Long-term efficacy and safety of ezetimibe 10 mg in patients with homozygous sitosterolemia: a 2-year, open-label extension study

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SUMMARY
Objective: To assess the long-term efficacy and safety profile of ezetimibe 10 mg/day in patients with homozygous sitosterolemia. Methods: This was an extension of a multi-centre, randomised, double-blind, placebo-controlled base study in which patients with homozygous sitosterolemia and plasma sitosterol concentrations > 5 mg/dl were randomised 4:1 to ezetimibe 10 mg/day (n = 30) or placebo (n = 7) for 8 weeks. Patients who successfully completed the base study with > 80% compliance to study medication were eligible to enter two, successive, 1-year extension studies in which ezetimibe 10 mg/day was administered in an open-label manner. Patients remained on their current treatment regimen (e.g. bile salt-binding resins, statins and low-sterol diet) during the base and extension studies. Patients had to be off ezetimibe therapy for ≥ 4 weeks prior to entering the first extension. Efficacy and safety/tolerability parameters were evaluated every 12 and 26 weeks in the first and second years respectively. The primary efficacy end-point was mean percentage change in plasma sitosterol from baseline to study end for the cohort of patients (n = 21) who successfully completed the second extension study. Results: Treatment with ezetimibe 10 mg/day led to significant mean percentage reductions from baseline in plasma concentrations of sitosterol (−43.9%; p < 0.001), campesterol (−50.8%; p < 0.001), low-density lipoprotein (LDL) sterols (−13.1%; p < 0.050), total sterols (−10.3%; p < 0.050) and apolipoprotein (apo) A-1. Maximal reductions in sitosterol and campesterol occurred within the first 52 weeks of treatment and were sustained for the duration of the study. For LDL sterol, total sterols and apo A-1, maximal reductions were achieved early (by weeks 4 or 16) and waned slightly through the remainder of the study. Overall ezetimibe 10 mg was well tolerated. Conclusion: In patients with homozygous sitosterolemia, long-term treatment with ezetimibe 10 mg/day for 2 years was effective in reducing plasma plant sterol concentrations with an overall favourable safety and tolerability profile.

Introduction
Sitosterolemia is a recessively inherited metabolic disease characterised by markedly (> 30-fold) increased tissue and plasma plant sterol concentrations (1). Sitosterolemia is caused by a mutation in one of two ATP-binding cassette (ABC) transporters, ABCG5 or ABCG8, responsible for pumping sterols (plant sterols and cholesterol) from the brush border of enterocytes into the intestinal lumen and from the liver into bile (2–6). Patients with this disease present with hyperabsorption and diminished biliary excretion of sterols leading to tendinous and/or tuberous xanthomas, aortic stenosis, premature atherosclerosis and coronary heart disease (7–12). Additional clinical features of sitosterolemia include the occurrence of haemolysis, presumably caused by the increased osmotic fragility of erythrocytes, anaemia, thrombocytopenia, as well as hepatocellular injury (7,13,14).
Historically, treatment of sitosterolemia included dietary restriction of sterol intake, use of bile-acid sequestrants, ileal bypass surgery and low-density lipoprotein (LDL) immunoadsorption (13). More recently, ezetimibe has become the primary treatment choice for patients with sitosterolemia (15–17). Ezetimibe operates at the level of the intestinal enterocyte to inhibit the absorption of dietary and biliary sterols, including cholesterol and phytosterols, primarily through the Niemann-Pick C1-Like 1 transporter (15,17–20). Standard enzymatic techniques are unable to distinguish cholesterol from sterols; therefore, use of gas–liquid chromatography (GLC) is necessary for diagnosing and evaluating treatment effects in patients with sitosterolemia.

A previous double-blind, placebo-controlled clinical study showed that ezetimibe 10 mg, when combined with bile-acid sequestrants, statins and/or a low-sterol diet, was effective at lowering plasma phytosterol concentrations in sitosterolemic patients after 8 weeks of treatment (15). In that study, plasma concentrations of sitosterol and campesterol, the most prevalent phytosterols in human plasma, were significantly reduced by 21.0% and 24.3% respectively (p < 0.001 vs. baseline). Phytosterol concentrations declined progressively throughout the 8-week base study, but remained elevated above the normal range at study end-point. This report describes the results of two, successive, 1-year open-label extension studies designed to determine if longer-term treatment (2 years) with ezetimibe 10 mg/day results in more pronounced changes in plasma phytosterol concentrations in patients with sitosterolemia.

