The development of the oral macrocyclic peptide enlicitide (MK-0616), for the treatment of hypercholesterolemia

Doug Johns, PhD, FAHA
Clinical Director, Translational Medicine
Merck & Co., Inc. Rahway NJ USA

CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
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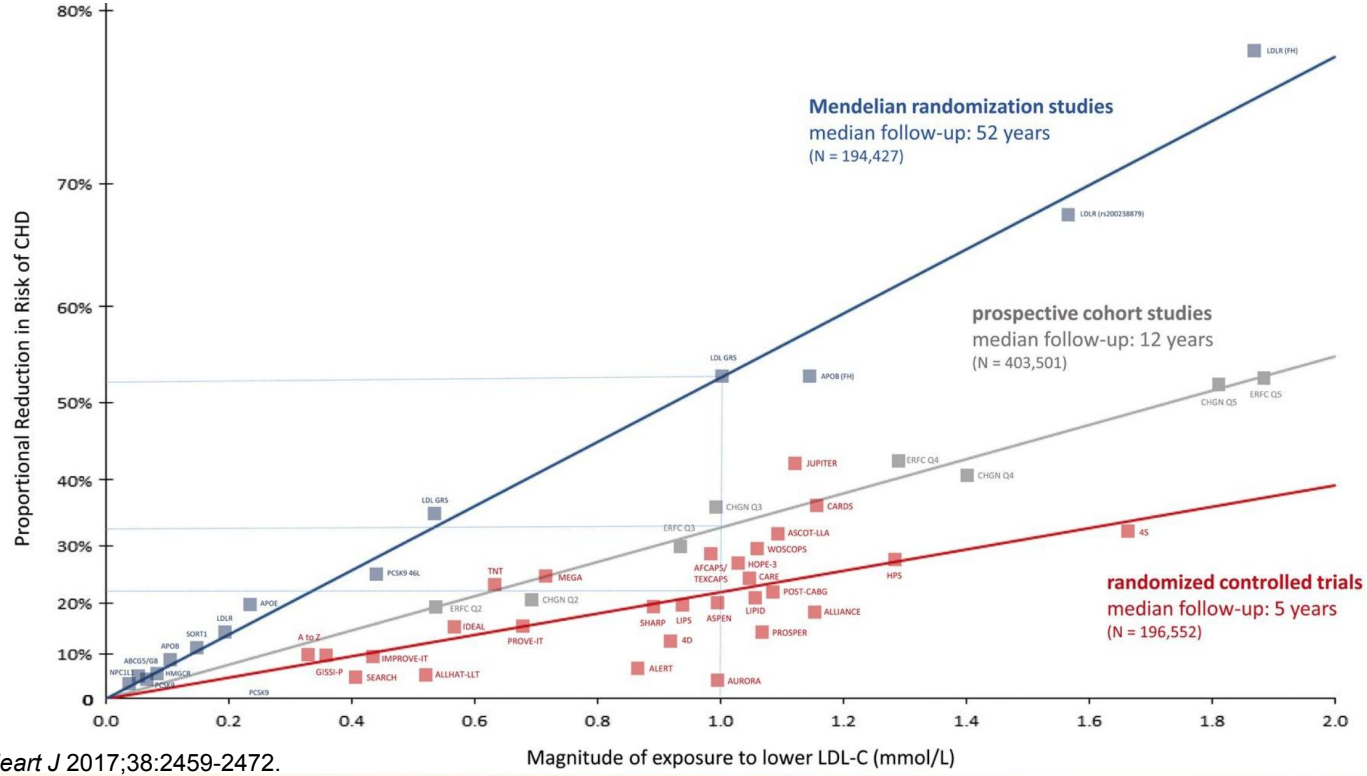
NEXT-GENERATION DELIVERY INNOVATIONS

LDL-C causes atherosclerosis; lowering it reduces risk of CHD



Ference et al, *Eur Heart J* 2017;38:2459-2472.

PCSK9 (Proprotein convertase subtilisin/kexin type 9) as a therapeutic target

Out of **~165 million** treated, **~60 million** patients not at LDL-C goal (US)

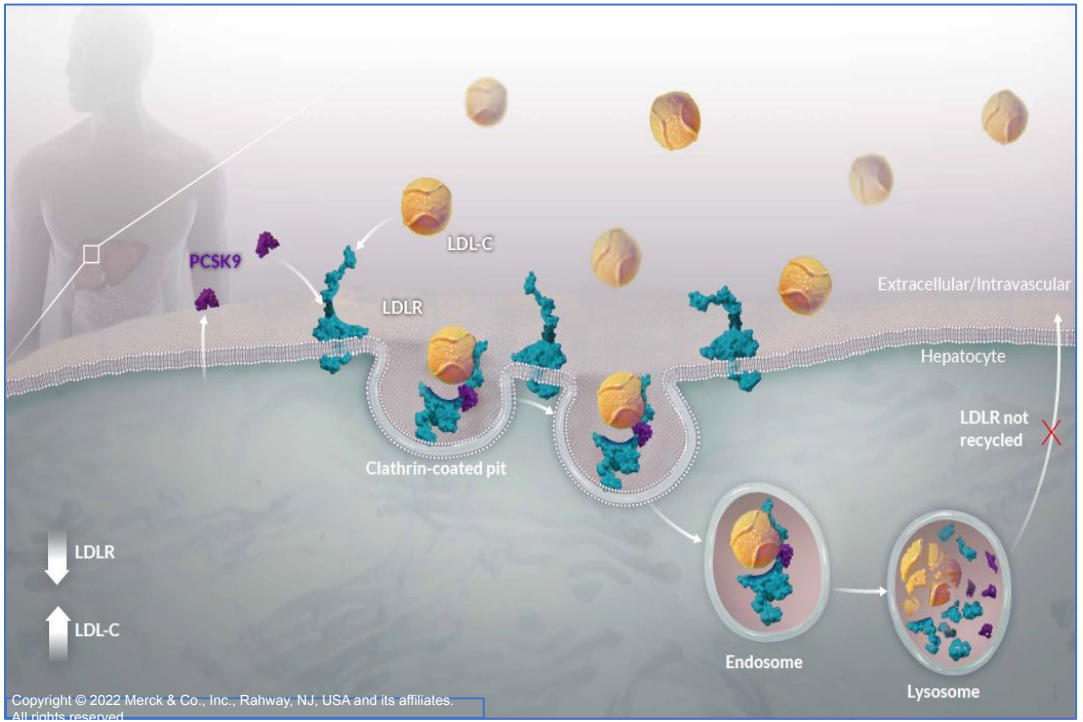
PCSK9 Genetic evidence

- Loss-of-function mutants

↓LDL-C, ↓CV risk

- Gain-of-function mutants

↑LDL-C, ↑CV risk



1. Lestavel-Dellatre et al. *Metabolism* 1994;43:397. Varret et al. *Am J Hum Genet 1999;*64:1378. Hunt et al. *Arterioscler. Thromb. Vasc. Biol.* 2000;20: 1089. 2. Abifadel et al. *Nat Genetic 2003;*34:154. 3. Cohen et al. *Nat Genet*. 2005;37:161 . 4. Cohen et al. *N Engl J Med 2006;*354:1264.

