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ORIGINAL RESEARCH ARTICLE

Orally Bioavailable Macrocyclic Peptide That Inhibits Binding of PCSK9 to the Low Density Lipoprotein Receptor

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BACKGROUND: Inhibition of PCSK9 (proprotein convertase subtilisin/kexin type 9)-low density lipoprotein receptor interaction with injectable monoclonal antibodies or small interfering RNA lowers plasma low density lipoprotein-cholesterol, but despite nearly 2 decades of effort, an oral inhibitor of PCSK9 is not available. Macrocyclic peptides represent a novel approach to target proteins traditionally considered intractable to small-molecule drug design.

METHODS: Novel mRNA display screening technology was used to identify lead chemical matter, which was then optimized by applying structure-based drug design enabled by novel synthetic chemistry to identify macrocyclic peptide (MK-0616) with exquisite potency and selectivity for PCSK9. Following completion of nonclinical safety studies, MK-0616 was administered to healthy adult participants in a single rising-dose Phase 1 clinical trial designed to evaluate its safety, pharmacokinetics, and pharmacodynamics. In a multiple-dose trial in participants taking statins, MK-0616 was administered once daily for 14 days to characterize the safety, pharmacokinetics, and pharmacodynamics (change in low density lipoprotein cholesterol).

RESULTS: MK-0616 displayed high affinity (K_i = 5pM) for PCSK9 in vitro and sufficient safety and oral bioavailability preclinically to enable advancement into the clinic. In Phase 1 clinical studies in healthy adults, single oral doses of MK-0616 were associated with >93% geometric mean reduction (95% CI, 84–103) of free, unbound plasma PCSK9; in participants on statin therapy, multiple–oral-dose regimens provided a maximum 61% geometric mean reduction (95% CI, 43–85) in low density lipoprotein cholesterol from baseline after 14 days of once-daily dosing of 20 mg MK-0616.

CONCLUSIONS: This work validates the use of mRNA display technology for identification of novel oral therapeutic agents, exemplified by the identification of an oral PCSK9 inhibitor, which has the potential to be a highly effective cholesterol lowering therapy for patients in need.

Key Words: atherosclerosis ■ cholesterol ■ clinical trial ■ hypercholesterolemia ■ low density lipoprotein ■ macrocyclic peptide ■ mRNA display technology ■ PCSK9 inhibitors

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For Sources of Funding and Disclosures, see page 157.

Clinical Perspective

What Is New?

- The work reported here demonstrates the de novo invention of a novel macrocyclic peptide, MK-0616, capable of achieving the potency and selectivity of an antibody in a form that can be taken orally.
- This provides validation and guidance for the use of mRNA display technology, a major advancement in the medicinal chemistry underlying drug discovery.

What Are the Clinical Implications?

- As an oral inhibitor of proprotein convertase subtilisin/kexin type 9, MK-0616 exhibited robust lowering of low density lipoprotein cholesterol when added to statin treatment.
- As such, MK-0616 could be a highly effective cholesterol-lowering therapy for patients who require low density lipoprotein cholesterol reduction.
- MK-0616 has the potential to overcome numerous barriers to treatment, allowing more patients to achieve low density lipoprotein cholesterol goals and reduce cardiovascular risk earlier in their treatment journey.

Nonstandard Abbreviations and Acronyms

AE	adverse event
AF	AlexaFluor
$AUC_{0-\infty}$	area under the concentration versus time curve from pre-dose to infinity
AUC_{0-24}	area under the concentration from pre-dose to 24 hours post-dose
AUC_{0-last}	area under the concentration from pre-dose to last measurement taken
C_{24}	plasma concentration observed 24 hours post-dose
C_{max}	maximum observed plasma concentration
EDTA	ethylenediaminetetraacetic acid
LDL	low density lipoprotein
LDL-C	low-density lipoprotein cholesterol
LDLR	LDL receptor
mAb	monoclonal antibodies
MK-0616	macrocyclic peptide
PCSK9	proprotein convertase subtilisin/kexin type 9
PE	permeation enhancer
T_{max}	time when C_{max} was first observed

Until recently, targets of therapeutic pharmaceutical disease intervention were limited to proteins like enzymes, G-protein coupled receptors, and ion channels, which function by interacting with relatively small substrates. Such targets can often be engaged by 300–500 Dalton synthetic molecules that mimic a natural substrate, occupying and blocking a highly specific active site. These molecules have the advantages of being not only selective and potent, but also orally bioavailable, making them amenable to the simplicity of dosing as a pill. With increasing understanding of complex biological regulatory mechanisms, it has become apparent that this approach to therapy leaves a wide array of disease mediators unavailable to traditional therapeutic intervention. These disease mediators often function through protein-protein interactions in which formation of a complex is dependent on interactions spread over a relatively large surface area. The development of monoclonal antibodies (mAb) as a class of therapeutic agents that can disrupt pathological protein complexes has demonstrated a successful alternative to the small-molecule paradigm.[1] However, therapeutic antibodies generally have higher production costs compared with small molecules and cannot be dosed orally, instead requiring regular (daily/weekly/monthly) subcutaneous or intravenous administration, limiting patient choice. Thus, there is a broad medical need to identify therapeutic agents that combine the ability of an antibody to bind a large protein surface with high affinity, with the ease of oral administration.

There are precedents for such an intermediate class of agents among peptides approved for clinical use,[2,3] including the orally administered molecules cyclosporin, desmopressin, and semaglutide. However, cyclosporin is a fungal natural product, while desmopressin and semaglutide were designed based on naturally occurring peptides with physiological activity. Given the intrinsic challenges to chemical synthesis of macrocyclic peptides, in the absence of such natural product leads, it has been impractical to develop similar therapies on a broad scale because it was impossible to create molecular libraries of sufficient size and diversity to provide a collection to be screened for leads against the many pathologic interactions of clinical interest.

Recent developments in cyclic peptide discovery now provide a practical route to access such a library: mRNA display (Figure 1), an evolution-based affinity-selection platform,[4] combined with an array of novel, unnatural amino acid residues,[4] can yield an unprecedented chemical diversity of metabolically stable macrocyclic peptide lead molecules. These leads can be efficiently optimized by medicinal chemists using state-of-the-art solid-phase peptide synthesis.[5] Further, because of advances in formulation and delivery strategies, it is theoretically possible to make such compounds (which, although orders of magnitude smaller than antibodies, are larger and less cell permeable than traditional small molecules) orally bioavailable to patients.[6] The potential for architectural complexity in these molecules enables access to diverse,

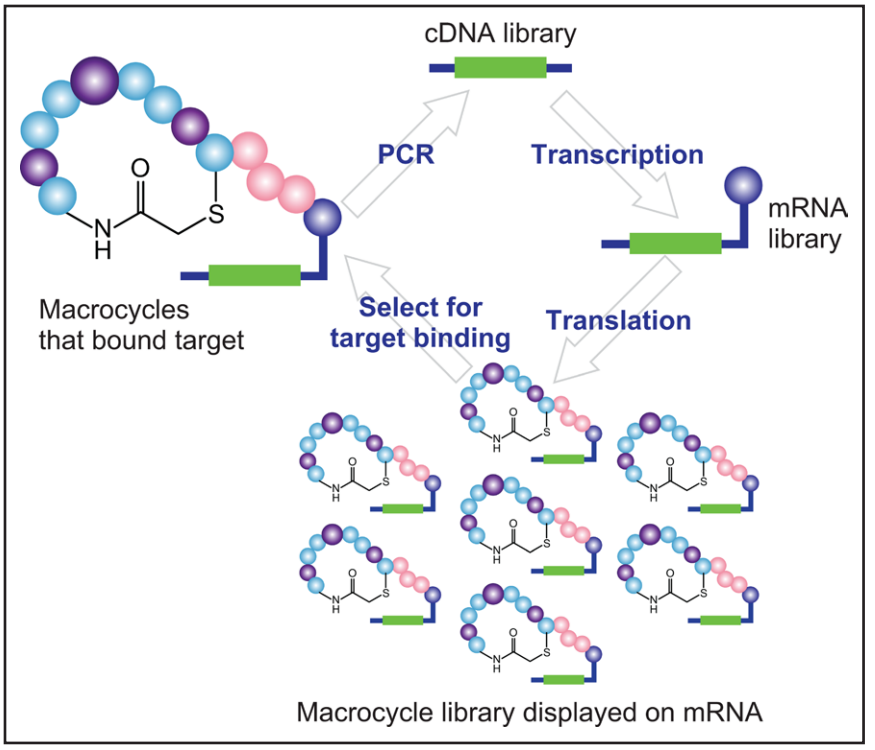

Figure 1. Messenger ribonucleic acid (mRNA) display screening platform. mRNA display is a powerful in vitro affinity selection platform that allows screening of large, genetically encoded peptide libraries. The platform takes advantage of the transcription/translation machinery and post-translation cyclization, under cell-free conditions to prepare the libraries. A key feature is that the mRNA is covalently linked to its encoded peptide which enables amplification of desired peptides shown to interact with a target of interest. cDNA indicates complementary DNA (deoxyribonucleic acid); and PCR, polymerase chain reaction.