Patients and methods

Study population

Males and females, ≥ 10 years of age, with a diagnosis of homozygous sitosterolemia who had plasma sitosterol concentrations > 5.0 mg/dl (normal range < 1.0 mg/dl) despite ongoing treatment were eligible to participate in the original randomised clinical trial (15). To confirm the homozygous nature of mutations in the ABCG gene, genetic analyses were performed on 29 consenting patients prior to enrolment in the base study protocol. In addition to meeting the original entry criteria for the base study, patients were required to have been ≥ 80% compliant with therapy during the base study to be eligible for participation in the extension phase. Three patients who were not enrolled in the base study were allowed to enter the extension study so as to increase the sample size. These patients did not undergo genetic testing.

To ensure a stable baseline measurement, patients had to be on a stable regimen of diet and/or medica-

Sample statistics for the treatment of sitosterolemia (including bile salt-binding resins and statins) and off ezetimibe base study therapy for at least 4 weeks prior to the initiation of the first extension study. If it was considered clinically appropriate, bile salt-binding resins were discontinued prior to entry in the extension study because of the possibility that resins may lower ezetimibe concentrations (21). If it was not considered clinically appropriate to modify the dose of the bile acid-binding resin, patients were enrolled and instructed to take ezetimibe ≥ 2 h before or ≥ 4 h after the resin. All other treatments (e.g. diet, statins) were continued and expected to remain stable during the base and extension studies. Patients were advised to strictly avoid all phytosterol-containing products (e.g. margarines, yogurt etc.) during the base and extension studies. Ingestion of these products has been shown to increase plasma concentrations of sitosterol and campesterol in patients with sitosterolemia (22).

Study design

This was an extension of a multi-centre, randomised, double-blind, placebo-controlled base study (Merck-Schering Plough Protocol numbers 002 and 003; National Clinical Trial Registry number NCT00092807) in which patients were randomised 4 : 1 to ezetimibe 10 mg/day (n = 30) or placebo (n = 7) for 8 weeks (15). Following successful completion of the base study, eligible patients entered two, successive, 1-year extension studies in which ezetimibe 10 mg/day was administered in an open-label manner. The first open-label extension study was initiated only after an analysis of the double-blind base study showed ezetimibe 10 mg to be safe and effective in reducing plasma sitosterol concentrations (≥ 15% reduction). Therefore, it was anticipated that all patients continuing from the base into the extension studies would experience a large gap of up to 6 months prior to their enrolment in the first extension study. Given the relative rarity of this disorder and the need to ensure sufficient power to assess within-group differences in plant sterols, the first extension study protocol was amended to allow for the participation of patients who were not

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enrolled in the base study. In total, 36 patients were enrolled in these extension studies (Merck-Schering Plough Protocol numbers 00 2-10 and 003-10; National Clinical Trial Registry number NCT00092807) between 6 November 2001 and 30 November 2004. A total of 11 sites in the USA and 11 international sites in eight countries (Canada, Finland, France, Germany, Netherlands, Norway, South Africa and Sweden) participated in these studies.

Efficacy and safety/tolerability parameters were evaluated every 12 weeks in the first year and every 26 weeks (i.e. 6 months) in the second year. Plasma specimens collected with ethylenediaminetetraacetic acid (EDTA) were obtained after an overnight fast (at least 12 h from the last meal) at weeks 0 (screening/baseline; visit 1), 4 (visit 2), 16 (visit 3), 28 (visit 4), 40 (visit 5), 52 (visit 6), 78 (visit 7) and 104 (visit 8) of the open-label extension period. A follow-up phone call was made 14 days after each patient took their last dose of extension study medication to collect information regarding potential serious adverse experiences. Patients could be withdrawn from the extension studies at any time for the following predefined reasons: (i) increases in liver function tests of > 3× the patient’s baseline value and > 3× upper limit of normal (ULN) or ≥ 10 × ULN; (ii) a positive result on pregnancy testing; (ii) patients requiring chronic (> 2 weeks) treatment with systemic corticosteroids and (iv) clinically relevant abnormalities in laboratory parameters.

