

ORIGINAL ARTICLE

## Inhibition of Microsomal Triglyceride Transfer Protein in Familial Hypercholesterolemia

Marina Cuchel, M.D., Ph.D., LeAnne T. Bloedon, M.S., R.D.,  
Philippe O. Szapary, M.D., Daniel M. Kolansky, M.D., Megan L. Wolfe, B.S.,  
Antoine Sarkis, M.D., John S. Millar, Ph.D., Katsunori Ikewaki, M.D.,  
Evan S. Siegelman, M.D., Richard E. Gregg, M.D., and Daniel J. Rader, M.D.

### ABSTRACT

#### BACKGROUND

From the University of Pennsylvania School of Medicine, Philadelphia (M.C., L.T.B., P.O.S., D.M.K., M.L.W., J.S.M., E.S.S., D.J.R.); Hôtel Dieu de France Hospital, St. Joseph University, Beirut, Lebanon (A.S.); Jikei University School of Medicine, Tokyo (K.I.); and Bristol-Myers Squibb Pharmaceutical Research Institute, Lawrenceville, NJ (R.E.G.). Address reprint requests to Dr. Rader at the University of Pennsylvania Medical Center, 654 BRBII/III Labs, 421 Curie Blvd., Philadelphia, PA 19104, or at rader@mail.med.upenn.edu.

N Engl J Med 2007;356:148-56.  
Copyright © 2007 Massachusetts Medical Society.

Patients with homozygous familial hypercholesterolemia have markedly elevated cholesterol levels, which respond poorly to drug therapy, and a very high risk of premature cardiovascular disease. Inhibition of the microsomal triglyceride transfer protein may be effective in reducing cholesterol levels in these patients.

#### METHODS

We conducted a dose-escalation study to examine the safety, tolerability, and effects on lipid levels of BMS-201038, an inhibitor of the microsomal triglyceride transfer protein, in six patients with homozygous familial hypercholesterolemia. All lipid-lowering therapies were suspended 4 weeks before treatment. The patients received BMS-201038 at four different doses (0.03, 0.1, 0.3, and 1.0 mg per kilogram of body weight per day), each for 4 weeks, and returned for a final visit after a 4-week drug washout period. Analysis of lipid levels, safety laboratory analyses, and magnetic resonance imaging of the liver for fat content were performed throughout the study.

#### RESULTS

All patients tolerated titration to the highest dose, 1.0 mg per kilogram per day. Treatment at this dose decreased low-density lipoprotein (LDL) cholesterol levels by 50.9% and apolipoprotein B levels by 55.6% from baseline ( $P < 0.001$  for both comparisons). Kinetic studies showed a marked reduction in the production of apolipoprotein B. The most serious adverse events were elevation of liver aminotransferase levels and accumulation of hepatic fat, which at the highest dose ranged from less than 10% to more than 40%.

#### CONCLUSIONS

Inhibition of the microsomal triglyceride transfer protein by BMS-201038 resulted in the reduction of LDL cholesterol levels in patients with homozygous familial hypercholesterolemia, owing to reduced production of apolipoprotein B. However, the therapy was associated with elevated liver aminotransferase levels and hepatic fat accumulation.

**H**OMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA is caused by loss-of-function mutations in both alleles of the low-density lipoprotein (LDL) receptor gene.<sup>1-3</sup> Patients with the disease have plasma cholesterol levels of more than 500 mg per deciliter (12.9 mmol per liter); if untreated, patients have cardiovascular disease before 20 years of age and generally do not survive past 30 years of age.<sup>1-3</sup> Patients with homozygous familial hypercholesterolemia also have a poor response to conventional drug therapy,<sup>1-3</sup> which generally lowers LDL cholesterol levels through up-regulation of the hepatic LDL receptor. The current standard of care for these patients is LDL apheresis. This procedure can transiently reduce LDL cholesterol levels by more than 50%<sup>4,5</sup> and may delay the onset of atherosclerosis,<sup>6-8</sup> but it must be repeated frequently (every 1 to 2 weeks) and is not widely available. Thus, new therapies are needed for patients with homozygous familial hypercholesterolemia, as well as for other patients with severe refractory hypercholesterolemia who are candidates for LDL apheresis.