biologically relevant molecular conformations, thereby creating mAb-like potential to block the interactions upon which protein-protein complexes depend, while being synthetically tractable to the introduction of pharmacologic characteristics normally designed into smaller molecules.[7]

Therapeutic agents that reduce plasma low-density lipoprotein cholesterol (LDL-C) lower risk of atherosclerotic cardiovascular disease.[8–10] However, despite decades of research and treatment, atherosclerotic cardiovascular disease remains a leading cause of death globally,[11] and improved therapy is still needed. PCSK9 (proprotein convertase subtilisin/kexin type 9) is a plasma protein with a well-documented role in maintaining plasma levels of LDL-C. There are strong correlations between plasma PCSK9 and cardiovascular events.[12,13] PCSK9 regulates the concentration of LDL-C through a protein-protein interaction with the LDL receptor (LDLR), facilitating its degradation and reducing clearance of LDL particles.[14] Reduction of PCSK9 levels with therapeutic small inhibitory RNA, or inhibition of PCSK9-LDLR complex formation with a therapeutic mAb results in decreased plasma LDL-C concentration and improved cardiovascular outcomes.[15,16]

Although either PCSK9-directed small interfering RNA or mAb therapy can reduce LDL-C in patients with hypercholesterolemia, both types of therapy are dosed by injection. Despite years of effort,[17] identification of orally available small-molecule inhibitors of the PCSK9-LDLR interaction has proved elusive. Herein we describe the invention and clinical assessment of a macrocyclic peptide (MK-0616), reporting evidence of PCSK9 inhibition, and robust plasma LDL-C reduction in human participants dosed orally with the molecule. This work demonstrates both a novel approach to improving patient access to a powerful LDL-C-lowering mechanism and a generalizable strategy for the de novo generation of macrocyclic peptides with the potential to be oral therapeutic agents having affinity and specificity comparable to mAbs.

METHODS

Preclinical Methods

Complete chemical synthesis of MK-0616 and characterization has been described as Example 25 in patent application WO 2019/246349 Al.

Alexa Fluorescence Resonance Energy Transfer PCSK9 Binding Assay

The PCSK9 time-resolved fluorescence resonance energy transfer Alexa fluorescence resonance energy transfer Standard assay[18] measures the interaction between PCSK9 and an AlexaFluor647 (AF) tagged cyclic peptide, Reagent A (K_D = 83 nM). A solution containing 1 nM biotinylated PCSK9 + 2.5 nM Lance Streptavidin Europium (Strep-Eu) was made in 50 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) pH 7.4, 0.15 M NaCl, 5 mM $CaCl_2$, 0.01% bovine serum albumin (BSA), and 0.01% Surfactant P20. A separate solution containing 40 nM of the AF tagged cyclic peptide was made in the same buffer system. An Echo Acoustic Liquid Handler (Beckman) was used to transfer 0.750 µl of test compound to an assay plate followed by the addition of 15 µl of PCSK9+Stept-Eu and 15 µl of AF peptide. The final assay volume was 30.750 µl containing 0.5 nM PCSK9,

1.25 nM Strep-Eu, and 20 nM AF cyclic peptide. The reaction was incubated at room temperature for at least 2 hours prior to fluorescence measurements using an Envision Multilabel Reader. IC_{50} values were determined by fitting data to a sigmoidal dose-response curve using nonlinear regression. K_i was then calculated from the IC_{50} and the K_D of AF cyclic peptide (Table 1). Counts (B-counts) of the europium-labeled PCSK9 were followed to observe if compounds were adversely affecting PCSK9. A reduction in B-counts would suggest a false positive of inhibition of binding.

In Vitro Titration of PCSK9 in Human Plasma

To evaluate the in vitro potency of PCSK9 inhibitors to bind with PCSK9 in human plasma (MRL Volunteer Blood Donor Program for research; n=20 human controls), increasing concentrations (0, 0.3, 1, 3, 5, 10, 50, 100, 500, and 1000 nM) of test compounds 44[18] and MK-0616 (each starting at 0.1 mM in dimethylsulfoxide) were prepared in 150 µL pooled ethylenediaminetetraacetic acid (EDTA) anticoagulated plasma (human or cynomolgus monkey) using an HP D300 digital dispenser. The plasma samples titrated with compound 44 or MK-0616 were incubated for 30 minutes at 25°C and 50 µL aliquots of each sample were used to assess PCSK9 target engagement using the affinity capture, enzymatic digestion, and liquid chromatography with tandem mass spectrometry procedure described.

Molecular model images were created with the PyMOL Molecular Graphics System, Version 2.2.3 Schrödinger, LLC.

Passive permeability was determined according to a previously described method.[19] Briefly, MDCKII cells were cultured in 96-well transwell culture plates. The area of membrane was 0.11 cm^2. MDCKII cells were originally obtained from P. Borst, MD, The Netherlands Cancer Institute (Amsterdam, Netherlands) and used under a license agreement. Solutions with [^{3}H]MK-0616 (5 µM), [^{3}H]verapamil (1 µM) or [^{3}H]mannitol (5 µM) were prepared in Hanks balanced salt solution with 0.1% weight/volume bovine serum albumin, 10 mM HEPES (pH 7.4), 10 µM cyclosporin (to inhibit transporter activity), and 1.2 µM dextran Texas red. Substrate solution (150 µL) was added to either the apical or the basolateral compartment of the culture plate and buffer (150 µL; Hanks balanced salt solution, with 0.1% weight/volume BSA,10 mM HEPES, 10 µM cyclosporin, pH 7.4) was added to the opposite compartment. A 50 µL aliquot was taken from both sides at 3 hours, and radioactivity was determined by liquid scintillation counting in a MicroBeta Wallac Trilux scintillation counter. The experiment was performed in triplicate. A mass balance ≥70% was considered acceptable. Dextran Texas red was used as a marker of paracellular flux to confirm monolayer integrity. The passive permeability of verapamil (P_{app} = 28.5*10^{-6} cm/s) and mannitol (P_{app} = 2.1*10^{-6} cm/s) confirmed the functionality of the assay. The reported apparent permeation (P_{app}) is the average of the P_{app} for transport from apical to basolateral and P_{app} for transport from basolateral to apical at t = 3 hours and is expressed as 10^{-6} cm/s.

Table 1. In Vitro Activity for Compound 44 and MK-0616

Compound	Alexa FRET Ki (pM)	IC_{50} in human plasma (nM)
44	2	2–3
MK-0616	5	2.5

FRET indicates fluorescence resonance energy transfer. Data derived from Tucker et al.[18]

Clinical Study Methods

Both studies reported here (Merck & Co., Inc., Rahway, NJ, protocols: MK-0616-001 and -003) were approved by Independent Ethics Committee Ziekenhuisnetwerk Antwerpen, Antwerp, Belgium and conducted at SGS Belgium NV, Antwerp, Belgium. Both studies were conducted in accordance with Good Clinical Practices guidelines and the ethical principles set forth by the Declaration of Helsinki. All participants gave written informed consent.

Study Objectives, Hypotheses, and End Points

The primary objectives of these studies were evaluation of the safety and plasma pharmacokinetics of single-rising (-001) or multiple (-003) oral doses of MK-0616 in fasted, healthy (-001) and statin-treated (-003) participants. Key secondary objectives were evaluation of the effects of a meal on the plasma pharmacokinetics of a single oral dose of MK-0616 compared with the fasted state (-001); estimation and comparison of the plasma pharmacokinetics of MK-0616 formulated alone, with Labrasol, or with sodium caprate (-001); and evaluation of the percentage reduction of free PCSK9 in healthy and statin-treated participants following a single oral dose of MK-0616 (-001) or of plasma LDL-C from baseline in statin-treated participants following multiple oral doses of MK-0616 (-003).

The -001 study hypothesis was that a dose of MK-0616 with acceptable safety and tolerability results in an 80% mean (geometric mean) maximum inhibition in free PCSK9 in healthy participants. The -003 study hypothesis was that following 14 days of multiple oral doses of MK-0616, at least 1 dose with acceptable safety and tolerability results in at least a 50% true mean (geometric mean) reduction of LDL-C from baseline in statin-treated participants.

Study safety end points were adverse events (AEs), physical examination findings, laboratory safety assessments, 12-lead electrocardiogram, and vital signs. MK-0616 pharmacokinetic end points for both studies were area under the concentration (AUC) versus time curve from pre-dose to infinity ($AUC_{0-\infty}$), AUC from predose to 24 hours post-dose (AUC_{0-24}), AUC from predose to last measurement taken (AUC_{0-last}), maximum observed plasma concentration (C_{max}), plasma concentration observed 24 hours post-dose (C_{24}), apparent terminal half-life ($t_{1/2}$), and time when C_{max} was first observed (T_{max}). MK-0616 pharmacokinetic end points for -003 also included estimated accumulation ratio (R_{ac}: Day 14/Day1), the apparent total plasma clearance, and the apparent volume of distribution during the terminal phase. Apparent total plasma clearance and volume of distribution during the terminal phase were not calculated for this study. Efficacy end points were plasma concentration of free PCSK9 (for -001) and plasma LDL-C concentration (for -003).