Analytical methods

Standard assays for the measurement of lipids and lipoproteins [i.e. triglycerides (TG), apolipoprotein (apo) B and apo A-1] were performed by technicians at Medical Research Laboratories, Highland Heights, KY, or its subsidiary, Clinical Research Laboratories, Brussels, Belgium according to previously published procedures (15,23). In addition, the same laboratory performed standard enzymatic cholesterol assays (which do not distinguish between cholesterol and plant sterols) to obtain a measurement of total sterols, high-density lipoprotein (HDL)-sterols and LDL-sterols (i.e. cholesterol + plant sterols). (HDL)-sterols were measured by enzymatic methods following heparin manganese precipitation of apo B-containing lipoproteins [i.e. LDL and very LDL (VLDL)]. In patients with TG values < 350 mg/dl, Friedewald’s equation was used to calculate LDL-sterols: LDL-sterols (mg/dl) = total sterols (mg/dl) – [HDL-sterols (mg/dl) + TG (mg/dl)/5] (24). While Friedewald’s equation has been used in previous studies to provide an estimate of LDL-sterols in sitosterolemia patients (25), to our knowledge, no studies have specifically validated the accuracy of ‘TG/5’ as an approximation of VLDL-sterols in this patient population. For patients with TG values ≥ 350 mg/dl, LDL-sterols were directly measured following isolation by ultracentrifugation (i.e. β-quantification). The standard enzymatic technique used to measure total cholesterol demonstrates 50% and 65% sensitivity for campesterol and sitosterol, respectively, with 100% sensitivity for cholesterol (26).

The remaining sterol and cholesterol measurements [i.e. sitosterol, campesterol, lathosterol, total cholesterol and HDL-cholesterol (C)] were analysed at a separate laboratory by capillary GLC. GLC-flame-ionisation detection was used to measure total cholesterol and HDL-C following heparin manganese precipitation. The chromatographic quantifications of sitosterol, campesterol and lathosterol were performed by GLC-mass spectrometry-selected ion monitoring (27). Plasma concentrations of LDL-C were to be imputed from the density > 1.006 fraction obtained following β-quantification and HDL-C using the following equation: LDL-C = cholesterol in density > 1.006 – HDL-C. Technical issues associated with the GLC measurement of cholesterol in the density > 1.006 and/or HDL-C fractions prevented the assessment of LDL-C in this study. Therefore, HDL-C and LDL-C are not reported in this paper.

Achilles tendon thickness (mm) was measured by lateral foot radiography; x-ray films were centrally read by a radiologist blinded to sequence (baseline vs. end of study). Paired baseline (base study week 0) and on-treatment radiographs were only available on a subset of patients (n = 18 first extension study; n = 12 second extension study) because radiography was generally not performed in patients younger than 18 years. An independent radiologist was hired to evaluate the x-ray films for those subjects entering the extension studies. Thus, the baseline measurements closely matched, but were not identical to that reported in the base study (15). In addition to Achilles tendon thickness, the size of cutaneous xanthomas (length × width; cm²) was measured directly at baseline and at end of study.

Statistical analysis

The primary objective of this extension study was to evaluate the efficacy of ezetimibe 10 mg following 104 (extension study 2) weeks of treatment. The primary efficacy variable was mean percentage change in plasma sitosterol concentrations from baseline (extension week 0) to end-point (week 104). Key secondary efficacy variables were the percentage change from baseline in concentrations of campesterol and LDL-C (GC). Tertiary variables were percentage changes in plasma lathosterol, total sterols, LDL sterols, HDL-sterols, TG, apo A-1 and apo B relative to
baseline. As previously noted, LDL-C and HDL-C were not reported in this study because of technical issues.

For all efficacy variables, the within group percentage change was assessed by using summary statistics and 95% confidence intervals (CIs). For reasons of the variability and small sample sizes, non-parametric analyses were reported where appropriate. No multiplicity adjustment was planned for supportive secondary efficacy variables. The descriptive statistics for the primary efficacy end-point, percentage change in plasma sitosterol from baseline to study end and corresponding 95% CIs were computed for gender, bile salt-binding resin stratum, concomitant statin use and baseline sitosterol subgroups. The assumption of normality was assessed by the Shapiro–Wilk statistic.

For reasons of varying lengths of patient exposure times in the extension studies, the primary efficacy analysis was performed on the completers population \((n = 21)\), which consisted of all patients who had valid efficacy measurements at baseline (extension week 0) and week 104. An additional, supportive efficacy analysis was performed on the all-patients-treated (APT) population \((n = 36)\) that consisted of all patients who received at least one dose of extension study medication and who had both baseline (extension week 0) and at least one efficacy measurement taken after the initiation of the extension study using the data carried over method to impute missing values (note: when necessary, values were carried over from the first to second extension studies). The APT population was provided as supportive analysis for the primary (sitosterol) and key secondary [campesterol and LDL-C (GC)] efficacy parameters, only.

A predefined, exploratory analysis of Achilles tendon thickness was performed on all patients \((n = 12)\) with paired baseline (base week 0) and week 104 (extension study 2) radiographs.

Safety and tolerability were assessed in the all-patients-as-treated (APaT) population, which consisted of all patients who received at least one dose of extension study medication \((n = 36)\). Physical examinations, vital signs, 12-lead ECGs and safety laboratory measurements comprising haematology, serum chemistry [including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and alkaline phosphatase] and urinalysis were performed throughout the study. Adverse experiences were monitored throughout the study and the severity and relationship to study drug for any adverse experience were determined by the study site investigator.