A potentially effective therapy for homozygous familial hypercholesterolemia would be to reduce LDL production. The microsomal triglyceride transfer protein is responsible for transferring triglycerides onto apolipoprotein B within the liver in the assembly of very-low-density lipoprotein (VLDL), the precursor to LDL.<sup>9</sup> In the absence of functional microsomal triglyceride transfer protein, as in the rare recessive genetic disorder abetalipoproteinemia, the liver cannot secrete VLDL, leading to the absence of all lipoproteins containing apolipoprotein B in the

plasma.<sup>10-12</sup> Thus, the pharmacologic inhibition of microsomal triglyceride transfer protein might be a strategy for reducing LDL production and plasma LDL cholesterol levels.

Preclinical studies in animal models lacking LDL receptors have shown that the inhibition of microsomal triglyceride transfer protein significantly reduces serum cholesterol levels.<sup>13,14</sup> We evaluated the cholesterol-lowering efficacy of the microsomal triglyceride transfer protein inhibitor BMS-201038 in patients with homozygous familial hypercholesterolemia and determined the mechanism of cholesterol reduction, the tolerability, and the effects on hepatic fat, using magnetic resonance imaging (MRI).

## METHODS

### STUDY PATIENTS

Six patients with homozygous familial hypercholesterolemia (three men and three women), 18 to 40 years of age, were enrolled in and completed the study. A diagnosis of homozygous familial hypercholesterolemia was suspected on clinical grounds and was confirmed by genetic analysis. Exclusion criteria were major surgery in the previous 3 months, congestive heart failure, history of liver disease or aminotransferase levels of more than three times the upper limit of the normal range, a serum creatinine level of more than 2.5 mg per deciliter (221  $\mu$ mol per liter), cancer within the past 5 years, or history of alcohol abuse or drug abuse. Two patients had known, clinically significant cardiovascular disease; both had undergone prosthetic-valve replacement and were receiving anticoagulation therapy. Our study was

Table 1. Baseline Characteristics of the Study Patients.

Patient No.	Sex	Age yr	Weight kg	Body-Mass Index*	Cardiovascular Disease†	LDL-Receptor Gene Mutations
1	F	18	56.1	24.3	Absent	delEx3-6/delEx3-6
2	F	18	59.0	25.3	Absent	1877delA/?
3	M	35	85.4	27.7	Present	652delGGT/652delGGT
4	F	40	77.3	30.1	Present	Ser156Leu/Ser156Leu
5	M	22	60.1	18.5	Absent	Cys660Xaa/Cys660Xaa
6	M	21	64.0	23.2	Absent	Cys660Xaa/Cys660Xaa

\* The body-mass index is the weight in kilograms divided by the square of the height in meters.

† Patients 3 and 4 had symptomatic coronary artery disease that was confirmed by coronary angiography. Patients 1, 2, 5, and 6 had no symptoms of cardiovascular disease and were regularly evaluated with the use of noninvasive testing (and, if appropriate, coronary angiography), without evidence of obstructive coronary disease.

Table 2. Lipid and Lipoprotein Levels at Baseline, after Receipt of One of Four Doses of BMS-201038 for 4 Weeks, and after the 4-Week Washout Period.\*

Measure	Patient No.						Percent Change from Baseline	P Value
	1	2	3	4	5	6		
Total cholesterol (mg/dl)								
Baseline	756	837	903	684	711	1212		
0.03 mg	660	840	717	717	684	1248	-4.8±9.9	0.29
0.1 mg	627	858	585	774	648	1086	-9.3±16.6	0.23
0.3 mg	482	714	591	504	424	891	-29.8±9.2	<0.001
1.0 mg	284	410	443	340	236	379	-58.4±8.6	<0.001
Washout	993	1053	1023	714	714	738	6.0±25.1	0.58
LDL cholesterol (mg/dl)								
Baseline	480	789	609	637	534	636		
0.03 mg	505	748	585	668	442	597	-3.7±8.3	0.32
0.1 mg	558	753	483	718	481	403	-7.1±20.1	0.42
0.3 mg	348	642	498	436	387	478	-24.7±5.3	<0.001
1.0 mg	224	383	403	301	201	306	-50.9±9.3	<0.001
Washout	804	883	858	518	559	478	13.6±35.4	0.39
VLDL cholesterol (mg/dl)								
Baseline	256	21	270	12	153	549		
0.03 mg	135	69	114	15	220	627	34.4±103.3	0.45
0.1 mg	48	84	75	24	138	642	42.3±142.4	0.50
0.3 mg	108	48	57	29	28	372	3.3±103.7	0.94
1.0 mg	34	5	18	8	14	44	-78.7±23.1	<0.001
Washout	153	138	138	162	129	216	273.6±535.1	0.27
Triglycerides (mg/dl)								
Baseline	285	130	362	82	233	605		
0.03 mg	248	84	279	110	416	502	4.1±43.5	0.83
0.1 mg	194	68	139	113	105	658	-24.9±39.7	0.19
0.3 mg	200	87	148	88	126	340	-34.1±22.8	0.02
1.0 mg	51	56	102	46	69	206	-65.2±13.3	<0.001
Washout	226	119	210	234	135	288	3.3±90.6	0.93