Quantification of MK-0616

MK-0616 and a stable-isotope labeled internal standard, [$^{13}C_3$,$^2H_9$]MK-0616, were extracted from a 100 µL aliquot of human K2-EDTA plasma using an automated protein precipitation procedure with 400 µL of 0.1% formic acid in acetonitrile as the precipitation solvent. The drug and the stable-isotope labeled internal standard were chromatographed using reverse phase liquid chromatography with a Waters Acquity HSS T3 (50

x 2.1 mm, 1.8 µm) and a gradient mobile phase consisting of 2 mM NH_4F in H_2O and acetonitrile. Detection was achieved with tandem mass spectrometric detection employing a turbo ionspray interface in the positive ion mode. The multiple reaction monitoring transitions monitored were a mass-to-charge ratio of 775.5 à 761.5 for the drug and a mass-to-charge ratio of 781.6 à 767.6 for the internal standard. The lower limit of quantitation for this method is 0.5 ng/mL with a linear calibration range from 0.5 to 300 ng/mL. Intra-day percent bias found during validation ranged from −0.7–9.6% with a precision ≤4.6 (%coefficient of variation) while inter-day percent bias was 3.3–5.3% with a %coefficient of variation ≤4.7. Incurred sample reproducibility of the assay was assessed in study -001 with 127 out of 127 (100%) samples meeting the ±20% acceptance criteria.

Quantification of Free and Total-PCSK9

A validated, fit-for-purpose assay, based on the assay previously described,[18] was used to quantify plasma levels of free PCSK9 (ie, PCSK9 not bound to MK-0616). Briefly, a biotinylated analog of MK-0616 conjugated to streptavidin magnetic beads was incubated with 500 µL K2-EDTA plasma collected at baseline and at post-dose time points indicated. PCSK9 not bound by study drug in each plasma sample (free PCSK9) was captured and eluted from the beads with acid. Following neutralization, a stable-isotope labeled surrogate PCSK9 tryptic peptide (GTVSGTLIGLEIFR^; R^ = $^{13}C_6$, $^{15}N_4$) was added as an internal standard, along with a mass spectrometry-compatible surfactant, and the samples were digested overnight with trypsin and quenched the next day. The resulting signature peptides were analyzed via ultra-high performance liquid chromatography-mass spectrometry. The response ratio of endogenous PCSK9 tryptic peptide (GTVSGTLIGLEIFR) signal to internal standard signal was used to calculate the concentration of free PCSK9 in samples from a 12-point standard curve ranging from 0.00977 to 20 nmol/L (nM). The standard curve was generated using recombinant PCSK9 protein serially diluted into 5% BSA in Dulbecco's phosphate-buffered saline + 0.05% sodium azide, to which biotinylated MK-0616 streptavidin magnetic beads were added. After incubation, standard samples were processed as above in parallel with the study samples.

Similarly, a validated, fit-for-purpose assay was used to quantify total-PCSK9. Briefly, stable-isotope labeled ($^{13}C_6$, $^{15}N_4$ arginine and $^{13}C_6$, $^{15}N_2$ lysine, Promise Proteomics) internal standard recombinant PCSK9 protein was added to 100 µL of K2-EDTA plasma collected at baseline and at post-dose time points indicated, and incubated with anti-PCSK9 monoclonal antibodies ([EPR7627(2)] ab238995, Abcam) conjugated to tosylactivated magnetic beads. Endogenous PCSK9 and stable-isotope labeled internal standard PCSK9 protein in each plasma sample was captured and eluted from the beads with acid. Following neutralization, a mass spectrometry-compatible surfactant was added, and the samples were digested overnight with trypsin and quenched the next day. The resulting signature peptides were analyzed via ultra-high performance liquid chromatography-mass spectrometry. The response ratio of endogenous surrogate PCSK9 tryptic peptide (GTVSGTLIGLEIFR) signal to internal standard surrogate PCSK9 peptide (GTVSGTLIGLEIFR^; R^ = $^{13}C_6$, $^{15}N_4$) signal was used to calculate the concentration of total-PCSK9 in samples from an 8-point standard curve ranging from 0.156 to 20 nmol/L (nM). The standard curve was generated using recombinant PCSK9 protein serially diluted in 5% BSA in Dulbecco's phosphate-buffered saline + 0.05% sodium azide + stable-isotope labeled internal standard PCSK9 protein, incubated with anti-PCSK9 tosylactivated magnetic beads, and processed as above in parallel with the study samples.

Pharmacokinetic and Statistical Analyses

The population for safety analyses in both studies consisted of all participants who received at least 1 dose of investigational treatment. The populations for pharmacokinetics and pharmacodynamics analyses in both studies consisted of those participants who complied with the protocol sufficiently to ensure that generated data would be likely to exhibit the effects of treatment, according to the underlying scientific model.

For both studies, the incidence of AEs was descriptively summarized, and summary statistics and plots (as clinically appropriate) were generated for raw laboratory safety tests, electrocardiograms, and vital signs. In summary statistics, participants who were randomized to placebo more than 1 time were counted only once in the summary, but this single count included all the AEs reported for an individual.

All derived plasma pharmacokinetics parameter values were calculated using the software Phoenix WinNonlin (Version 6.3). AUCs were calculated using the linear trapezoidal method for ascending concentrations and the log trapezoidal method for descending concentrations (linear up, log down) calculation method. For each pharmacokinetic parameter, individual values of MK-0616 were natural log transformed and analyzed using a linear mixed-effects model containing a fixed effect for dose level (both studies), day (for -003: Day 1, Day 14), the interaction of dose and day (for -003), and a random effect for participant (both studies). Kenward and Roger's method was used to calculate the denominator degrees of freedom for the fixed effects. Ninety-five percent CIs for the least squares means for each treatment were constructed on the natural log scale and referenced the t-distribution. Exponentiation of the least-squares means, and lower and upper limits of these CIs, yielded estimates for the population geometric means and CIs about the geometric means on the original scale. Geometric mean ratios and 95% CIs were provided for selected comparisons using the model described above.

To test the -001 study hypothesis, the posterior probability that the true geometric mean of maximum percent target reduction (inhibition) is at least 80% was calculated for each MK-0616 dose using the estimates from the mixed model. The normality of natural log-transformed values of maximum inhibition was assumed and a noninformative prior was used. A 30% posterior probability for at least 1 dose level that also exhibited an acceptable safety and tolerability profile satisfied the pharmacodynamic hypothesis.

In study -003, percent reduction from baseline in LDL-C was evaluated using natural log-transformed fold change from baseline values, dose level by day interaction, and a random effect for patient. Least squares means and 95% CIs for each dose level and day were constructed. Exponentiating the least squares means, and lower and upper limits of these CIs yielded estimates for the population geometric means and confidence intervals about the geometric means on the original scale.

To test the -003 study hypothesis, the posterior probability that the true geometric mean of percent reduction in LDL-C at Day 14 (24-hours post-dose) is at least 50% was calculated for

each MK-0616 dose using the estimates from the linear model described above. The normality of natural log-transformed values of percent reduction was assumed and a noninformative prior was used. If this posterior probability exceeded 50% for at least 1 dose level that exhibited an acceptable safety and tolerability profile, the pharmacodynamic hypothesis was satisfied.

Study-Specific Design Details

MK-0616-001 – Single-Oral-Dose Study

This was a single-center, randomized, double-blind, placebo-controlled, single-ascending-dose clinical study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of MK-0616 in healthy adult males 18 through 50 years of age. The study was conducted between July 4, 2019, and October 23, 2020.

The study was designed with 6 panels, each with 12 participants who received a single oral dose of MK-0616 (n=9) or placebo (n=3) in a single dosing period, followed by subsequent dosing periods separated by a washout period ≥7 days. For the single-dose dose-escalation assessment of MK-0616, after a dosing group completed treatment, participants were re-randomized for the next dose, and in some cases, participants who had been randomized to placebo may have been randomized to placebo again or to active treatment, and vice versa. For this reason, the total number of participants for this trial was n=60; however the number of instances of dosing of MK-0616 is greater than 60 due to re-randomization of participants from period to period, within certain panels of the study (a trial diagram is included in Supplemental Material [Table S1]). Dose escalation beyond 100 mg did not initiate until after preliminary pharmacokinetics of doses up to 35 mg and available safety of doses up to 100 mg had been reviewed. Except as noted, all doses were administered in a fasted state. In 1 dosing group, participants received treatment following a high-fat breakfast (fat = 55.6 g, carbohydrate = 55 g, protein = 31.1 g). In another, participants received treatment approximately 30 minutes before a lower-fat breakfast (fat = 8 g, carbohydrate = 65 g, protein = 13 g). The effects of various concentrations of 2 different permeation enhancers (PEs) on the pharmacokinetics of MK-0616 (MK-0616 + Labrasol, MK-0616 + sodium caprate) were also evaluated. There was an interval of ≥4 days between dose escalations across panels and ≥7-day washout between consecutive dosing for any given participant. At each MK-0616 dose level, all available safety data through at least 24 hours post-dose were reviewed by the sponsor and the investigator before dose escalation. The decision to proceed to the next-higher dose level was based upon acceptable safety of MK-0616. Two planned groups that would have assessed MK-0616 in participants who were receiving statin therapy were not used. Not all pharmacokinetic data for every dosing group are reported here (ie, comparison of pharmacokinetics in immediate release/hard gelatin capsule versus enteric coated capsule, and varying doses of PE), but safety data from all dosing groups are included in the summary and listing of specific AEs (Table S2).