PCSK9 inhibitors lower LDL-C and reduce cardiovascular risk

Advancements in drugging PCSK9: 3 injectable agents approved

2015. Two monoclonal antibody inhibitors for PCSK9 are approved for subcutaneous injection: Regeneron and Sanofi's PRALUENT® (alirocumab) and Amgen's REPATHA® (evolocumab).

Praluent® (alirocumab) Injection 75mg/mL 150mg/mL

Repatha® (evolocumab) injection 140 mg/mL

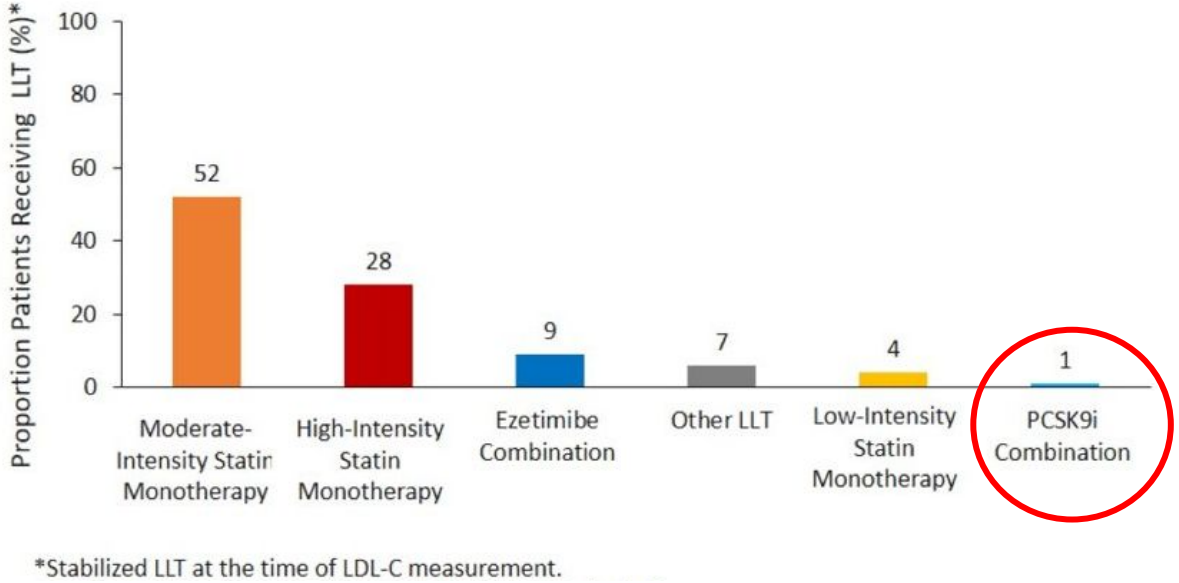
- **~60% ↓ LDL-C**
- **~15% ↓ cardiovascular events (MI, stroke, CV death)**
- **~52% ↓ LDL-C**

Studies to demonstrate reduction in

2021. An siRNA inhibitor of PCSK9 is approved: Novartis' LEQVIO® (inclisiran).

LEQVIO® (inclisiran) injection 284 mg/1.5 mL

Despite being highly effective, PCSK9 inhibitors are underutilized



*Stabilized LLT at the time of LDL-C measurement.

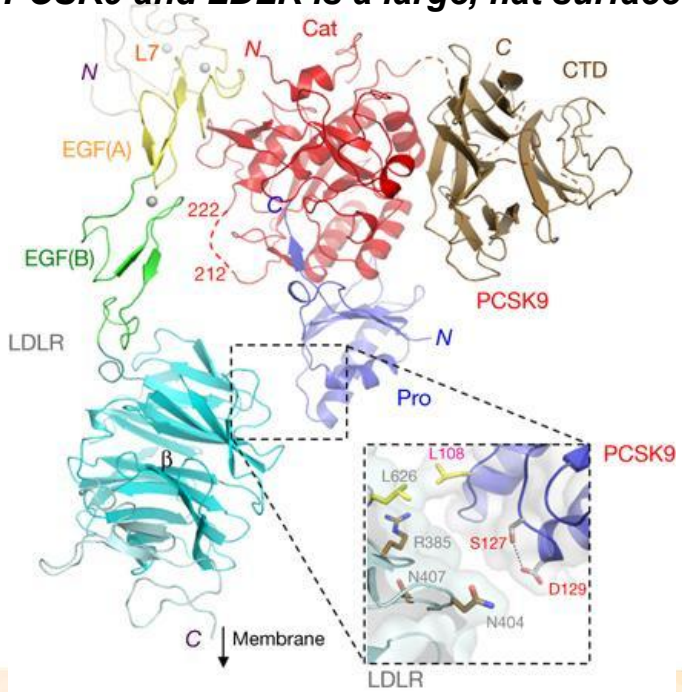
Barriers to use

- Cost
- Payer restrictions
- Clinical inertia
- Injectables only

Ray et al. *Eur J Prev Cardiol.* 2021;28:1279-1289

Why is it difficult to develop an oral inhibitor of PCSK9?

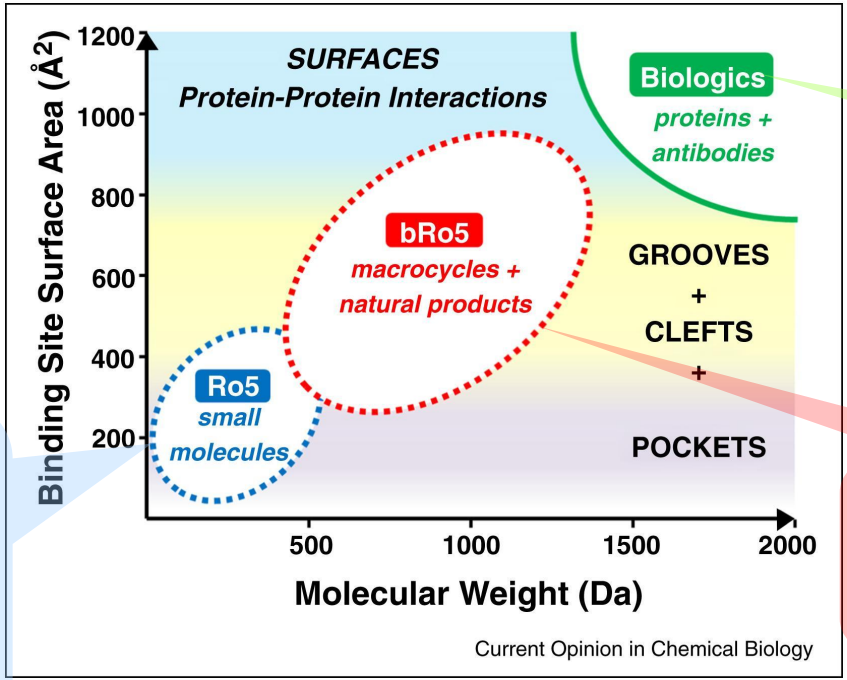
The protein-protein interaction between PCSK9 and LDLR is a large, flat surface