**Results**

**Patients**

Of the 37 patients who successfully completed the base study (15), four \((4/37; 11\%)\) did not meet protocol eligibility requirements and the remaining 33 \((33/37; 89\%)\) patients were enrolled in the first, 52-week, open-label, extension study (Figure 1). Three additional patients who were not enrolled in the base study were permitted to enter the extension study (see ‘Patients and methods’ section). In total, 36 patients enrolled in the first extension study, with one patient \((1/36; 3\%)\) discontinuing therapy during the first year for reasons unrelated to study medication and one patient \((1/36; 3\%)\) was lost to follow-up. Of the 34 patients who completed the first extension study, 12 \((12/34; 35\%)\) were excluded.

**Figure 1** Study design schematic
because the sites and/or patients decided not to participate in the second, 52-week, open-label extension study and the remaining 22 (22/34; 65%) patients were enrolled. One patient discontinued therapy during the second year because of two non-serious adverse experiences of libido decrease and fatigue. Both these adverse events were mild in intensity and deemed to be possibly related to study medication by the study investigator. Thus, a total of 21 (21/36; 58%) patients successfully completed the second extension study. These 21 patients comprised the completers population for the efficacy analysis (primary end-point). All patients \((n = 36)\) who took at least one postbaseline dose of extension study medication were included in the APaT population for the safety and tolerability analyses.

The extension study population included 36 patients (67% male; 86% Caucasian) clinically diagnosed with homozygous sitosterolemia with a mean age of 37 years (range: 8–72 years) and a mean weight of 66 kg (range: 19–103 kg). Most patients were undergoing drug treatment prior to enrolment, including the use of bile salt-binding resins in 13 patients (all of whom continued such therapy during the extension phase), statins in 10 patients (all of whom continued such therapy during the extension phase), fibrates in one patient (this patient continued fibrate therapy during the first extension study, but subsequently discontinued from the study prior to enrolment in the second year) and apheresis in one patient (this patient continued to undergo regular apheresis procedures throughout the first extension study, but subsequently discontinued from the study prior to enrolment in the second year). Four of the patients enrolled in this study underwent prior ileal bypass surgery to treat their disease. The patient demographic and anthropomorphic characteristics of the entire randomised cohort and completers populations were generally similar (Table 1). Furthermore, the base and extension study populations were comparable with regard to patient characteristics (data not shown) (15). For both the base and extension study populations, plant sterol showed dramatic elevations in sitosterol and campesterol with relatively normal concentrations of cholesterol.

Complications of sitosterolemia, including elevated liver enzyme concentrations, haemolysis, and abnormally low platelet count, haemoglobin, and haematocrit are common findings in affected individuals. Abnormal liver enzyme levels at baseline were relatively common, with 13 patients (36.1%) having elevations in one or more liver function tests; 11 (30.6%), 12 (33.3%) and eight (22.2%) patients experienced elevations in AST, ALT and \(\gamma\)-glutamyl transferase respectively. Low platelet count (<100,000) was observed in 10 patients (27.8%), and low haemoglobin and haematocrit values were seen in five (13.9%) and four (11.1%) patients respectively.

In these extension studies, compliance to study drug was > 85% over the entire 2-year extension study period for 33 of 36 enrolled patients (96%).

| Table 1 | Baseline characteristics of the entire study population and patients who successfully completed both extension studies |
|---------|-------------------------------------------------------------------------------------------------------------------|
| Gender, n (%) | Entire study population \((n = 36)\) | Completers population \((n = 21)\) |
| Age subgroup, n (%) | | |
| Female | 24 (66.7%) | 13 (61.9%) |
| Male | 12 (33.3%) | 8 (38.1%) |
| Race, n (%) | | |
| Caucasian | 31 (86.1%) | 17 (81.0%) |
| Black | 1 (2.8%) | 0 (0%) |
| Asian | 1 (2.8%) | 1 (4.8%) |
| Hispanic | 3 (8.3%) | 3 (14.3%) |
| Body weight, kg | | |
| Mean | 65.9 | 66.3 |
| Range | 18.9–103.3 | 18.9–103.3 |
| Use of concomitant sitosterolemia therapies, n (%) | | |
| None | 16 (44.4%) | 8 (38.1%) |
| Statins | 10 (27.7%) | 7 (33.3%) |
| Bile salt-binding resin | 13 (36.1%) | 8 (38.1%) |
| Fibrates | 1 (2.8%) | 0 |
| Ileal bypass surgery | 4 (11.1%) | 1 (4.8%) |
| Apheresis | 1 (2.8%) | 0 |
| Baseline xanthoma measurement (cm²) | | |
| Mean (SD for mean) | 2.3 (3.1) | 2.7 (3.5) |
| Median (SD for median) | 0.7 (2.6) | 1.1 (2.6) |
| Range | 0.1–10.5 | 0.2–10.5 |
| Baseline Achilles tendon thickness (mm) | | |
| Mean (SD for mean) | 17.8 (5.7) | 16.7 (6.0) |
| Median (SD for median) | 18.5 (7.7) | 18.0 (8.8) |
| Range | 9.0–30.0 | 9.0–30.0 |