MK-0616-P003 – Multiple-Oral-Dose Study

This was a single-center, randomized, double-blind, placebo-controlled, multiple-rising-dose clinical study to evaluate the safety, pharmacokinetics, and pharmacodynamics of MK-0616 in statin-treated men and in statin-treated women of nonchild-bearing potential. The study was conducted between June 19, 2020, and January 5, 2021.

Eligible participants were 18 through 65 years of age and on a stable dose of any statin therapy for at least 2 months prior to screening, with LDL-C ≥70 and ≤160 mg/dL at screening. Participants in the group that had a meal 30 minutes before dosing had LDL-C values ≥60 and ≤160 mg/dL at screening. Participants were otherwise in good health based on laboratory safety tests.

The study was designed with 4 panels, each with 12 participants who received multiple doses of MK-0616 (n=9) or placebo (n=3). Except as indicated, all doses of MK-0616 were administered to participants in a fasted state. In 1 dosing group, participants consumed a lower-fat breakfast 30 minutes prior to study drug administration on prespecified days.

The decision to proceed from the first MK-0616 dose level (20 mg MK-0616/360 mg sodium caprate once/day) to the second dose (10 mg MK-0616/360 mg sodium caprate once/day) was based on review of all available safety data by the sponsor and investigator through 24 hours following the seventh dose of 20 mg MK-0616/360 mg sodium caprate. After dosing with 10 mg MK-0616/360 mg sodium caprate, preliminary pharmacokinetics (through 24 hours following the Day 14 dose) and all available safety data, including all available plasma LDL-C data, were reviewed prior to initiating other dosing regimens.

RESULTS

Invention and Characterization of MK-0616

MK-0616 (Figure 2) is a macrocyclic peptide designed to bind PCSK9 and inhibit its interaction with the LDLR. MK-0616 was derived from the lead compound previously reported as 44 (Figure 2) in Tucker et al[18] which was identified through mRNA display screening combined with structure-based design and iterative medicinal chemistry.[18,20] While a detailed report of the derivation of MK-0616 is out of scope here, 2 primary limitations of compound 44 were addressed. First, 44 was synthesized by an inefficient process reliant on a low-yield combination of linear solid-phase peptide synthesis and sequential orthogonal cyclization reactions in solution, limiting molecule optimization through exploration of structure/activity relationships. Second, 44 is susceptible to chemical oxidation attributable to the sulfides incorporated during post-translational macrocyclization. Our synthetic approach was redesigned to develop a novel, convergent, solution-phase, fragment-based strategy, enabling us to design freely and optimize analogs. Important modifications of compound 44 found in MK-0616 are replacement of the thiol-based linker and central triazole (Figure 2, red to teal) to minimize oxidation susceptibility (44 = 98%, MK-0616 = 2.9%) while maintaining potency (44 K_i = 2 pM, MK-0616 K_i = 5 pM), and changing the northern olefin to an amide cross-linker to increase solubility (via reduction in lipophilicity) and to avoid isomers generated from the olefin formation (Figure 2, orange to teal).

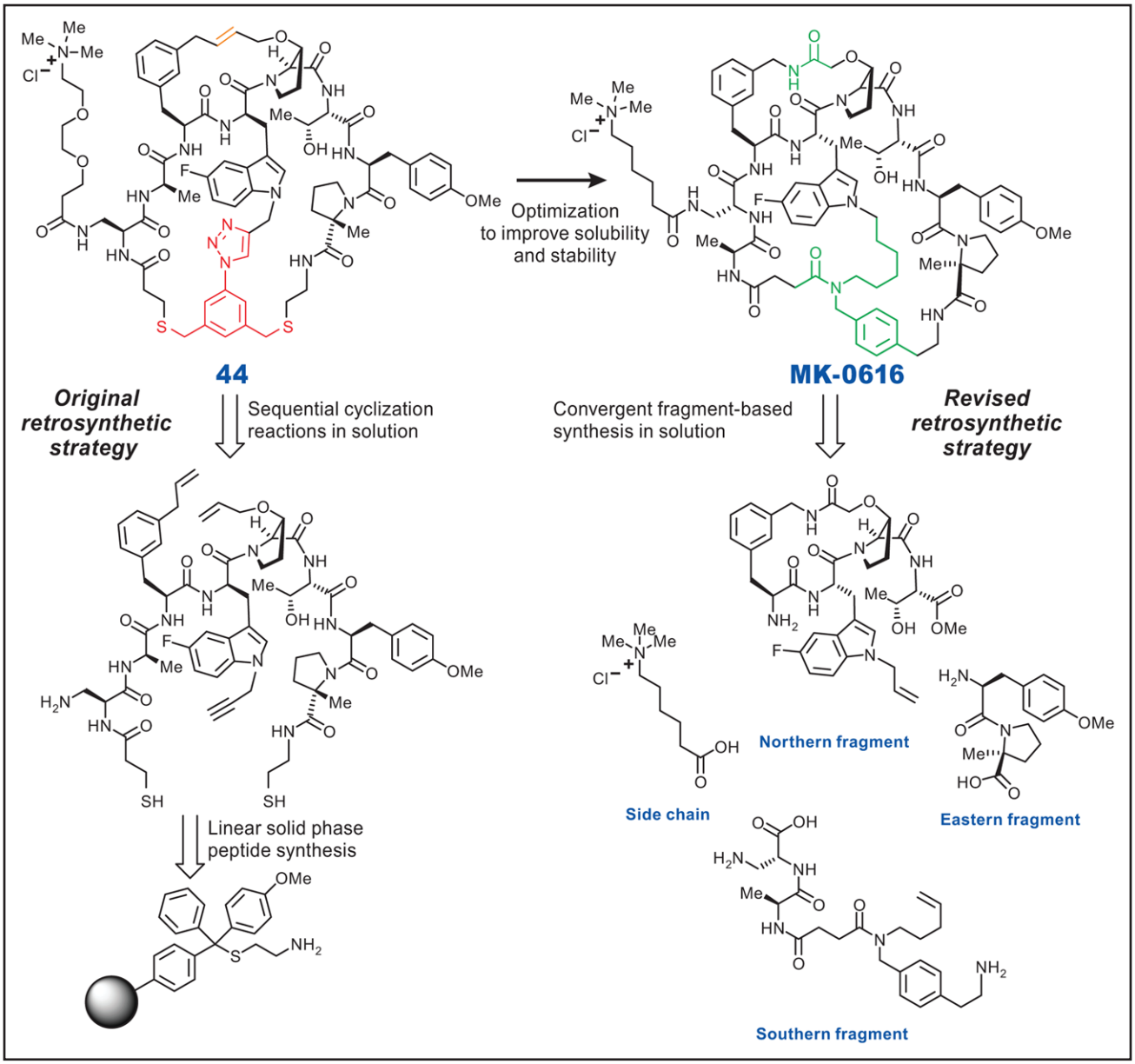

Figure 2. Redesigned synthesis and key features of MK-0616.
Critical modifications of 44 are replacement of the sulfide-based linker and central triazole (red to teal) and changing the northern olefin to an amide crosslinker (orange to teal).

Based on structural data from similar molecules,[18,20] we infer that MK-0616 binds to the LDLR binding domain of PCSK9[21] (Figure 3A-C). In human plasma it inhibited PCSK9 with an IC_{50} (2.5 ± 0.1 nM) consistent with the protein's typical plasma concentration. MK-0616 can be isolated as a stable, amorphous chloride salt with acceptable solubility (>7 mg/mL at pH7; 7.8 mg/ml in fasted-state simulated intestinal fluid).

In a passive permeability assay, the transcellular permeability (P_{app}) of MK-0616 was very low (0.71×10^{-6} cm/s). MK-0616 had no observed off-target activity when tested at a concentration of 10 μM in 122 receptor, ion channel, enzyme radioligand binding, and cellular assays in the Eurofins Cerep Panlabs screening panel. In standard assessments, MK-0616 was not genotoxic or mutagenic; it was well tolerated in safety pharmacology studies and 1-month repeat-dose toxicity studies in rats and monkeys. Similar to compound 44,[18] MK-0616 demonstrated oral bioavailability and robust reduction of free PCSK9 in cynomolgus monkeys when formulated with an established PE.