- PCSK9:LDLR interaction buries 500 Å^2 of solvent accessible surface
- Traditionally more suitable to disrupt a PPI with a large biologic
- **Therapeutic Concept**: *Develop a small molecule PCSK9 inhibitor that can be taken orally and that provides robust lowering of LDL-C and broad access to patients with elevated LDL-C*

Lo Surdo et al. EMBO Rep. 2011;*12*:1300

Cyclic peptides offer an opportunity to interrogate “undruggable” targets



Membrane permeant

- Size < 500 MW
- Polarity:
  < 5 hydrogen bond donors
  < 10 hydrogen bond acceptors
  < 5 cLogP (lipophilicity)
- Increased potential for good ADME

- **Bind to large surface areas**
- High affinity and selectivity
- Good safety profiles

Share properties of both small molecules and biologics

Naylor et al. *Current opinion in chemical biology* 2017;38;141-147

Cyclic peptides as therapeutic agents

Pros	Cons
• High target affinity and selectivity • Good safety profiles • Constrained conformation • New platforms to prepare novel peptide libraries	• Poor physical properties limit oral absorption • Proteolytic and transporter liabilities • Limited access to unnatural amino acids • Scalable syntheses

J Nat Prod, 2011, 74(3), 496-511
J Am Chem Soc, 2010, 132, 9259

Brief history of therapeutic peptides

- About 40 cyclic peptide therapeutics, primarily derived from natural products, have been approved
- “Natural products may not provide enough versatility for all inheritable human disease”

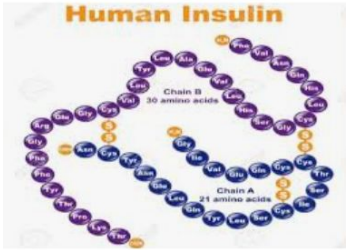
Insulin

Source: Natural product

Indication: Diabetes

Isolation/Approval: 1920s, 1982

ROA: injection



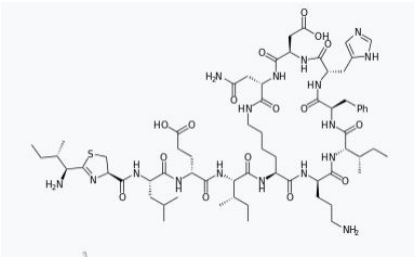
Bacitracin

Source: Natural product

Indication: Antibiotic

Isolation/Approval: 1943 , 1962

ROA: injection, topical



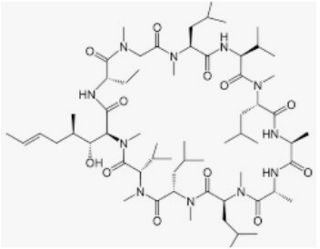
Cyclosporine

Source: Natural product

Indication: organ rejection

Isolation/Approval: 1969, 2000

ROA: oral



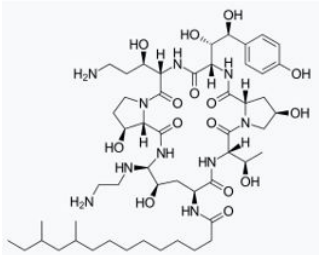
Caspofungin

Source: Natural product

Indication: Antifungal

Isolation/Approval: 1985, 2001

ROA: injection



J Nat Prod. 2011 March 25; 74(3): 496–511.
Dancik V, Seiler KP, Young DW, Schreiber SL, Clemons PL. J Am Chem Soc. 2010; 132:9259-

Using cellular machinery to

Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 12297–12302, November 1997
Biochemistry

RNA-peptide fusions for the *in vitro* selection of peptides and proteins

RICHARD W. ROBERTS* AND JACK W. SZOSTAK†

Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

Edited by Thomas Maniatis, Harvard University, Cambridge, MA, and approved September 2, 1997 (received for review July 25, 1997)

ABSTRACT **Covalent fusions between an mRNA and the peptide or protein that it encodes can be generated by *in vitro* translation of synthetic mRNAs that carry puromycin, a peptidyl acceptor antibiotic, at their 3′ end. The stable linkage between the informational (nucleic acid) and functional (peptide) domains of the resulting joint molecules allows a specific mRNA to be enriched from a complex mixture of mRNAs based on the properties of its encoded peptide. Fusions between a synthetic mRNA and its encoded *myc* epitope peptide have been enriched from a pool of random sequence mRNA-peptide fusions by immunoprecipitation. Covalent RNA-peptide fusions should provide an additional route to the *in vitro* selection and directed evolution of proteins.**

In vitro selection experiments using RNA and DNA have shown that nucleic acid molecules with specific molecular recognition and catalytic properties can be isolated from complex pools of random sequences by repeated rounds of selection and amplification (for reviews see refs. 1–5). Directed *in vitro* evolution, in which mutagenic amplification is combined with continued selective pressure, has been widely used to select for nucleic acids with altered or improved binding or catalytic properties (e.g., refs. 6 and 7). Because proteins carry out a wider range of structural and catalytic roles in biology and are much more extensively used in diagnostic, therapeutic, and industrial applications, great interest has been generated in the development of methods for the *in vitro* selection and directed evolution of proteins. The main barrier to the development of effective methods for protein evolution has been the difficulty of recovering the information encoding a protein sequence after the protein has been translated. Until recently, most approaches to this problem have involved a step in which the DNA is transcribed and translated *in vivo*, and the resulting protein is expressed in such a way as to remain physically linked to the encoding nucleic acid, which then is recovered for amplification and further selection. Examples of such approaches include phage display (ref. 8 and recently reviewed in refs. 9 and 10), plasmid display (11), and completely *in vivo* genetic approaches (12–14). In general, such approaches are limited by the complexity of the sequence libraries that can be generated; for example, phage display libraries typically are limited by transfection efficiency to less than 10^9 independent members.

More recently, selection schemes based on the display of the nascent peptide chain on the surface of the ribosome have been developed (15–17). This approach has the advantage of being fully *in vitro* and potentially allowing larger libraries ($>10^{15}$) to be explored; however, selections must be performed under conditions that preserve the integrity of the ribosome·mRNA·peptide ternary complex.