*Two patients who were younger than the protocol-specified age range, one 8 and the other 9 years of age, were granted waivers to participate in the extension phase. Patients were allowed to take more than one concomitant pharmacological therapy for the treatment of sitosterolemia during the two extension studies. Thus, a single patient could be included in more than one category shown.
Three of 22 (14%) patients were enrolled in study centres that experienced delays in obtaining institutional review board approvals for conducting the second extension study. These patients received marketed Zetia™ (Merck/Schering-Plough Pharmaceuticals, North Wales, PA, USA) during the interim period to ensure consistency of study treatment. The delays between the first and second extension studies ranged from 20 to 95 days (i.e. 1–3 months).

### Efficacy analyses

Treatment with ezetimibe 10 mg/day for 2 years resulted in significant (p < 0.001 vs. baseline) mean percentage reductions in plasma sitosterol concentrations from baseline of −43.9% (95% CI: −52.2 to −35.6) (Table 2). Progressively larger reductions in plasma sitosterol were observed during the first 40 weeks of the open-label extension phase, with maximal reductions achieved by 52 weeks of treatment (Figure 2). The reductions in plasma sitosterol observed at week 52 (−47.6%; 95% CI: −50.9 to −44.4) were generally sustained through the end of the extension study. The analysis of mean percentage change over time in plasma sitosterol for the APT population was generally similar to that observed for the completers population (Figure 2). The only exception was the finding of generally slightly smaller reductions in sitosterol over time relative to the completer’s population, with a similar magnitude of mean percentage reduction from baseline to extension study end-point of −41.2% (95% CI: −45.7 to −36.8; p < 0.001 vs. baseline).

All 21 patients in the completers population and 35 patients (35/36 = 97%) in the APT population demonstrated reductions in plasma sitosterol at study end-point. A similar proportion of patients in the completers (19/21 = 91%) and APT populations (35/36 = 97%) had reductions in plasma sitosterol concentrations of at least 25% at study end-point. The magnitude of the sitosterol reductions achieved with ezetimibe 10 mg was generally consistent across subgroups defined by patient baseline characteristics, including gender, concomitant usage of bile salt-binding resins or statins, and median baseline sitosterol (Figure 3).

Treatment with ezetimibe 10 mg also led to significant (p < 0.001 vs. baseline) reductions in plasma campesterol of −50.8% (95% CI: −58.8 to −42.7) at study end-point (Table 2). Plasma campesterol progressively declined over the first 40 weeks of the study reaching a maximum reduction of −53.6% (95% CI: −56.9 to −50.3) at week 52 (Figure 4). After week 52, plasma concentrations of campesterol remained generally stable for the remainder of 104 weeks treatment period. As with plasma sitosterol, the analysis of mean percentage change over time in plasma campesterol for the APT population was generally similar to that observed for the

| Parameter (mg/dl) | n | Mean (SD) | Range | % Change (SE) | 95% Confidence interval | p-value† |
|------------------|---|-----------|-------|--------------|-------------------------|---------|
| Sitosterol (GLC) | 21 | 20.95 (7.97) | 7.94–38.36 | −43.9 (4.0) | −52.2 to −35.6 | < 0.001 |
| Campesterol (GLC) | 21 | 11.56 (4.73) | 4.87–23.99 | −50.8 (3.9) | −58.8 to −42.7 | < 0.001 |
| Lathosterol (GLC) | 21 | 0.41 (0.50) | 0.06–1.93 | 0.0 (12.3) | −25.6 to 25.6 | 0.999 |
| LDL sterols | 20 | 149.8 (54.7) | 70.0–269.0 | −13.1 (5.7) | −25.0 to −1.2 | 0.032 |
| HDL sterols | 21 | 55.1 (9.4) | 40.0–69.0 | −4.2 (3.1) | −10.8 to 2.3 | 0.193 |
| Total cholesterol (GLC) | 21 | 186.9 (59.9) | 100.9–299.6 | 2.0 (5.3) | −9.1 to 13.2 | 0.709 |
| Total sterols | 21 | 227.8 (56.0) | 149.0–355.0 | −10.3 (3.1) | −16.7 to −3.9 | 0.003 |
| Triglycerides‡ | 21 | 115.0 (65.1) | 63.0–454.0 | −7.9 (6.9) | −22.3 to 6.4 | 0.239 |
| Apo A-1 | 21 | 156.0 (21.3) | 116.0–190.0 | −3.7 (2.7) | −9.2 to 1.9 | 0.187 |
| Apo B | 21 | 135.2 (39.0) | 70.0–222.0 | −10.1 (4.0) | −18.4 to −1.9 | 0.019 |
| Lathosterol:cholesterol ratio (μg/mg) | 21 | 0.25 (0.31) | 0.04–1.18 | 1.5 (11.2) | −21.9 to 24.9 | 0.893 |