Clinical Validation of MK-0616

Single-Dose Study in Humans (Study-001)

A single-center, randomized, double-blind, placebo-controlled, single-ascending-dose clinical study in 60 healthy male participants was used to initiate evaluation

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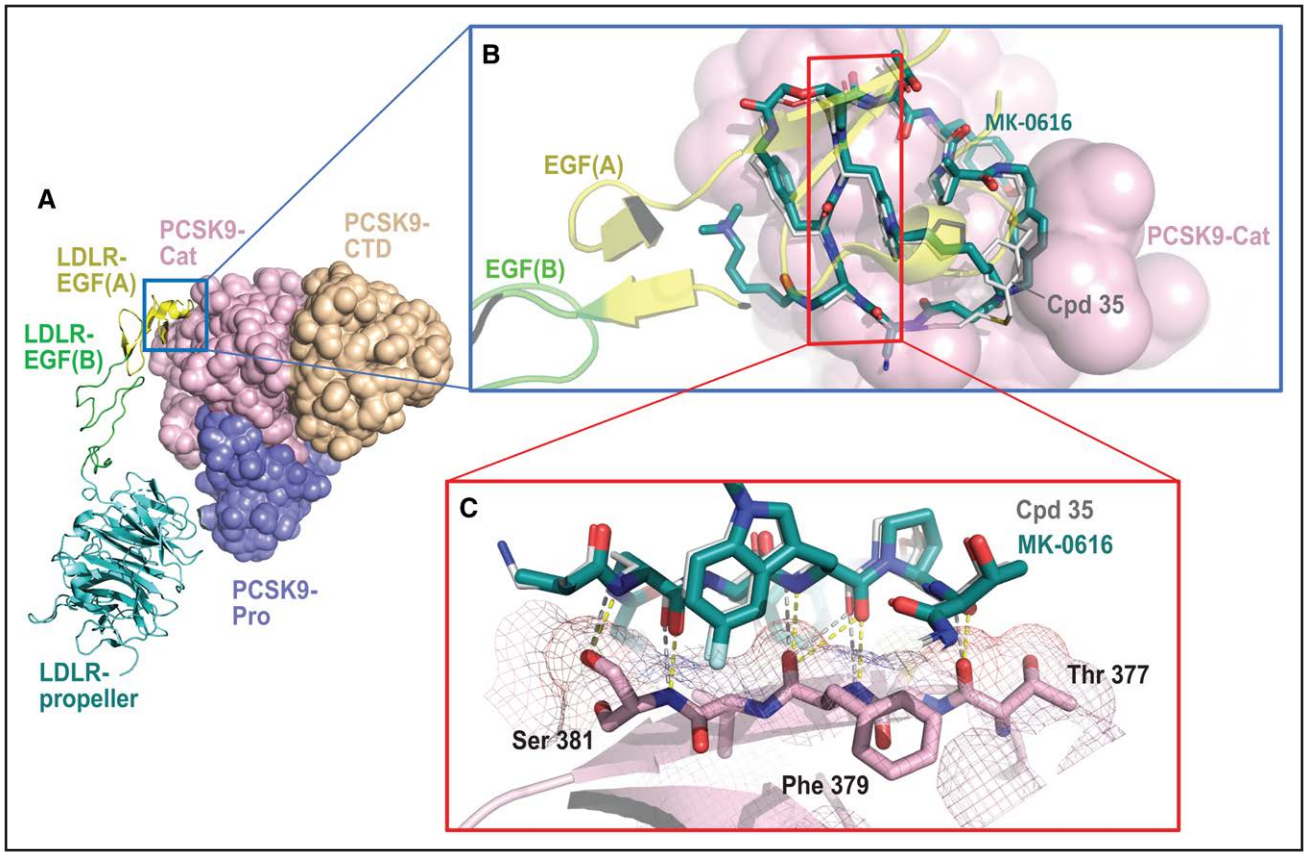

Figure 3. MK-0616-PCSK9 (proprotein convertase subtilisin/kexin type 9) complex.
A, Interaction of PCSK9 catalytic domain and prodomain with the LDLR epidermal growth factor and β-propeller domains, respectively, from PDB ID: 3P5B.[21] **B**, Modeling MK-0616 from related crystal structures (PDB IDs: 6XID, 6XIE, 75SG, and 75SH)[18,20] suggests MK-0616 (deep teal) interacts with a flat surface on the PCSK9 catalytic domain (pink surface), interrupting the interaction with the EGF(A) domain (transparent yellow) of LDLR. Compound 35 (PDB ID: 7S5H), a closely related analog of Compound 44,[18,20] is shown in white sticks, overlaid with MK-0616 to illustrate the binding site similarity. **C**, MK-0616 and Compound 35[18,20] can form key hydrogen bonds with T377, F379, and S381 of PCSK9-Cat domain. Cpd 35 indicates Compound 35; EGF, epidermal growth factor; LDLR-EGF, LDL receptor-EGF; PCSK9-Cat, PCSK9-catalytic doman; PCSK9-CTD, PCSK9-C-terminal domain; Phe 379, phenylalanine 379; Ser 381, serine 381; and Thr 377, threonine 377.

of the safety, tolerability, pharmacokinetics, and pharmacodynamics of orally dosed MK-0616. The study was designed with 6 dosing groups, each with 12 participants who received a single oral dose of MK-0616 (n=9) or placebo (n=3).

Participants

Study participants ranged in age from 19 to 50 years (mean age ± SD = 38.1 years ± 9.2; mean age across dosing panels ranged from 35.3 to 40.3 years). Participant races were American Indian or Alaska Native (n=1), Black or African American (n=1), and White (n=58). Ethnicity of participants was Hispanic or Latino (n=1), not Hispanic or Latino (n=57); and unknown (n=2).

Safety and tolerability

MK-0616 was generally well tolerated following single-dose, oral administration. Six participants discontinued study medication: 3 due to AEs, 2 due to protocol violations, and 1 voluntarily withdrew from the study. No deaths or serious AEs were reported during the study. Of the n=51 participants administered MK-0616, n=28 (54.9%) were reported to have experienced ≥1 AE; of the n=23 administered placebo, n=11 (47.8%) were reported to have experienced ≥1 AE. A listing of all AEs reported is provided in Table S2. No dose-limiting tolerability issues or dose-dependent pattern of drug-related AEs were observed. Of the AEs resulting in discontinuation from study intervention, 1 (rash maculo-papular) was assessed by the investigator to be drug related. With the exception of 1 severe AE that did not resolve by study completion (back pain, not assessed as drug related by the investigator), all AEs were transient, considered by the investigator to be mild or moderate in intensity, and resolved by study completion. There were no events of clinical interest reported. Other than LDL-C, which exhibited geometric mean reduction from baseline of up to approximately 30% at the highest single doses of MK-0616, there were no clinically meaningful trends observed as a function of dose for laboratory safety tests, vital signs, or electrocardiograms.

Pharmacokinetics

When fasted participants were dosed orally with 10 to 300 mg of MK-0616 formulated with the PE Labrasol, the increases in the area under the concentration versus time curve from pre-dose to infinity ($AUC_{0-\infty}$) and in C_{24} were less than dose proportional; the increase in C_{max} was generally dose proportional (Figure 4A and Table 2). The $t_{1/2}$ ranged from 35 hours at the lowest dose to 130 hours at the highest dose (Table 2). The apparent total plasma clearance of drug and the apparent volume of distribution during the terminal phase increased with increasing dose of MK-0616 (Table 2). The observed median collection times at T_{max} ranged from 1.50 to 2.02 hours (Table 3).

The C_{max} of a 200 mg dose of MK-0616 in the absence of 1,800 mg Labrasol was >5-fold lower compared with the same dose in the PE; the difference in $AUC_{0-\infty}$ was less pronounced (Table S3). At a 100 mg dose of MK-0616, Labrasol and sodium caprate formulations exhibited similar exposures of drug (Table S3).

The geometric mean ratios (fed/fasted) of $AUC_{0-\infty}$ and C_{max} for a 40 mg dose given 30 min after a high-fat breakfast (55.6 g fat, 55g carbohydrate, 31.1 g protein) compared with the same dose given under fasted conditions (an overnight fast of at least 8 hours duration, and no food for 4 hours post-dose) were 0.33 and 0.25, respectively; the T_{max} of the dose given after a meal was delayed compared with that of the dose given while fasting (Table S4). The geometric mean ratios (fed/fasted) of $AUC_{0-\infty}$ and C_{max} for a 40 mg dose when a lower-fat breakfast (8 g fat, 65g carbohydrate, 13 g protein) was eaten 30 min post-dose, compared with the same dose given under fasting conditions described above were 0.89 and 0.79, respectively; the T_{max} was comparable with or without a meal 30 min after dose (Table S4).

Effect on plasma PCSK9

For all doses of MK-0616 evaluated, geometric mean (95% CI) free plasma PCSK9 was reduced >93% (84, 103) from baseline (Figure 4B). With MK-0616 doses 35 mg and higher formulated with PEs, geometric mean reduction from baseline of free plasma PCSK9 >80% was maintained for >24 hours.