We sought to develop a simpler and more robust system in which an mRNA would become directly attached to the peptide or protein it encodes by a stable covalent linkage. We reasoned that because both the message and the adapters in protein synthesis are nucleic acids, it might be possible to generate an RNA that could act as both, i.e., a chimeric message-adapter (Fig. 1*A*). When translated, the adapter end would enter the ribosome and accept the nascent peptide (Fig. 1*B*) to generate a covalent mRNA-peptide fusion (Fig. 1*C*). In this way, the sequence information present in the peptide would be encoded in the covalently attached mRNA. This joint molecule then could act as a molecular Rosetta stone, allowing the unreadable information in the protein portion to be read and recovered via the attached mRNA.

The use of an mRNA with a charged tRNA linked to its 3′ end could, in principle, lead to the synthesis of the desired mRNA-peptide fusion molecules during translation; however, the resulting linkage would be an unstable aminoacyl ester. Puromycin, an antibiotic that mimics the aminoacyl end of tRNA, acts as a translation inhibitor by entering the ribosomal A site and accepting the nascent peptide as a result of the peptidyl transferase activity of the ribosome (18, 19). The resulting peptidyl-puromycin molecule contains a stable amide linkage between the peptide and the *O*-methyl tyrosine portion of the puromycin. The *O*-methyl tyrosine is, in turn, linked by a stable amide bond to the 3′-amino group of the modified adenosine portion of puromycin. Thus a synthetic mRNA with puromycin at its 3′ end would be expected to generate stable mRNA-peptide fusions.

MATERIALS AND METHODS

Synthesis of Controlled Pore Glass (CPG)-Puromycin. Puromycin·(HCl)$_2$ was converted to the free base by dissolution in H_2O, mixing with basic carbonate buffer, and extraction into chloroform. *N*-trifluoroacetyl puromycin was made by mixing the dried free base in 50/50 (vol/vol) dry pyridine (Fluka)/acetonitrile (Millipore) with an excess of trifluoroacetic anhydride (Fluka) for 1 hr at 25°C followed by workup with dilute ammonium hydroxide. *N*-trifluoroacetyl 5′-dimethoxytrityl (DMT) puromycin was made using DMT-Cl (Sigma) (20), and attached to an aminohexyl CPG (Sigma) support via the 2′OH using the standard protocol for attachment of DNA through its 3′OH (21) with the exception that the coupling step was carried out in the presence of ≈50 μmol activated puromycin per gram of CPG.

Synthesis of 3′ Puromycin Oligonucleotides and Templates. CPG-puromycin was used as a solid support for automated synthesis of 30-P (5′-dA$_{25}$dC$_5$CP) and 43-P (the RNA sequence 5′-GGAGGACGAAAUG linked to 30-P) according

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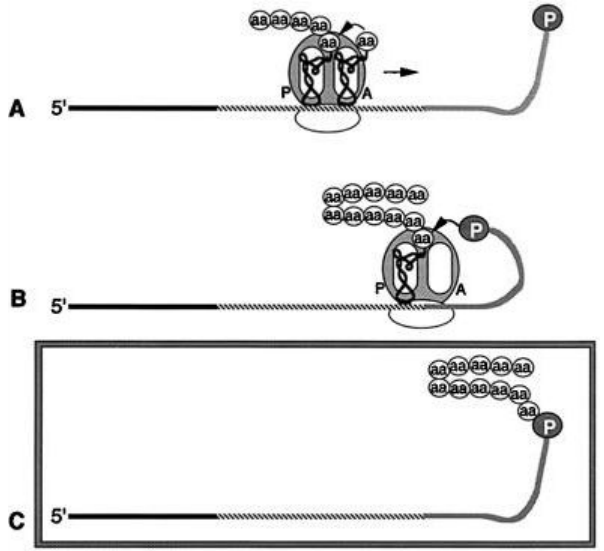
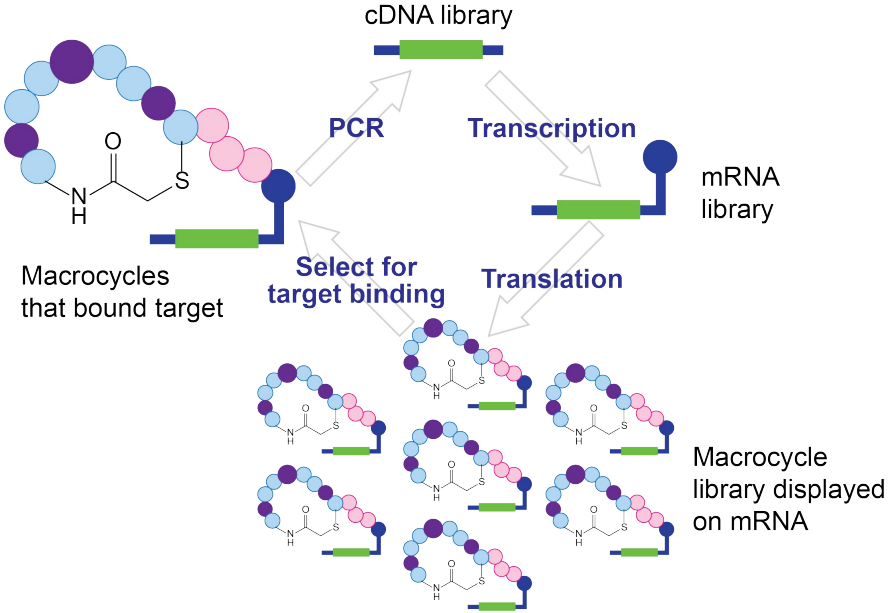
This paper was submitted directly (Track II) to the *Proceedings* office.
Abbreviations: CPG, controlled pore glass.
*Present address: Department of Chemistry, California Institute of Technology, Mail Code 147-75CH, Pasadena, CA 91125.
†To whom reprint requests should be addressed at: Department of Molecular Biology, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114-2696. e-mail: szostak@molbio.mgh.harvard.edu.

Jack Szostak, Harvard Medical School and Mass General Hosp, Boston, MA



RNA-peptide fusion

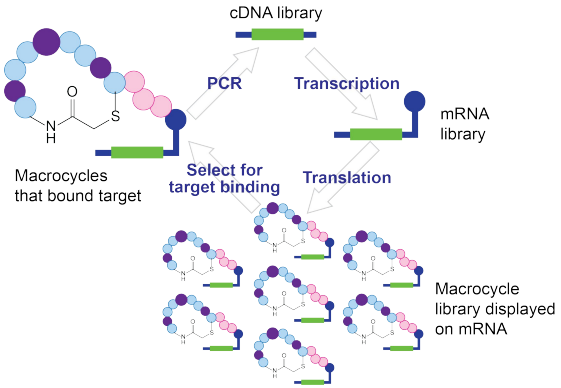
mRNA display selection



- Affinity-based selection platform enabling screening of ultra-large libraries (10^{14})
- Platform permits inclusion of unnatural amino-acids (N-Me, D-amino acids, heterocycles) to create cyclic peptides with improved properties

