Vasc Biol*, 2006; 26:2433-2438
- 25) Clarenbach JJ, Reber M, Lutjohann D, von Bergmann K, and Sudhop T: The lipid-lowering effect of ezetimibe in pure vegetarians. *J Lipid Res*, 2006; 47:2820-2824
 - 26) Dujovne CA, Ettinger MP, McNeer JF, Lipka LJ, LeBeaut AP, Suresh R, Yang B, Veltri EP, and Ezetimibe Study Group: Efficacy and safety of a potent new selective cholesterol absorption inhibitor, ezetimibe, in patients with primary hypercholesterolemia. *Am J Cardiol*, 2002; 90:1092-1097
 - 27) Knopp RH, Gitter H, Truitt T, Bays H, Manion CV, Lipka LJ, LeBeaut AP, Suresh R, Yang B, Veltri EP, and Ezetimibe Study Group: Effects of ezetimibe, a new cholesterol absorption inhibitor, on plasma lipids in patients with primary hypercholesterolemia. *Eur Heart J*, 2003; 24:729-741
 - 28) Knopp RH, Dujovne CA, LeBeaut A, Lipka LJ, Suresh R, and Veltri EP: Evaluation of the efficacy, safety, and tolerability of ezetimibe in primary hypercholesterolemia: a pooled analysis from two controlled phase III clinical studies. *Int J Clin Pract*, 2003; 57:363-368
 - 29) Pearson TA, Denke MA, McBride PE, Battisti WP, Brady WE, and Palmisano J: A community-based, randomized trial of ezetimibe added to statin therapy to attain NCEP ATP III goals for LDL cholesterol in hypercholesterolemic patients: the ezetimibe add-on to statin for effectiveness (EASE) trial. *Mayo Clin Proc*, 2005; 80:587-595
 - 30) Davidson MH, McGarry T, Bettis R, Melani L, Lipka LJ, LeBeaut AP, Suresh R, Sun S, and Veltri EP: Ezetimibe coadministered with simvastatin in patients with primary hypercholesterolemia. *J Am Coll Cardiol*, 2002; 40:2125-2134
 - 31) Ballantyne CM, Hourii J, Notarbartolo A, Melani L, Lipka LJ, Suresh R, Sun S, LeBeaut AP, Sager PT, Veltri EP, and Ezetimibe Study Group: Effect of ezetimibe coadministered with atorvastatin in 628 patients with primary hypercholesterolemia: a prospective, randomized, double-blind trial. *Circulation*, 2003; 107:2409-2415
 - 32) Kerzner B, Corbelli J, Sharp S, Lipka LJ, Melani L, LeBeaut A, Suresh R, Mukhopadhyay P, Veltri EP, and Ezetimibe Study Group: Efficacy and safety of ezetimibe coadministered with lovastatin in primary hypercholesterolemia. *Am J Cardiol*, 2003; 91:418-424
 - 33) Melani L, Mills R, Hassman D, Lipetz R, Lipka L, LeBeaut A, Suresh R, Mukhopadhyay P, Veltri E, and Ezetimibe Study Group: Efficacy and safety of ezetimibe coadministered with pravastatin in patients with primary hypercholesterolemia: a prospective, randomized, double-blind trial. *Eur Heart J*, 2003; 24:717-728
 - 34) Sager PT, Capece R, Lipka L, Strony J, Yang B, Suresh R, Mitchel Y, and Veltri E: Effects of ezetimibe coadministered with simvastatin on C-reactive protein in a large cohort of hypercholesterolemic patients. *Atherosclerosis*, 2005; 179:361-367
 - 35) Goldberg AC, Sapre A, Liu J, Capece R, and Mitchel YB: Efficacy and safety of ezetimibe coadministered with simvastatin in patients with primary hypercholesterolemia: a randomized, double-blind, placebo-controlled trial. *Mayo Clin Proc*, 2004; 79:620-629