Multiple-dose study in humans (Study-003)

This was a single-center, randomized, double-blind, placebo-controlled, multiple-rising-dose clinical study to evaluate the safety, pharmacokinetics, and pharmacodynamics of MK-0616 in statin-treated males (n=27) and in statin-treated females of nonchildbearing potential (n=13).

Participants

Participant age ranged from 19 to 65 years (mean age ± SD = 57.7 years ± 8.5; mean age across dosing panels ranged from 56.2–60.3 years). All participants were of White race and "not Hispanic or Latino" ethnicity. The majority of study participants (85%, n=34) were being treated with a stable dose of moderate- to high-intensity statins[22] for ≥2 months before initiating study medication; 15% of participants (n=6) were taking a low-intensity statin.[22] All participants continued statin treatment throughout study participation and completed study medication; 1 withdrew from the study.

Safety and tolerability

MK-0616 was generally well tolerated during 14 days of daily oral dosing. No deaths or serious AEs were reported during the study. Of the n=31 administered MK-0616, n=21 (67.7%) were reported as having experienced 1 or more AEs during the study; of the n=9 administered placebo, n=6 (66.7%) were reported as having experienced 1 or more AEs. A listing of all AEs is provided in Table S5. In the MK-0616 treatment group, n=7 participants (22.6%) reported 1 or more AEs assessed by the investigator as drug-related (gastroesophageal reflux disease [n=2, 6.5%], and dry mouth, dyspepsia, hunger, dizziness, insomnia, and flushing [each n=1, 3.2%]); in the placebo group n=2 (22.2%) reported 1 or more AEs assessed as drug-related (decreased appetite, dizziness, and headache [each n=1, 11.1%]). All AEs were considered by the investigator to be mild or moderate in intensity. Other than LDL-C, which exhibited significant reduction from baseline during treatment with MK-0616 (Figure 5B), there were no clinically meaningful trends for laboratory safety tests, vital signs, or electrocardiograms observed.

Pharmacokinetics

On dosing Days 1 and 14, increase of AUC_{0-24} and C_{max} for doses of MK-0616 at 10 and 20 mg (formulated in sodium caprate and given under fasting conditions) were less than dose proportional (Table 3). On Day 14, the T_{max} for all fasted doses was <2 hours and the $t_{1/2}$ was 178 to 244 hours (Table 3). The accumulation ratio (Day 14/Day 1) for AUC_{0-24}, C_{max}, and C_{24} ranged from 1.32 to 2.04 for all the dose regimens (Table 3). MK-0616 steady state was achieved by the end of the first week for all dosing regimens (Figure 5A).

A low-fat breakfast given 30 minutes prior to a 10 mg MK-0616 dose reduced the AUC_{0-24}, C_{max}, and C_{24} of MK-0616 by 40-55% on dosing Days 1 and 14 and delayed the Day 14 T_{max} by approximately 6 hours compared with the same dose given under fasting conditions (Table 3).

Effects on plasma PCSK9 and LDL-C

Plasma levels of free PCSK9 across the 14 days of dosing were >90% reduced from baseline by both the 10 and 20 mg doses of MK-0616 given under fasting conditions (data not shown). Reduction of free PCSK9 was less when a 10 mg dose was given 30 minutes post-meal (data not shown). For each dose of MK-0616, the maximum reduction of free PCSK9 was observed at time points corresponding to the T_{max} on Day 1 and Day

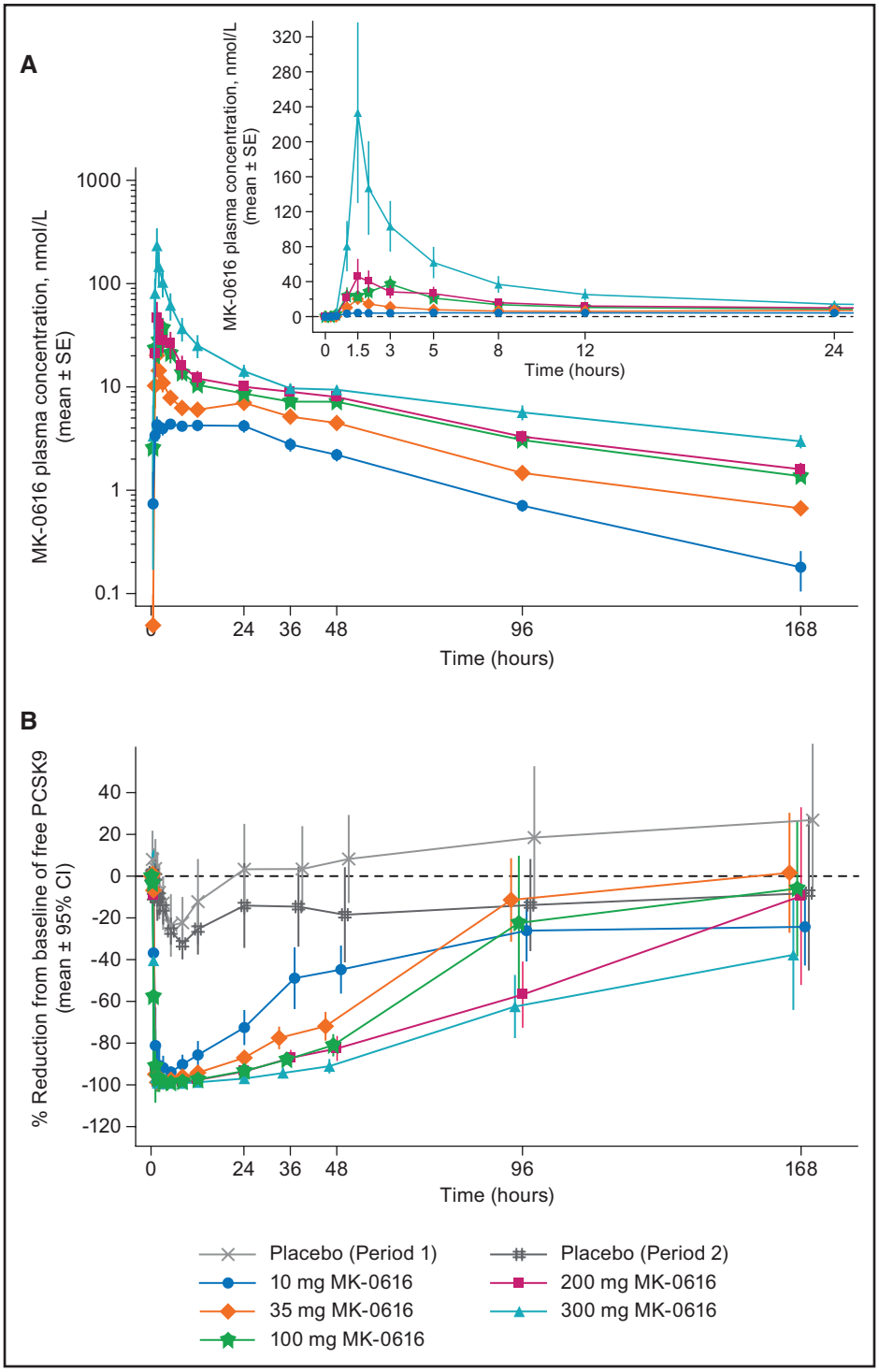

Figure 4. Single-dose pharmacokinetics and pharmacodynamics of MK-0616.
A, Mean ± SE plasma concentration-time profiles of MK-0616 (inset shows the data plotted on a semi-logarithmic scale) and (**B**) mean ± SE percentage of baseline free plasma PCSK9 (proprotein convertase subtilisin/kexin type 9)-time profiles following single oral doses of 10, 35, 100, 200, and 300 mg MK-0616 in Labrasol as indicated and, in (**B**), placebo Period 1 and placebo Period 2 as indicated. There were n=9 healthy participants per MK-0616 dose level and n=3 healthy participants in each placebo group. Randomized participants given placebo, or 10, 35, or 100 mg MK-0616 (Period 1) were re-randomized for treatment with placebo, 200, or 300 mg MK-0616 (Period 2), therefore a placebo group is shown for each period. Per the pre-specified statistical analysis of free PCSK9, as described in the Clinical Study Methods, the hypothesis that a dose of MK-0616 will result in a true geometric mean PCSK9 reduction from baseline of greater than 80% was supported for all doses of MK-0616 (10, 35, 100, 200, 300 mg; posterior probability >99%).