- Cyclic peptides are genetically encoded by a randomized DNA library, which is transcribed to mRNA library
- Each mRNA is ribosomally translated into a peptide and that peptide is linked to the encoding mRNA in the process (barcoded)
- The barcoded library is screened against an immobilized target, binders are eluted, amplified, sequenced by NGS, and the process is repeated for 6-7 rounds, “enriching” for high-affinity binders

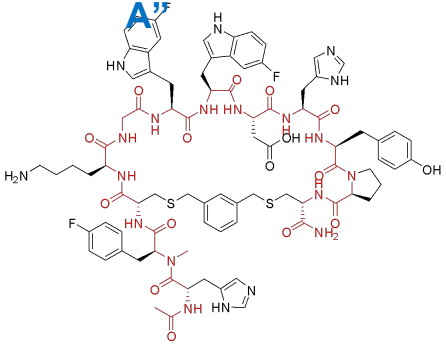
Macrocyclic peptide hits for PCSK9 through mRNA display



Collaboration wi Ra Pharma

mRNA display screen

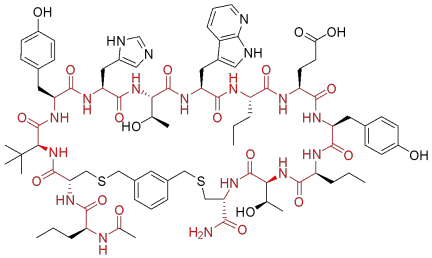
"Series A"



Lead A / compound 1
MW = 1889; 10-13 AA
TR-FRET IC_{50} = 483 nM

- Good potency
- Poor metabolic stability
- Poor physical properties
- No structural enablement
- No cellular permeability

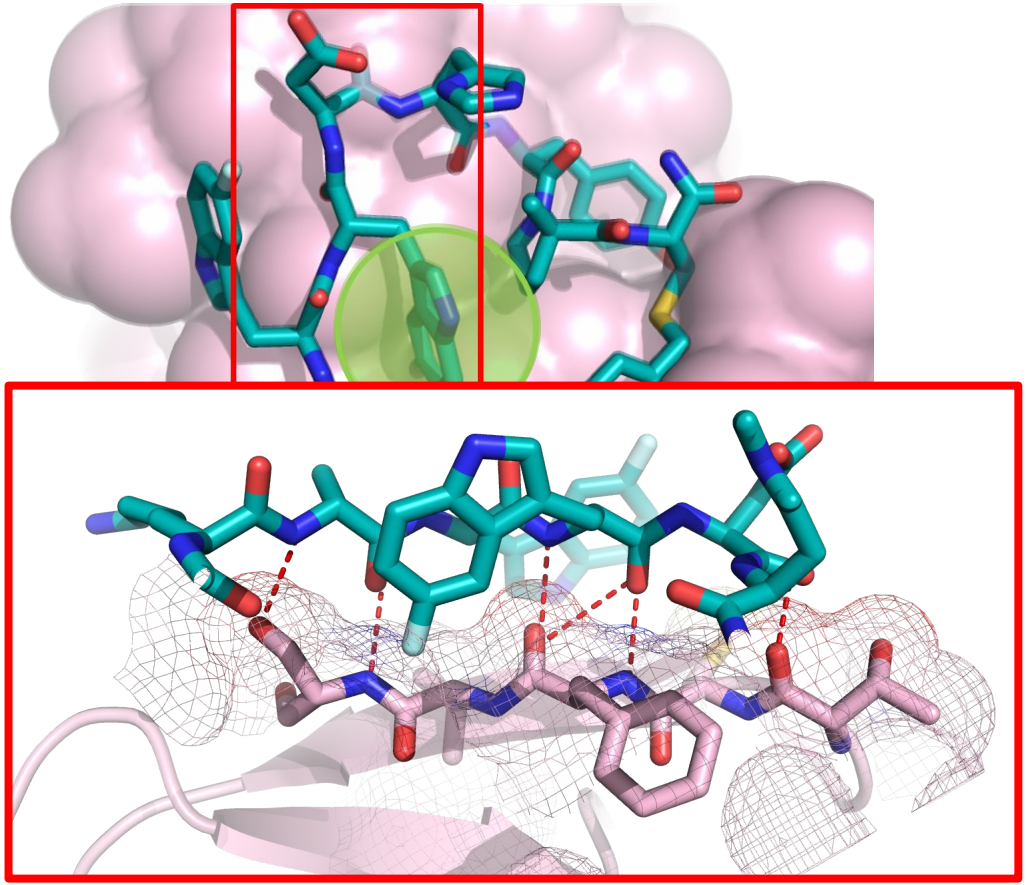
"Series B"

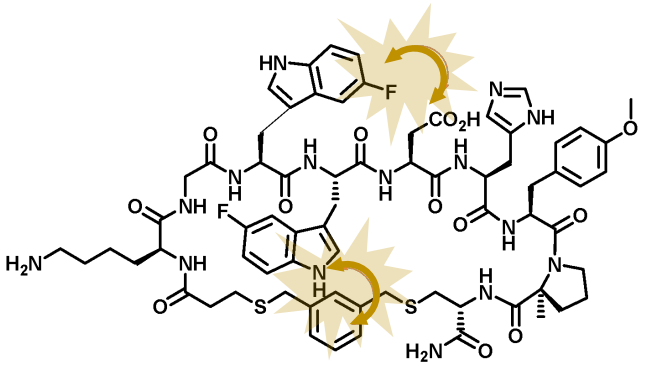


Lead B
MW = 1760; 10-12 AA
TR-FRET IC_{50} = hit (Ra)
De-prioritized

J. Med. Chem. **2020**, *63*, 13796;

Structure based drug design drives optimization of SAR

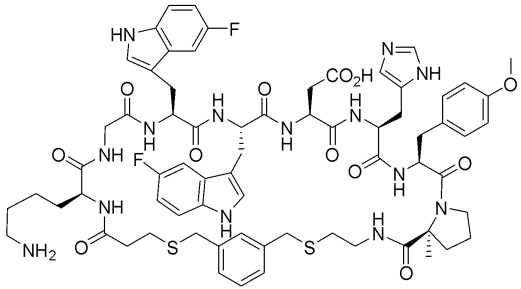




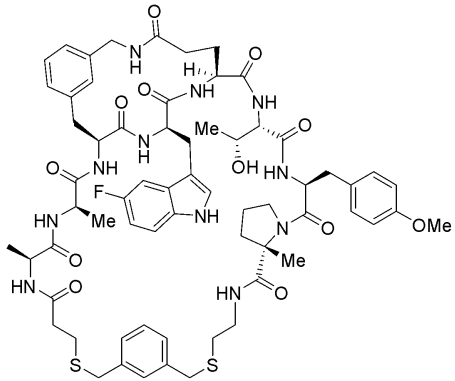
Compound
2
IC_{50} 4 nM

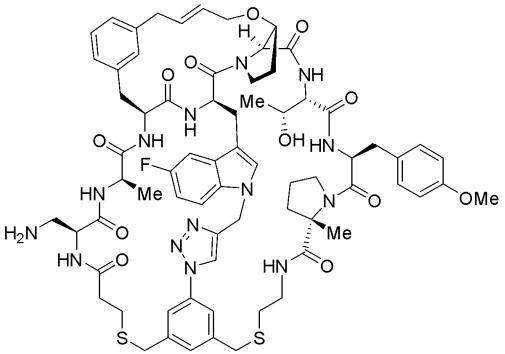
- Doughnut-like, flat contact surface (~190 Å^2)
- Key hydrophobic interactions
- 5F-TRP in center is a key interaction
- 5 H-bonds with beta-sheet
- Potential areas for cross linking