 - 36) Bays HE, Ose L, Fraser N, Tribble DL, Quinto K, Reyes R, Johnson-Levonas AO, Sapre A, Donahue SR, and Ezetimibe Study Group: A multicenter, randomized, double-blind, placebo-controlled, factorial design study to evaluate the lipid-altering efficacy and safety profile of the ezetimibe/simvastatin tablet compared with ezetimibe and simvastatin monotherapy in patients with primary hypercholesterolemia. *Clin Ther*, 2004; 26:1758-1773
 - 37) Ballantyne CM, Blazing MA, King TR, Brady WE, and Palmisano J: Efficacy and safety of ezetimibe coadministered with simvastatin compared with atorvastatin in adults with hypercholesterolemia. *Am J Cardiol*, 2004; 93:1487-1494
 - 38) Ballantyne CM, Abate N, Yuan Z, King TR, and Palmisano J: Dose-comparison study of the combination of ezetimibe and simvastatin (Vytorin) versus atorvastatin in patients with hypercholesterolemia: the Vytorin Versus Atorvastatin (VYVA) study. *Am Heart J*, 2005; 149:464-473
 - 39) Goldberg RB, Guyton JR, Mazzone T, Weinstock RS, Poliss A, Edwards P, Tomassini JE, and Tershakovec AM: Ezetimibe/simvastatin vs atorvastatin in patients with type 2 diabetes mellitus and hypercholesterolemia: the VYTAL study. *Mayo Clin Proc*, 2006; 81:1579-1588
 - 40) Catapano AL, Davidson MH, Ballantyne CM, Brady WE, Gazzara RA, Tomassini JE, and Tershakovec AM: Lipid-altering efficacy of the ezetimibe/simvastatin single tablet versus rosuvastatin in hypercholesterolemic patients. *Curr Med Res Opin*, 2006; 22:2041-2053
 - 41) Ballantyne CM, Weiss R, Moccetti T, Vogt A, Eber B, Sosef F, Duffield E, and EXPLORER Study Investigators: Efficacy and safety of rosuvastatin 40 mg alone or in combination with ezetimibe in patients at high risk of cardiovascular disease (results from the EXPLORER study). *Am J Cardiol*, 2007; 99:673-680
 - 42) Farnier M, Volpe M, Massaad R, Davies MJ, and Allen C: Effect of co-administering ezetimibe with on-going simvastatin treatment on LDL-C goal attainment in hypercholesterolemic patients with coronary heart disease. *Int J Cardiol*, 2005; 102:327-332
 - 43) McKenney JM, Farnier M, Lo KW, Bays HE, Perevozskaya I, Carlson G, Davies MJ, Mitchel YB, and Gumbiner B: Safety and efficacy of long-term co-administration of fenofibrate and ezetimibe in patients with mixed hyperlipidemia. *J Am Coll Cardiol*, 2006; 47:1584-1587
 - 44) Farnier M, Roth E, Gil-Extremera B, Mendez GF, Macdonell G, Hamlin C, Perevozskaya I, Davies MJ, Kush D, Mitchel YB, and Ezetimibe/Simvastatin + Fenofibrate Study Group: Efficacy and safety of the coadministration of ezetimibe/simvastatin with fenofibrate in patients with mixed hyperlipidemia. *Am Heart J*, 2007; 153:335 e1-8
 - 45) Kastelein JJ, Sager PT, de Groot E, and Veltri E: Comparison of ezetimibe plus simvastatin versus simvastatin monotherapy on atherosclerosis progression in familial hypercholesterolemia. Design and rationale of the Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE) trial. *Am Heart J*, 2005; 149:234-239
 - 46) Rossebo AB, Pedersen TR, Allen C, Boman K, Chambers J, Egstrup K, Gerds E, Gohlke-Barwolf C, Holme I, Kesaniemi VA, Malbecq W, Nienaber C, Ray S, Skjaerpe T, Wachtell K, and Willenheimer R: Design and Baseline Characteristics of the Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) Study. *Am J Cardiol*, 2007; 99:970-973