Table 2. Pharmacokinetic Parameter Values of MK-0616 in Healthy Participants After Single Doses of 10, 35, 100, 200, and 300 mg (n=9/Dose)*

Parameter	MK-0616, single dose (mg)				
	10 (n=9)	35 (n=9)	100 (n=9)	200 (n=9)	300 (n=9)
$AUC_{0-\infty}$, h•nmol/L†	259 (205, 328)	540 (427, 683)	1080 (852, 1360)	1260 (984, 1260)	2260 (1790, 2850)
AUC_{0-24}, h•nmol/L†	93.0 (72.1, 120)	165 (128, 213)	309 (239, 398)	339 (259, 443)	778 (603, 1000)
AUC_{0-last}, h•nmol/L†	235 (185, 297)	501 (396, 634)	1020 (805, 1290)	1170 (910, 1500)	2000 (1580, 2540)
C_{max}, nmol/L	5.21 (3.48, 7.80)	17.8 (11.9, 26.7)	46.2 (30.9, 69.0)	45.3 (29.5, 69.5)	149 (99.5, 223)
C_{24}, nmol/L‡	3.90 (3.28, 4.65)	6.98 (5.86, 8.32)	8.47 (7.11, 10.1)	9.91 (8.23, 11.9)	13.3 (11.2, 15.9)
T_{max}, h‡	1.50 (0.50, 24.08)	1.50 (1.50, 1.50)	1.50 (1.00, 3.00)	2.02 (1.07, 5.00)	1.50 (1.00, 1.98)
$t_{1/2}$, h§	35.14 (17.5)	42.86 (5.8)	81.52 (16.9)	95.47 (27.7)	129.95 (40.5)
CL/F, L/h§	24.98 (40.5)	42.96 (26.7)	59.83 (29.9)	103.17 (34.3)	84.64 (52.2)
Vz/F, L§	1266.52 (39.4)	2656.39 (26.0)	7037.04 (24.0)	14209.96 (42.3)	15868.31 (65.9)

Values shown are geometric mean (95% confidence interval) unless otherwise indicated.

$AUC_{0-\infty}$ indicates area under the concentration versus time curve from pre-dose to infinity; AUC_{0-24}, area under the curve from pre-dose to 24 hours post-dose; AUC_{0-last}, area under the curve from pre-dose to last measurement taken; C_{24}, plasma concentration observed 24 hours post-dose; CL/F, apparent total plasma clearance; C_{max}, maximum observed plasma concentration; $t_{1/2}$, apparent terminal half-life; T_{max}, time when C_{max} was first observed; and Vz/F, apparent volume of distribution during the terminal phase.

*All doses were formulated with 1800 mg Labrasol.

†Back-transformed least squares mean and 95% confidence interval from linear mixed-effects model performed on natural log-transformed values.

‡Median (min, max).

§Geometric mean and percent geometric CV.

14. Plasma levels of total PCSK9 were increased during the 14 day dosing period. The greatest increase in total PCSK9 was observed at the 20 mg dose, where plasma levels were increased by 122 ± 45% from baseline (mean±SEM) 24 hours after the 14th dose.

Both 10 and 20 mg once-daily dosing of MK-0616 administered under fasting conditions were associated with continuous, gradual reductions of plasma LDL-C during the 14-day dosing period (Figure 5B). The geometric mean percent reductions from baseline in LDL-C of 58.2% (95% CI, 41.1–82.6) ± 8% and 60.5% (95% CI, 42.9–85.3) for 10 and 20 mg daily doses, respectively, were observed by Day 14 of dosing. Dosing of 10 mg MK-0616 30 minutes after a standard meal resulted in a geometric mean percent reduction from baseline in LDL-C of 11.6% (95% CI, 7.5–17.8) at Day 14 of dosing. Dosing of placebo resulted in a geometric mean percent reduction from baseline in LDL-C of 6.9% (95% CI, 4.7–10.2) at Day 14 of dosing.

DISCUSSION

Here we describe the invention of a macrocyclic peptide (MK-0616) that has a high affinity for the LDLR-binding domain of PCSK9 and displays oral absorption in human participants. The clinical data we report indicate that a single oral dose of MK-0616 should inhibit the interaction of plasma PCSK9 and the LDLR for >24 hours, and that a multiple-oral-dose regimen lasting 14 days provides clinically meaningful reduction from baseline in LDL-C in participants being treated with intermediate- or high-intensity statin. These data provide support for further development of MK-0616 as a novel oral therapy for atherosclerotic cardiovascular disease, a leading cause of morbidity and mortality globally.[11] In addition, the data provide proof of concept for the identification and development of molecules that can disrupt other, previously undruggable pathophysiological protein-protein complexes.

While significant improvements in the optimization of traditional oral small-molecule drug candidates have led to reduced attrition in clinical studies due to poor pharmacokinetics,[23] the translation of these learnings to beyond Rule of Five molecules like MK-0616 remains challenging. Generally, oral absorption of therapeutic molecules requires properties described by Lipinski's Rule of Five,[24] facilitating passive, transcellular permeability. While these rules are not immutable (cyclosporine, which is cell permeable, breaks most of them), the beyond Rule of Five macrocyclic peptides developed from mRNA display leads, and as exemplified by MK-0616 are not cell permeable. In preclinical studies, oral bioavailability required coformulation with a PE like the medium-chain fatty acid sodium caprate, allowing paracellular absorption of these

Table 3. Pharmacokinetic Parameter Values of MK-0616 on Days 1 and 14 of Daily Doses of 10 (n=8) and 20 (n=9) mg Under Fasting Conditions and 10 mg 30 Minutes After a Meal (n=6)*

Parameter	MK-0616, multiple daily doses (mg)		
	10, fasted (n=8)	20, fasted (n=9)	10, post-meal (n=6)
Day 1			
$AUC_{0\text{-}24}$, h•nmol/L†	122 (91.8, 162)	142 (108, 185)	55.9 (40.2, 77.6)
C_{max}, nmol/L†	7.05 (5.15, 9.65)	7.87 (5.85, 10.6)	3.29 (2.29, 4.72)
C_{24}, nmol/L†	5.30 (3.96, 7.10)	6.13 (4.65, 8.08)	2.02 (1.44, 2.83)
Day 14			
$AUC_{0\text{-}24}$, h•nmol/L†	178 (134, 236)	280 (211, 371)	106 (76.6, 148)
C_{max}, nmol/L†	10.6 (7.78, 14.6)	16.1 (11.8, 21.9)	5.28 (3.67, 7.58)
C_{24}, nmol/L‡	7.01 (5.24, 9.40)	11.1 (8.32, 14.8)	4.06 (2.90, 5.69)
T_{max}, h‡	1.3 (0.50, 5.02)	1.8 (1.00, 5.00)	8.0 (2.00, 8.03)
$t_{1/2}$,h§	242 (32.1)	244 (22.9)	178 (60.2)
Accumulation ratio (Day 14/Day 1)			
$AUC_{0\text{-}24}$, h•nmol/L‖	1.46 (1.13, 1.88)	1.97 (1.54, 2.54)	1.90 (1.42, 2.55)
C_{max}, nmol/L‖	1.51 (1.15, 1.99)	2.04 (1.56, 2.68)	1.60 (1.17, 2.21)
C_{24}, nmol/L‖	1.32 (1.03, 1.70)	1.81 (1.42, 2.31)	2.01 (1.51, 2.67)

Values shown are geometric mean (95% confidence interval) unless otherwise indicated.

$AUC_{0\text{-}24}$ indicates area under the concentration versus time curve from predose to 24 hours post-dose; C_{24}, plasma concentration observed 24 hours post-dose; C_{max}, maximum observed plasma concentration; $t_{1/2}$, apparent terminal half-life; and T_{max}, time when C_{max} was first observed.

*All doses were formulated with 360 mg sodium caprate.

†Back-transformed least squares mean and 95% confidence interval from linear mixed-effects model performed on natural log-transformed values.

‡Median (min, max).

§Geometric mean and percent geometric CV.

‖Back-transformed least squares mean difference and 90% confidence interval from linear mixed-effects model performed on natural log-transformed values.

peptides despite the presence of potentially labile peptide bonds and their relatively large size. PEs afford the absorption of cell-impermeable compounds by promoting size-limited passage through tight junctions between intestinal epithelial cells.[3] The mechanism as to how caprate modulates the opening of tight junctions to enable paracellular absorption of molecules that are otherwise impermeable has been studied.[25–27] Coformulation with a PE takes advantage of the paracellular mode of absorption allowing for oral delivery of compounds that are not passively permeable. In this work there were 3 features identified as prerequisites for co-formulation with PEs. In order for macrocyclic peptides to pass through tight junctions they needed to be (1) stable to proteases present in the gastrointestinal tract; (2) soluble in the gastrointestinal tract; and (3) of a macrocyclic peptide size that allows passage through tight junctions. The work reported here demonstrates that the use of a PE can sufficiently enhance human oral bioavailability of a beyond Rule of Five macrocyclic peptide generated from mRNA display to make it clinically effective as a once-daily oral 10 mg dose, without requiring extensive chemical modification to enable cellular permeability.

There can be a significant advantage to a molecule that cannot passively diffuse into cells. Once out of the gastrointestinal tract, the molecule is confined to extracellular space due to inherently poor cell permeability. This precludes many possible unintended interactions, increasing the likelihood of the molecule to have an acceptable safety profile. Consistent with this expectation, MK-0616 was well tolerated in human participants at all doses and durations of treatment evaluated. The overall incidence of AEs in the MK-0616 groups in both studies reported here were similar to the placebo groups. No dose- or duration-related patterns of AEs were noted in either study. However, it should be noted that our approach, while ideal for an extracellular target, would not directly apply to intracellular targets given limited inherent cell permeability.