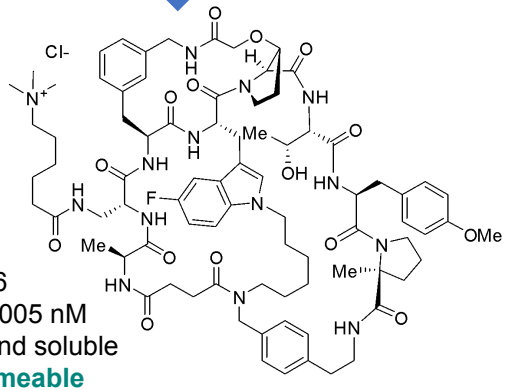
Structure based drug design to yield enlicitide



2 (IC_{50} = 4 nM)



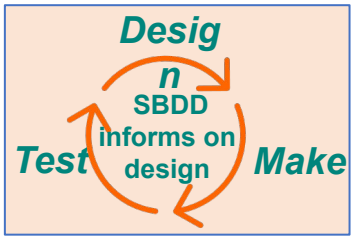




MK-0616
IC_{50} = 0.005 nM
Stable and soluble
Not permeable

Design principles

- GI protease and/or Transporter stability
- Potency
- Solubility / Phys-Chem



~ 3117 analogs
~ 5 years

- Transcellular permeability (P_{app}) of MK-0616 was very low in MDCKII (0.71 x 10^{-6} cm/s; reference mannitol 2.1 x 10^{-6} cm/s)
- Sufficient solubility (BCS 3, >7 mg/mL at pH7; 7.8 mg/ml in fasted-state simulated intestinal fluid for amorphous chloride salt)
- Demonstrated oral bioavailability and robust reduction of free PCSK9 in cynomolgus monkeys when formulated with an established permeation enhancer

Tucker TJ, et al. J Med Chem. 2021 Nov 25;64(22):16770-16800. doi: 10.1021/acs.jmedchem.1c01599.
Johns DG, et al. Circulation. 2023 Jul 11;148(2):144-158. doi: 10.1161/CIRCULATIONAHA.122.063372.

Cyclic peptide binding mode mimics that of the antibodies

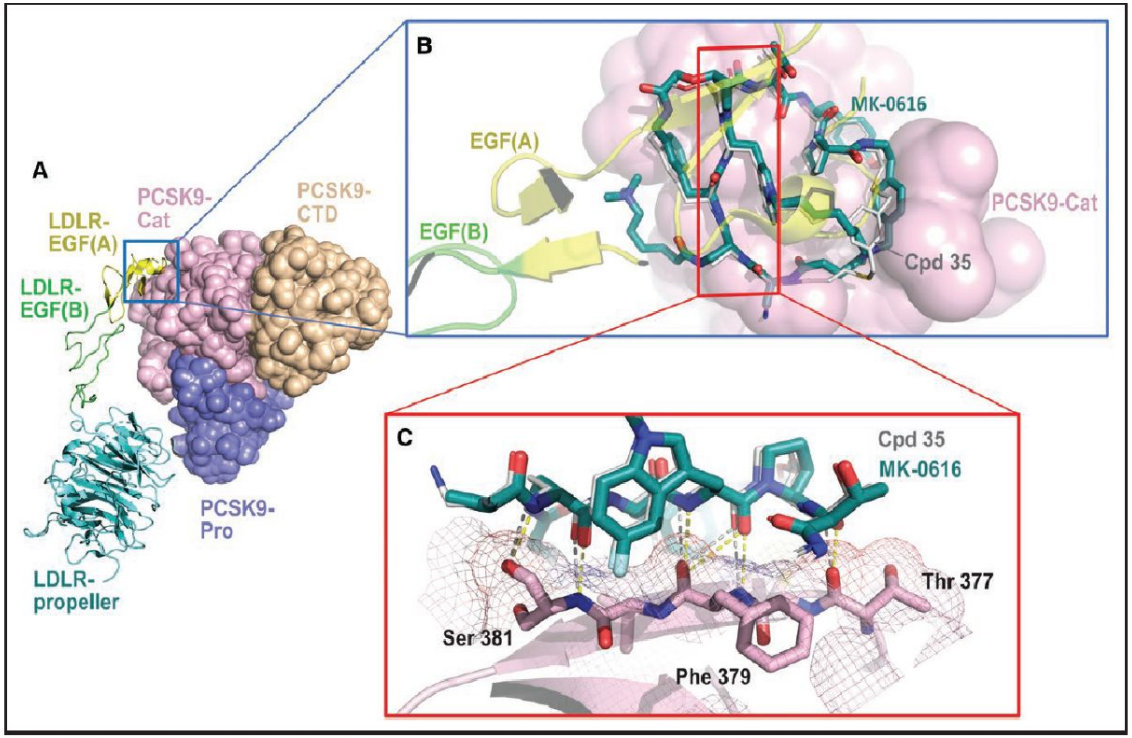
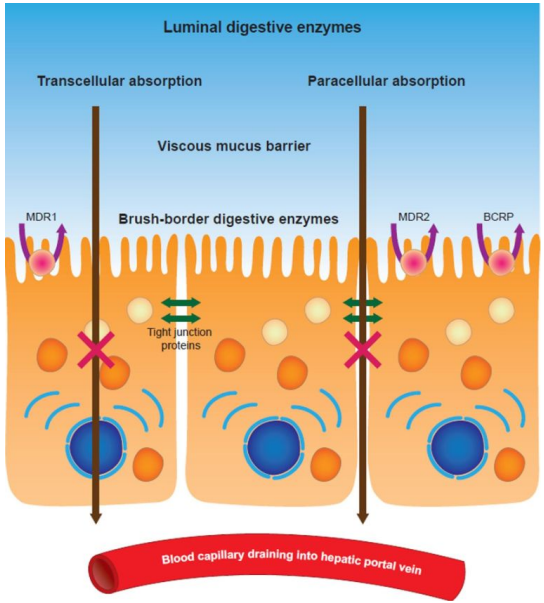


Figure 3. Macrocyclic peptide (MK-0616)–PCSK9 (proprotein convertase subtilisin/kexin type 9) complex.