approved by the institutional review board and the General Clinical Research Center of the University of Pennsylvania and was monitored by the Office of Human Research of the University of Pennsylvania. The study protocol was fully explained to all six patients, each of whom provided written, informed consent.

#### STUDY PROTOCOL

The authors designed the study and generated, held, and analyzed the data. The study drug, BMS-201038, was provided by Bristol-Myers Squibb.

This was an open-label study to evaluate the safety, tolerability, and efficacy of BMS-201038 for the treatment of patients with homozygous familial hypercholesterolemia. During an initial screening visit, the eligibility of the six patients was verified, their health status was evaluated, and a very-low-fat diet was initiated. All lipid-lowering treatments, including apheresis, were suspended at least 4 weeks before the baseline visit and continued to be suspended until the study was completed. No other drug treatment was suspended. BMS-201038 was administered,

Table 2. (Continued.)

Measure	Patient No.						Percent Change from Baseline	P Value
	1	2	3	4	5	6		
Apolipoprotein B (mg/dl)								
Baseline	315	273	342	240	303	387		
0.03 mg	306	354	336	300	330	396	10.2±14.0	0.13
0.1 mg	276	336	288	276	225	375	-3.2±18.8	0.70
0.3 mg	228	312	273	216	213	330	-14.7±16.0	0.08
1.0 mg	112	149	216	121	91	127	-55.6±13.5	<0.001
Washout	324	345	432	324	282	312	10.7±21.7	0.28
HDL cholesterol (mg/dl)								
Baseline	20	27	24	35	24	27		
0.03 mg	20	23	18	34	22	24	-10.4±9.0	0.04
0.1 mg	21	21	27	32	29	41	9.9±25.6	0.39
0.3 mg	26	24	36	39	9	41	11.6±43.5	0.54
1.0 mg	26	22	22	31	21	29	-2.2±18.0	0.77
Washout	36	32	27	34	26	44	29.9±33.4	0.08
Apolipoprotein A-I (mg/dl)								
Baseline	68	79	83	76	62	30		
0.03 mg	67	67	63	80	77	95	34.2±90.9	0.40
0.1 mg	69	64	79	80	65	74	22.4±61.5	0.41
0.3 mg	78	70	80	88	64	94	38.7±86.2	0.32
1.0 mg	62	64	64	67	49	44	-6.1±26.4	0.59
Washout	100	81	82	96	76	104	57.3±94.4	0.20

\* Plus-minus values are means ±SD. To convert values for cholesterol to millimoles per liter, multiply by 0.02586. To convert values for triglycerides to millimoles per liter, multiply by 0.01129. P values are for the levels during the study versus those at baseline.

beginning at the baseline visit, at four increasing doses — 0.03, 0.1, 0.3, and 1.0 mg per kilogram of body weight per day — each for 4 weeks. The patients returned to the General Clinical Research Center every 7, 14, and 28 days after the start of a new dose, and 28 days after the last dose of the study drug, for safety and pharmacodynamic evaluations.

The most recent Common Terminology Criteria for Adverse Events of the National Cancer Institute (initially version 2 and subsequently version 3) were used to assign a severity grade to all adverse events. According to protocol, if a patient had a confirmed grade 3 (severe) adverse event, the dose was decreased to 1.5 times the previous dose for 4 weeks (with visits at 7, 14, and 28 days during that period). If there was no evidence of adverse events of grade 3 or higher during that period, the dose was increased to the next-high-

est dose and treatment proceeded per protocol. Adverse events were judged by one of the investigators as not related to treatment with the study drug, unlikely to be related, possibly related, probably related, or definitely related, and these judgments were reviewed by a data and safety monitoring board.