In the single-rising-dose study (-001), all doses of MK-0616 were associated with a >90% geometric mean reduction of free plasma PCSK9 from baseline, supporting the primary study hypothesis. Absorption of MK-0616 was rapid, while elimination of the single-dose pharmacokinetics profile was biphasic, with an initial, rapid clearance phase followed by a slower terminal phase. The rapid clearance is thought to be due to excretion of unbound MK-0616, while the slower terminal phase likely represents MK-0616 bound to PCSK9. The kinetics of the clearance of bound MK-0616 could, in part, explain the lack of dose proportionality of $AUC_{0\text{-}\infty}$ and C_{24}, despite the C_{max} being relatively dose proportional. The oral bioavailability of MK-0616 formulated with a PE was estimated to be approximately 2%, based upon the plasma exposure achieved at a given oral dose. This estimated oral bioavailability is similar to the absolute oral bioavailability observed during preclinical studies with nonhuman primates.

In Study-001, there was measurable MK-0616 in the plasma of participants given a single 200 mg dose of the drug in the absence of a PE, but overall exposure increased 2- to 3-fold when the same dose was given with either sodium caprate or Labrasol. The PEs also increased MK-0616 C_{max} and decreased T_{max}. Together the effects of a PE reduce the daily dose that would be required for maximum PCSK9 engagement across 24 hours and provide a more desirable 24-hour pharmacokinetics profile.

In Study-003, once-daily doses of MK-0616 for 14 days resulted in a maximum mean reduction from baseline plasma LDL-C of ≈66%, supporting the primary

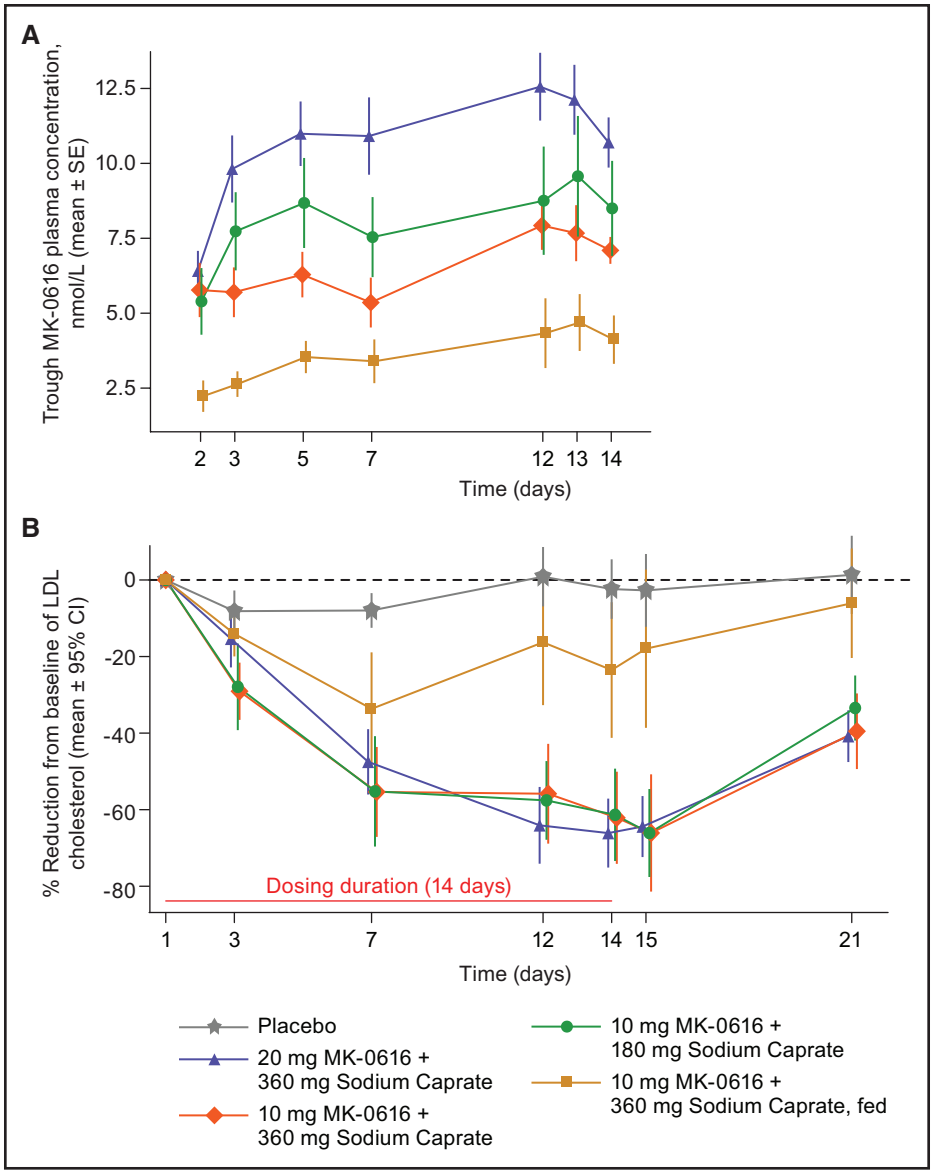

Figure 5. Multiple-dose pharmacokinetics and pharmacodynamics of MK-0616.
A, Mean ± SE trough plasma concentration-time profiles of MK-0616 and (**B**) geometric mean percentage (95% CI) of baseline LDL-C-time profiles during and after multiple, oral doses (once daily for 14 days) of MK-0616 at 20 mg (n=9 [pharmacokinetics], n=8 [pharmacodynamics] participants) or 10 mg (n=8) under fasting conditions, or 10 mg administered 30 minutes post-meal (n=6 [pharmacokinetics], n=5 [pharmacodynamics]) as indicated, and, in (**B**), placebo (n=9) as indicated. Per the pre-specified statistical analysis of LDL-C, as described in the Clinical Study Methods, the hypothesis that the true geometric mean LDL-C reduction from baseline was greater than 50% was supported for all doses of MK-0616 (posterior probability >50%), except for the dose administered under fed conditions (posterior probability <1%).

study hypothesis. In the absence of a lower-dosage form of MK-0616, the observation of a negative food effect in Study-001 provided the opportunity to titrate plasma MK-0616 and establish a sub-maximal effect on LDL-C. When 10 mg of MK-0616 was dosed 30 minutes following a standard breakfast, post-meal exposure was sufficiently low to compromise its effect on LDL-C.

All MK-0616 doses evaluated in Study -003 were associated with approximately 2-fold plasma accumulation, reaching an apparent steady state after 1 week. The reduction in LDL-C was gradual, declining steadily throughout the treatment period, in contrast to the rapid reduction of free PCSK9 observed after a single dose. The kinetics of the observed reduction in LDL-C are similar to those reported for mAbs that inhibit PCSK9-LDLR interaction.[28] These kinetics are somewhat expected, given that the effect of MK-0616 on PCSK9 is direct, while its effect on LDL-C is indirect, requiring first the establishment of a new equilibrium of LDLR concentration on cells after PCSK9 is inhibited and the subsequent establishment of a second new equilibrium in the rate of absorption of LDL-C. While the reduction in plasma LDL-C seemed to approach a plateau by Day 14,

it is possible that some additional LDL-C reduction would occur with longer-term dosing.

Study-003 was performed with participants taking statins to control plasma LDL-C. Statins stimulate expression of the PCSK9 gene,[29] and multiple statin clinical trials have demonstrated an increase in plasma PCSK9.[30] Therefore, it was important to evaluate the effect of MK-0616 on LDL-C under conditions in which circulating levels of PCSK9 were increased. The ~66% maximum reduction in LDL-C observed under such conditions indicates not only the efficacy of this therapy under the most challenging conditions, but also suggests that such a treatment could be provided in addition to current standard of care treatment for hypercholesterolemia.

The strengths of the Phase 1 clinical trials reported here include the wide range of single doses of MK-0616 evaluated, including doses that are higher than expected clinically relevant doses. Study limitations are the relatively small number of participants in each study, the relatively short duration of dosing, and the relative racial/ethnic homogeneity of the study population, which highlight the need for confirmation in larger scale clinical trials.

In summary, the work reported here demonstrates the de novo invention of a novel macrocyclic peptide capable of achieving the potency and selectivity of an antibody in a form that can be taken orally. This charts the course for and validates the use of mRNA display technology as a major advance in the medicinal chemistry underlying drug discovery. As an oral inhibitor of PCSK9, MK-0616 exhibited robust lowering of LDL-C when added to statin treatment. As such, MK-0616 could be a highly effective cholesterol-lowering therapy for patients who require LDL-C reduction. MK-0616 has the potential to overcome numerous barriers to treatment, allowing more patients to achieve LDL-C goals and reduce cardiovascular risk earlier in their treatment journey. While encouraging, the safety and efficacy of MK-0616 requires confirmation and more comprehensive evaluation in larger-scale Phase 2[31] and Phase 3 trials.

ARTICLE INFORMATION

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Disclosures

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Supplemental Material

Tables S1–S5

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