*Cholesterol associated with the low-density lipoprotein and high-density lipoprotein fractions could not be measured in this study because of technical issues. †No multiplicity adjustments were made for secondary efficacy variables. ‡Triglycerides are expressed as median values (standard deviation of the median) at baseline and median percentage change values (standard deviation of the median) from baseline because of non-normal distribution of results. SD, standard deviation of the mean (or median, where appropriate); LDL, low-density lipoprotein; HDL, high-density lipoprotein; GLC, measured by gas–liquid chromatography; apo, apolipoprotein.
**Figure 2** Mean percentage change from baseline (±SE) in plasma concentration of sitosterol over time in the completers and all-patient-treated populations.

**Figure 3** Mean percentage change (95% confidence interval) in plasma concentration of sitosterol from baseline to study end in various subgroups defined by baseline patient characteristics.
completer’s population. A significant ($p < 0.001$ vs. baseline) mean percentage reduction from baseline to extension study end-point of $-50.1\%$ (95% CI: $-55.7$ to $-44.5$) was seen for the APT population.

Peak reductions in total sterols ($-17.0\%;$ 95% CI: $-20.0$ to $-13.9$), LDL sterols ($-24.1\%;$ 95% CI: $-28.1$ to $-20.0$) and apo B ($-16.5\%;$ 95% CI: $-19.6$ to $-13.4$) were achieved early in the first extension study (week 16 for total sterols; week 4 for LDL sterols and apoB) and waned slightly through week 104 (Figure 5). At study end-point, plasma concentrations of total sterols, LDL sterols and apo B were significantly ($p \leq 0.050$ vs. baseline for all parameters) reduced by $-10.3\%$ (95% CI: $-16.7$ to $-3.9$), $-13.1\%$ (95% CI: $-25.0$ to $-1.2$) and $-10.1\%$ (95% CI: $-18.4$ to $-1.9$), respectively (Table 2). No signifi-

![Figure 4](image_url)  
**Figure 4** Mean percentage change from baseline ($\pm$SE) in plasma concentration of campesterol over time for the completers and all-patient-treated populations

![Figure 5](image_url)  
**Figure 5** Mean percentage change from baseline ($\pm$SE) in plasma concentrations of low-density lipoprotein (LDL)-sterols, total sterols and apolipoprotein (apo) B over time for the completers populations
cant changes from baseline were observed for lathosterol, lathosterol:cholesterol ratio, TG, HDL-sterols, total cholesterol (GLC) or apo A-1.

At baseline, the median Achilles tendon thickness measured by radiography was 16.7 mm (n = 20) (Table 1). After treatment with ezetimibe, a non-significant (p > 0.05) median percentage change in Achilles tendon thickness of −1.0% (95% CI: −5.4 to 3.3) was observed at week 104. The mean baseline subcutaneous xanthoma size measured directly was 2.7 cm² (n = 15) (Table 2). A borderline significant (p = 0.076) mean percentage reduction in xanthoma size of −25.3% (95% CI: −53.8 to 3.2) was observed at week 104.

Safety analyses

Ezetimibe was generally well-tolerated throughout the entire 104 weeks study. Overall, 30 (83%) patients reported one or more clinical adverse experiences (Table 3). The most common adverse events included upper respiratory infections (nine patients; 25%), dizziness (five patients; 14%), headache (five patients; 14%) and abdominal pain (four patients; 11%). Six (17%) patients reported treatment-related adverse experiences, none of which was considered serious by the study investigators. In total, five (14%) patients reported serious adverse experiences during these extension studies. All serious adverse experiences were considered unlikely related to study medication and none of these adverse events resulted in discontinuation from the study. Two patients discontinued from the first extension study; one for reason unrelated to the study medication (i.e. patient wished to conceive a child) and the second was lost to follow-up. One patient discontinued from the second extension study because of non-serious clinical adverse events of fatigue and decreased libido. This patient’s clinical adverse experiences were considered mild in intensity and were deemed possibly related to study medication.