#### DIET

All patients received detailed dietary counseling by a registered dietitian at the screening visit and at all subsequent visits until after the study drug was discontinued. The patients were advised to consume a diet containing less than 10% of energy from total dietary fat while consuming adequate calories to maintain weight or promote growth. All patients received a standard multivitamin that supplied 100% of the reference dietary intake for all vitamins and minerals.

**MRI OF THE LIVER**

MRI of the liver was conducted at baseline, after 4 weeks at each dose, and at 4 weeks after drug withdrawal, with the use of chemical-shift MRI techniques that have been shown to evaluate fat content of the liver accurately.<sup>15,16</sup> All quantitative MRI measurements of hepatic fat content were performed by a single radiologist, who was unaware of the patients' clinical status and liver-function results.

**LABORATORY ANALYSIS**

Blood was drawn at each visit, after a 12-hour fast. A standard metabolic panel, complete blood count, and standard urinalysis were also performed at each visit. Plasma lipid and lipoprotein analyses were performed in a lipid laboratory standardized by the Centers for Disease Control and Prevention. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured enzymatically on an autoanalyzer (Cobas Fara II, Roche Diagnostic Systems) with reagents from Sigma Chemical Co. VLDL and LDL cholesterol levels were determined with the use of beta-quantification and the standard Lipid Research Clinics protocol as modified by Cole et al.<sup>17</sup> Levels of apolipoproteins B and A-I were measured with the use of reagents from Wako Chemicals USA, and Lp(a) lipoprotein levels were measured with reagents from Diasorin on a Cobas Fara II autoanalyzer. Levels of lipoprotein subclasses were determined with the use of proton nuclear magnetic resonance spectroscopy, as previously described.<sup>18</sup>

**KINETICS STUDIES**

Before the study began, three patients (Patients 4, 5, and 6) had participated in a kinetics study to investigate the metabolism of lipoproteins containing apolipoprotein B in patients with homozygous familial hypercholesterolemia.<sup>19</sup> To investigate in vivo the mechanism of action of BMS-201038, we repeated the kinetic study in these patients at the end of the 4-week period at the highest dose (1.0 mg per kilogram per day), using identical methods (endogenous labeling with deuterated leucine).

**STATISTICAL ANALYSIS**

Statistical comparisons were performed with SAS software (version 8.2, SAS Institute). Continuous variables that were not normally distributed, such

as fasting triglyceride levels, were appropriately transformed to meet the assumptions of subsequent statistical tests. Continuous variables were analyzed using paired t-tests for changes over time or the Wilcoxon signed-rank test, as appropriate. Percentages were analyzed using the chi-square test or Fisher's exact test when expected cell counts were less than 5. For within-patient comparisons over time, we used McNemar's test, along with matched odds ratios and 95% confidence intervals. All P values were calculated from two-tailed tests, and P values less than 0.05 were considered to indicate statistical significance.

**RESULTS****STUDY PATIENTS**

The characteristics of the six study patients at screening are shown in Table 1. Four patients (Patients 1, 3, 5, and 6) were found to be negative for the LDL receptor on the basis of homozygosity for known loss-of-function LDL-receptor mutations.<sup>1,2</sup> A fifth (Patient 2) was found to be receptor-negative on the basis of phenotype and LDL-receptor activity in skin fibroblasts. The sixth patient (Patient 4) was found to have a defective LDL receptor on the basis of her LDL-receptor mutation.

**EFFECTS OF BMS-201038 ON PLASMA LIPID AND LIPOPROTEIN LEVELS**

The mean doses of BMS-201038 at each of the four titration steps were 2.0, 6.7, 20.1, and 67.0 mg per day. Table 2 shows the changes in lipid and lipoprotein levels during the study (additional lipoprotein results are available in Table A in the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org)). The mean total cholesterol level was 851 mg per deciliter (22.0 mmol per liter) at baseline. After 4 weeks of receiving the 0.3-mg-per-kilogram dose, the mean level was reduced to 601 mg per deciliter (15.5 mmol per liter), a 29.8% reduction from the baseline level ( $P < 0.001$ ). After 4 weeks of receiving the 1.0-mg-per-kilogram dose, the mean level was reduced to 349 mg per deciliter (9.0 mmol per liter), a 58.4% reduction from baseline ( $P < 0.001$ ).

The mean LDL cholesterol level was 614 mg per deciliter (15.9 mmol per liter) at baseline. After 4 weeks at the 0.3-mg-per-kilogram dose, the mean level was reduced to 465 mg per deciliter (12.0 mmol per liter), a 24.7% reduction from the



# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

## FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

## E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.