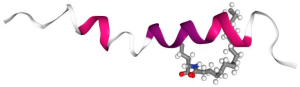
Johns et al. *Circulation 2023;148:144-158*

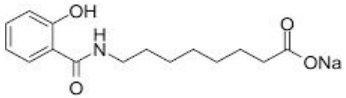
Oral bioavailability for peptides aided by permeation enhancers



Example using sodium salcaprozate (SNAC)

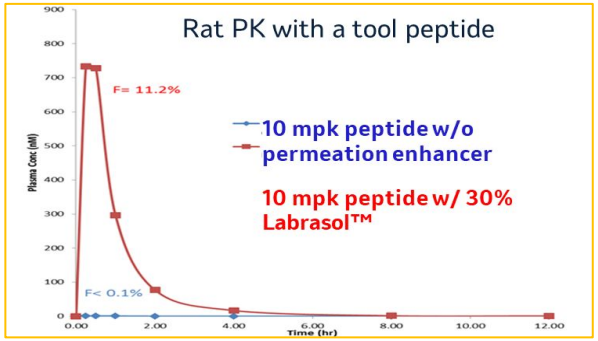
RYBELSUS® semaglutide tablets





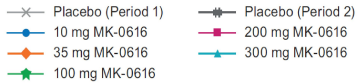
4,000 MW
0.4 – 1.0%F

Example at Merck using a mix of medium chain fatty acid triglycerides (Labrasol™)



Enlicitide Ph1 FIH SAD study

1. Is MK-0616 well-tolerated? ***YES (up to 300mg)***
2. Does MK-0616 get absorbed? ***YES***
3. Is PK variability high? ***NO***
4. Can we use sodium caprate? ***YES***



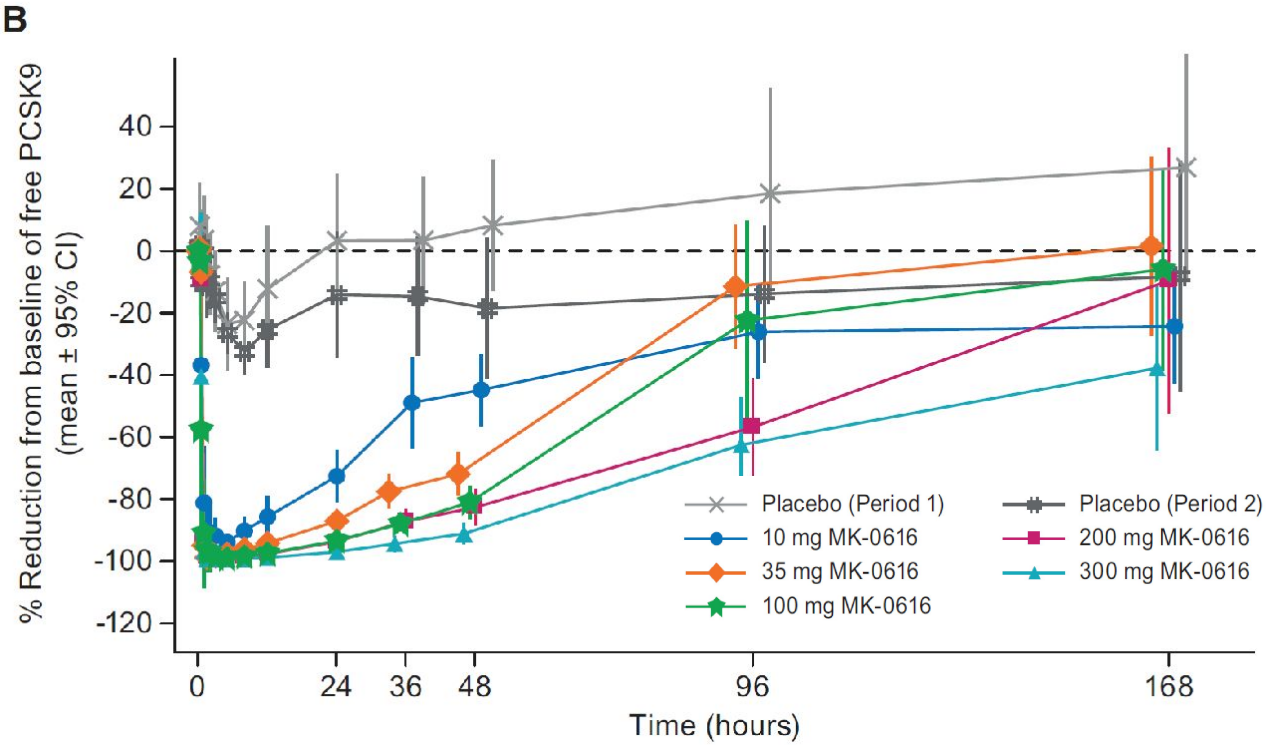
MK-0616 Treatment	AUC0-∞ (nM*hr)	AUC0-24 (nM*hr)	Cmax (nM)	C24 (nM)	Tmax (hr)	t1/2 (hr)
100 mg Labrasol	962 (29.1)	309 (44.6)	46.2 (91.1)	8.47 (20.8)	1.5 (1 - 3)	51.3 (7.25)
100 mg NaCap	978 (29.9)	281 (32.7)	41.3 (62.2)	8.22 (21)	1.5 (1 - 3)	56.8 (10.2)

Johns et al. *Circulation 2023;148:144-158*

Inhibition of free PCSK9

Evidence from marketed anti-PCSK9 mAbs:

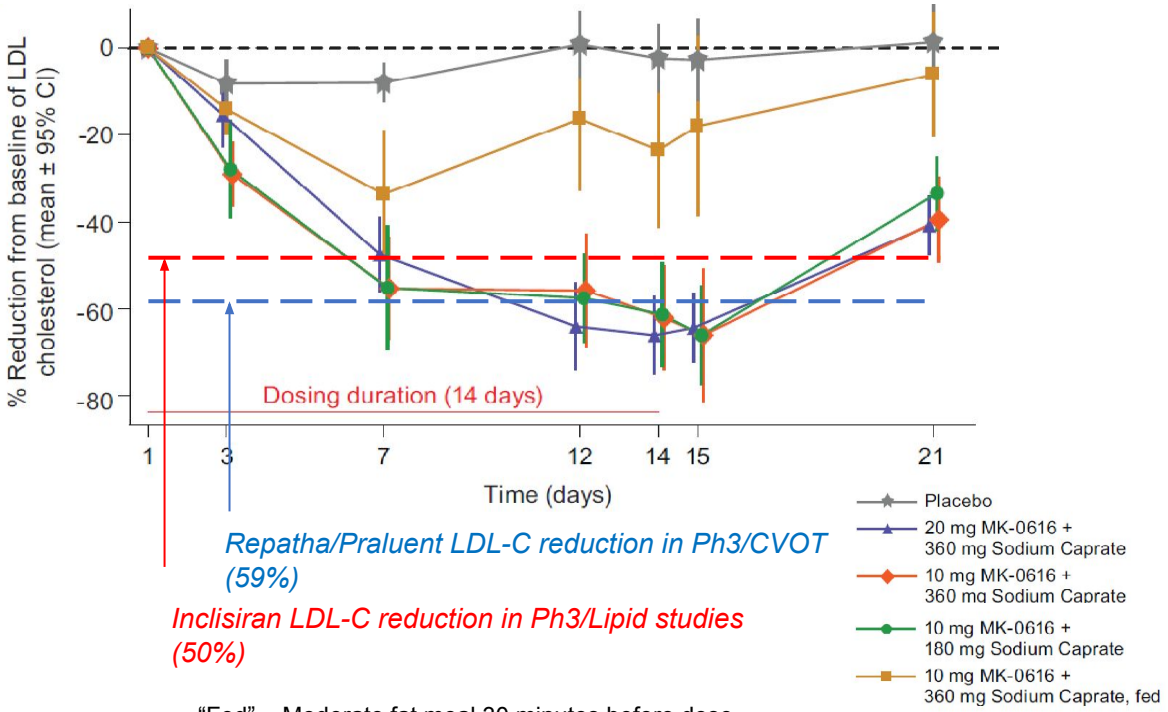
At least 80% reduction of free PCSK9 from baseline is required for maximum LDL-C reduction