During this study, special consideration was given to specific laboratory tests [namely ALT, AST and creatinine kinase (CK)], which have been traditionally evaluated with drugs of the statin class (28,29). Additionally, adverse events related to gallbladder, myopathy and rhabdomyolysis were carefully assessed. No patients in this study had consecutive increases in ALT or AST > 3 × ULN or CK elevations in excess of 5 or 10 × ULN. There were no reports of gallbladder- or hepatic-related adverse events and no cases of myopathy or rhabdomyolysis were reported. Safety laboratory tests for hematologic parameters (i.e. platelet count, haemoglobin and haematocrit) remained essentially stable during the treatment period.

Discussion

In a previously published, randomised, placebo-controlled, double-blind study conducted in patients with sitosterolemia, treatment with ezetimibe 10 mg/day for 8 weeks produced significant reductions in plasma concentrations of sitosterol and campesterol (−21% and −24% respectively; p < 0.001 vs. baseline and placebo) and was generally well tolerated (15). In that study, plasma phytosterol concentrations declined progressively over the entire 8-week treatment period. Plasma concentrations of sitosterol and campesterol did not plateau by the end of the 8-week base study, suggesting that longer treatment durations were required to reach maximal efficacy. Therefore two, successive, open-label extension studies were conducted to determine if longer-term treatment with ezetimibe 10 mg results in further improvements in plasma phytosterol concentrations.

In patients with homozygous sitosterolemia, long-term treatment with ezetimibe 10 mg for up to 2 years produced substantial mean percentage reductions from baseline in plasma sitosterol (−43.9%; p < 0.001 vs. baseline) and campesterol (−50.8%; p < 0.001 vs. baseline). Sitosterol and campesterol declined progressively during the first 40 weeks of the extension study and reached a nadir at week 52. The maximal reductions in plasma sterols seen at week 52 were generally sustained through the end of the extension study. Collectively, these findings

| Table 3 Summary of adverse events |
|----------------------------------|
| Safety parameter                | N = 36, n (%) |
| Clinical AE                     | 30 (83)       |
| Drug-related* clinical AE        | 6 (17)        |
| Serious AE                      | 5 (14)        |
| Drug-related* serious AE         | 0             |
| Death                           | 0             |
| Discontinued due to AE           | 1 (3)         |
| Discontinued due to drug-related* AE | 1 (3)     |
| Discontinued due to serious AE   | 0             |
| Single ALT/AST elevation ≥ 3 × ULN | 1 (3)       |
| Consecutive ALT/AST elevations ≥ 3 × ULN | 0    |
| Single CK elevation 3 × ULN to < 5 × ULN | 2 (6) |
| CK elevation ≥ 10 × ULN          | 0             |

* Determined by the investigator to be possibly, probably or definitely treatment-related laboratory adverse experiences.
† Defined as two or more consecutive values ≥ 3 × ULN or a single last value ≥ 3 × ULN with no follow-up value on record.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal; CK, creatine kinase; AE, adverse event.
confirm that chronic, long-term dosing of ezetimibe 10 mg/day is required to achieve maximal reductions in plasma sterols among patients with sitosterolemia.

Similar mean percentage reductions in plasma sitosterol were observed across prespecified patient subgroups defined by gender, concomitant use of lipid-altering medications (bile salt-binding resins or statins) and baseline plasma sitosterol concentrations (< 20 or ≥ 20 mg/dl). This finding suggests that a wide-range of patients with sitosterolemia may benefit from long-term therapy with ezetimibe 10 mg/day.

Further improvements from baseline in other lipid- and sterol-related parameters were observed over the course of this 2-year extension study. Small but significant reductions from baseline were seen for LDL-sterols (−13.1%; p < 0.032), total sterols (−10.3%; p < 0.003) and apo B (−10.1%; p < 0.019). In contrast to the plasma time profiles for sitosterol and campesterol, maximal reductions in LDL-sterols, total sterols and apo B were achieved early in the first extension study by week 4. These maximal reductions waned somewhat throughout the remainder of the first and second extension studies. This observation contrasts with the relatively stable reductions in LDL-sterols, total sterols and apo B seen over 8 weeks in the base study (15). Nevertheless, significant improvements in these parameters were observed at study end-point following long-term dosing for up to 2 years.

No significant changes from baseline were seen for the remaining efficacy end-points, including lathosterol, lathosterol:cholesterol ratio, TG, HDL-sterols, cholesterol (GLC) and apo A-1. Lathosterol is a precursor of cholesterol and the ratio of lathosterol:cholesterol has been used as a surrogate measure of cholesterol biosynthesis (30). Prior studies have shown significant increases in lathosterol:cholesterol ratio following ezetimibe 10 mg therapy in normo- and hypercholesterolaemic subjects (18,31). Results from the 8-week base study in sitosterolemic patients demonstrated a significant increase in the ratio of lathosterol:cholesterol relative to placebo (37%; p < 0.05) (15). The lack of a significant change in lathosterol and lathosterol:cholesterol seen at the end of this 2-year study suggests that long-term inhibition of intestinal cholesterol and phytosterol absorption with ezetimibe does not result in a compensatory increase in cholesterol biosynthesis in patients with sitosterolemia. Patients with sitosterolemia have been shown to have reduced cholesterol synthesis, associated with a marked reduction in the activity of critical enzymes involved the cholesterol biosynthesis pathway (32,33). Suppression of cholesterol synthesis as a result of the sitosterolemia phenotype may explain the apparent lack of ‘compensatory’ upregulation of cholesterol biosynthesis in these patients, in the face of reduced cholesterol absorption from the gut with chronic ezetimibe treatment.

One caveat worth noting is that the prespecified efficacy analysis was performed on the subset of patients with baseline and a week 104 efficacy measurement (i.e. completers population). Of the 36 patients enrolled in the first extension study, only 21 patients (21/36 = 58%) completed the entire 2-year study. Therefore, as a supportive analysis, the effects of ezetimibe 10 mg on plasma sitosterol (primary end-point) and campesterol (secondary end-point) were analysed for the more robust APT population, which included those patients who received at least one dose of extension study medication and who had paired baseline and at least one on-treatment efficacy measurement. The results of the APT population analysis were generally similar to that seen for the completers population, indicating that the completers population analyses provide a reliable estimate of the efficacy profile of ezetimibe 10 mg in this study.

In the 8-week base study, Achilles tendon thickness measured by radiography showed a slight reduction in size with ezetimibe 10 mg treatment relative to placebo (−0.6% vs. 8.0% respectively; p = 0.013 vs. placebo) (15). This initial finding suggested that tissue stores of plant sterols were significantly depleted with ezetimibe treatment after short treatment durations. This study evaluated the effect of ezetimibe 10 mg on Achilles tendon xanthoma thickness (measured by radiography; mm) and subcutaneous xanthoma size (measured directly; cm²) following 2 years of treatment. No significant changes in Achilles tendon thickness (−1.0%; p = ns vs. baseline) or xanthoma area (−25.3%; p = 0.076 vs. baseline) were observed at study end-point. Unlike the base study, the extension study did not include a placebo group; therefore, it is not possible to compare directly the Achilles tendon findings between the base and extension studies. However, the results of the 2-year study suggest that long-term treatment with ezetimibe 10 mg, when administered alone or in combination with bile-acid sequestrants, statins, and/or a low-sterol diet, does not produce substantial regression in xanthoma size. This finding contrasts with a recently published case report showing the complete regression of tuberous xanthomas in a paediatric patient with sitosterolemia following treatment with ezetimibe 10 mg and cholestyramine for up to 1 year (34).

One possible explanation is that xanthomas in older patients may be more recalcitrant to therapy relative to younger patients. It is also important to note that the interpretation of the xanthoma findings
in the extension study were hampered by small number of patients with paired baseline and week 104 radiography measurements (N = 12 in the second year) and large variability in xanthoma size at baseline (either by radiography or direct measurement).

Finally, long-term treatment with ezetimibe 10 mg was generally well tolerated when administered for up to 2 years in patients with sitosterolemia. A majority of adverse events were mild, transient and resolved despite continued treatment. Only one patient discontinued therapy during the study because of a non-serious adverse experience. Hepatobiliary and muscle adverse experiences were carefully evaluated in this study because of the known effects of lipid-lowering agents on these safety parameters (28,29). There were no incidences of consecutive elevations in liver enzymes ≥ 3 × ULN, and there were no reports of clinical hepatitis. Additionally, no cases of clinically important CK elevations or myopathy were reported.

In conclusion, long-term treatment with ezetimibe 10 mg/day produced progressive improvements in plasma plant sterol concentrations with an overall favourable safety and tolerability profile in patients with sitosterolemia. Maximal reductions in plasma sitosterol and campesterol, the two predominant sterols found in the plasma of patients with sitosterolemia, were achieved within the first year of treatment and remained generally stable throughout the second year. These findings are consistent with the observation that ezetimibe inhibits the intestinal absorption of plant sterols as well as cholesterol (19).

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