Johns et al. *Circulation 2023;148:144-158*

MAD study: LDL-C Reduction in Statin-Treated Participants

- Study participants
  - Statin-treated, Male, Female, Up to 65 y
  - Otherwise healthy
  - Statins can induce PCSK9 expression up to ~40%
- PK similar to FIH/SAD
  - Multiple dose accumulation ~2X
  - Steady state reached by Day 7
- LDL-C reduction similar/greater than what was observed with mAbs/siRNA



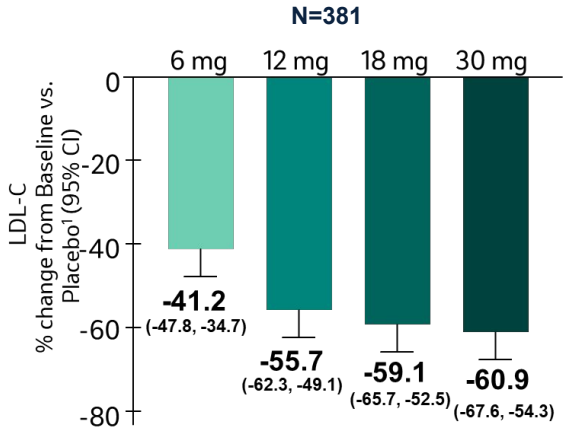
- "Fed" = Moderate fat meal 30 minutes before dose
- ~50-60% reduction in Cmax and AUC0-24
  - 10 mg dose performs more like 5 mg

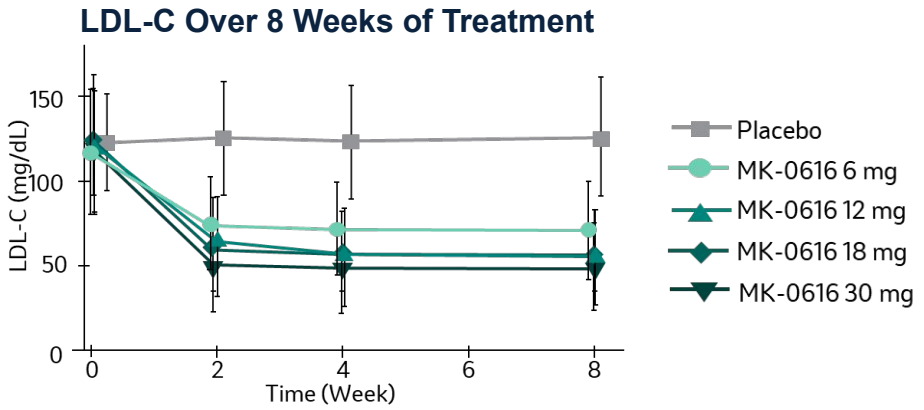
Johns et al. *Circulation 2023;148:144-158*

Ph2 study of enlicitide in adults with high cholesterol: results

- All doses of MK-0616 demonstrated statistically significant (P < 0.001) differences in percentage change in LDL-C from baseline to Week 8 vs placebo
- Near-complete efficacy for a given dose was reached by 2 weeks and the effect was persistent during the 8-week treatment period
- Results generally consistent across prespecified subgroups

Primary Endpoint: LDL-C Reduction from Baseline to Week 8





Efficacy Population: All participants who received ≥1 dose, had ≥1 observation for the analysis endpoint, and had baseline data for those analyses that require baseline data.
CI, confidence interval; LDL-C, low-density lipoprotein cholesterol
Ballantyne C, et al *J Am Coll Cardiol* 2023 Apr, 81 (16) 1553–1564

Enlicitide Phase 3 Clinical Program

CORALreef CLINICAL TRIALS	Purpose	Planned Sample Size	Status
Lipids (P013)	LDL-C reduction and safety in a broad population of hypercholesterolemic participants	2,760	Recruitment complete
HeFH (P017)	LDL-C reduction and safety in participants with Heterozygous Familial Hypercholesterolemia	270	Recruitment complete
AddOn (P018)	LDL-C reduction by MK-0616 versus other oral add-on therapies (ezetimibe or bempedoic acid or both)	300	Recruiting
Outcomes (P015)	Cardiovascular risk reduction in participants at high risk for atherosclerotic cardiovascular disease events	14,550	Recruiting
Extension (P019)	Open label extension study to provide access to MK-0616 for participants completing one of the lipid studies	~3000	Recruiting

CORALreef Lipids, HeFH, AddOn completion expected 2025

MERCK

Media > News releases > News release

Merck Announces Positive Topline Results From the First Two Phase 3 CORALreef Trials Evaluating Enlicitide Decanoate for the Treatment of Adults With Hyperlipidemia

June 9, 2025 6:45 am ET

Enlicitide demonstrated statistically significant and clinically meaningful reductions in LDL-C in both Phase 3 CORALreef HeFH and CORALreef AddOn trials

Enlicitide, a novel macrocyclic peptide, has the potential to be the first approved oral PCSK9 inhibitor

Thank you!

- Filippos Kesisoglou – Pharmaceutical Sciences
- Dennis Wolford – Translational Medicine Operations
- Susan Zhou – Quantitative Pharmacokinetics and Pharmacometrics
- Puja Banka – Global Clinical Development
- Enlicitide development team

Additional Biopharm studies: No effect of co-administered water volume on bioavailability

For some oral peptides, reduction in exposure as a function of water volume has been reported

- potentially due to reduction of stabilizing effect of excipients or dilution of permeation enhancer

Water Volume	AUCinf GMR point estimate	Cmax GMR point estimate
240 mL	Ref	Ref
120 mL	0.92	0.93
50 mL	1.02	0.95