Clinical Trials
What's Current and Why Participate

Seth J. Baum, MD, FACC, FAHA, FACPM, FNLA, FASPC

Founder, Excel Medical Clinical Trials: www.emclinicaltrials.com

President, The American Society for Preventive Cardiology

Director, Women's Preventive Cardiology BRRH

ExcelMedical
CLINICAL TRIALS

Disclosures: Research/Consulting/Advisory Boards/Speaking

- Amgen
- Sanofi/Regeneron
- BI/Lily
- Merck
- AstraZeneca
- Aralez
- Pfizer
- Esperion
- Madrigal
- Gemphire

ExcelMedical CLINICAL TRIALS

Phases of Clinical Research

- **Phase 1**
- Phase I trials assess a medication's safety. These trials also evaluate what the drug does to our bodies (pharmacodynamics) as well as what our bodies do to the drug (pharmacokinetics). This first phase of testing in human beings is performed in a very small number of healthy volunteers who and are ordinarily remunerated for their participation. A phase 1 trial will also investigate side effects and benefits that occur at various dosages. Phase 1 trials typically take several months to complete.

ExcelMedical
CLINICAL TRIALS

Phases of Clinical Research

- **Phase 2**
- After phase 1, when a medication has been shown to be safe, it is then further tested for efficacy. Phase 2 trials can take months to years to complete, and can involve several hundred subjects. These trials are typically randomized.
- Sometimes these trials are double blinded.

Phases of Clinical Research

- **Phase 3**
- In a phase 3 trial, a drug is tested in hundreds to thousands of subjects. Thus, both pharmaceutical companies and the FDA gain a better understanding of the medication's effectiveness, and safety. These trials are usually both randomized and double blinded, and can last several years. After a phase 3 trial has been successfully completed, pharmaceutical companies can request FDA approval to market the drug.
- When such a trial successfully gains FDA approval, it is often known as a "pivotal" phase 3 clinical trial.

ExcelMedical
CLINICAL TRIALS

Phases of Clinical Research

- **Phase 4**
- Phase 4 trials are performed with FDA approved medications in order to learn even more about an approved medication.

Top 5 Reasons people participate in Clinical Research

- **To advance medicine (33%)**
- **To help improve the lives of others (29%)**
- **To help improve personal conditions (15%)**
- **To earn extra money (5%)**.
- **To receive free medical care (3%).**

Center for Information and Study on Clinical Research Participation (CISCRP)

ExcelMedical CLINICAL TRIALS

Components of Clinical Research

- Research Site
  - Calibrated equipment
  - Locked storage for records and IP
  - Subjects
  - Principal Investigator
  - Sub-Investigators
  - Clinical Research Coordinators (CRC)
  - Clinical Research Assistants (CRA)
  - Regulatory Staff
- Contract Research Organization (CRO)
- Study Sponsor
- Pre-Site Visit (PSV)
- Site Initiation Visit (SIV)
- Frequent Interim Monitoring Visits (IMV)

ExcelMedical
CLINICAL TRIALS

20+ Active Clinical Studies (by indication)

- Hyperlipidemia (7)
- Statin Intolerance (2)
- Homozygous Familial Hypercholesterolemia (1)
- Hypertriglyceridemia (2)
- Familial Chylomicronemia Syndrome (1)
- Hyperlipoproteinemia(a) (1)
- Type 2 Diabetes (2)
- Heart Failure HFpEF & HFrEF (2)
- NASH/NAFLD (2)

ExcelMedical CLINICAL TRIALS

Why Evaluate Ways to Lower LDL, TG, Lp(a), and Inflammation? Because they ***Cause*** ASCVD.

The evolution of association to causation, the history of LDL and other biomarkers

ExcelMedical
CLINICAL TRIALS

LDL-C, from Hypothesis to fact

“You can only know where you're going if you know where you've been.”

- James Burke

ExcelMedical
CLINICAL TRIALS

LDL and Cholesterol

- **From Biology to Epidemiology to Randomized Clinical Outcomes Trials and Mendelian Randomization Studies**
  - Evidence from outcomes studies shows a consistent linear relationship between the extent of low-density lipoprotein cholesterol (LDL-C) lowering with statin Rx (and other methods) and the relative reduction in risk of ASCVD events.
- **Higher LDL-C = Higher CV Risk**
- **Lower LDL-C = Lower CV Risk**

[1]Laufs U, Descamps OS, Catapano AL, Packard CJ. Understanding IMPROVE-IT and the cardinal role of LDL-C lowering in CVD prevention. Eur Heart J 2014;35:1996-2000.

ExcelMedical CLINICAL TRIALS

Understanding Cholesterol Metabolism

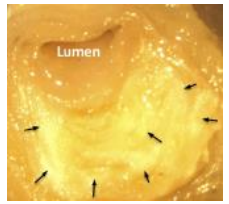
Where we've been

ExcelMedical
CLINICAL TRIALS

History of Cholesterol Understanding

1908–1913

Cholesterol related to atherosclerosis[1]

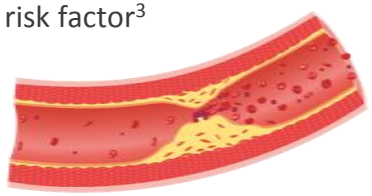


1948

Framingham Heart Study begins[2]

1961

Cholesterol identified as cardiovascular risk factor[3]

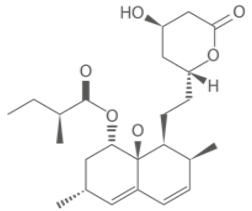


1985

Brown and Goldstein awarded nobel prize for cholesterol metabolism (LDL receptor)[4]

1987

First statin approved[5]

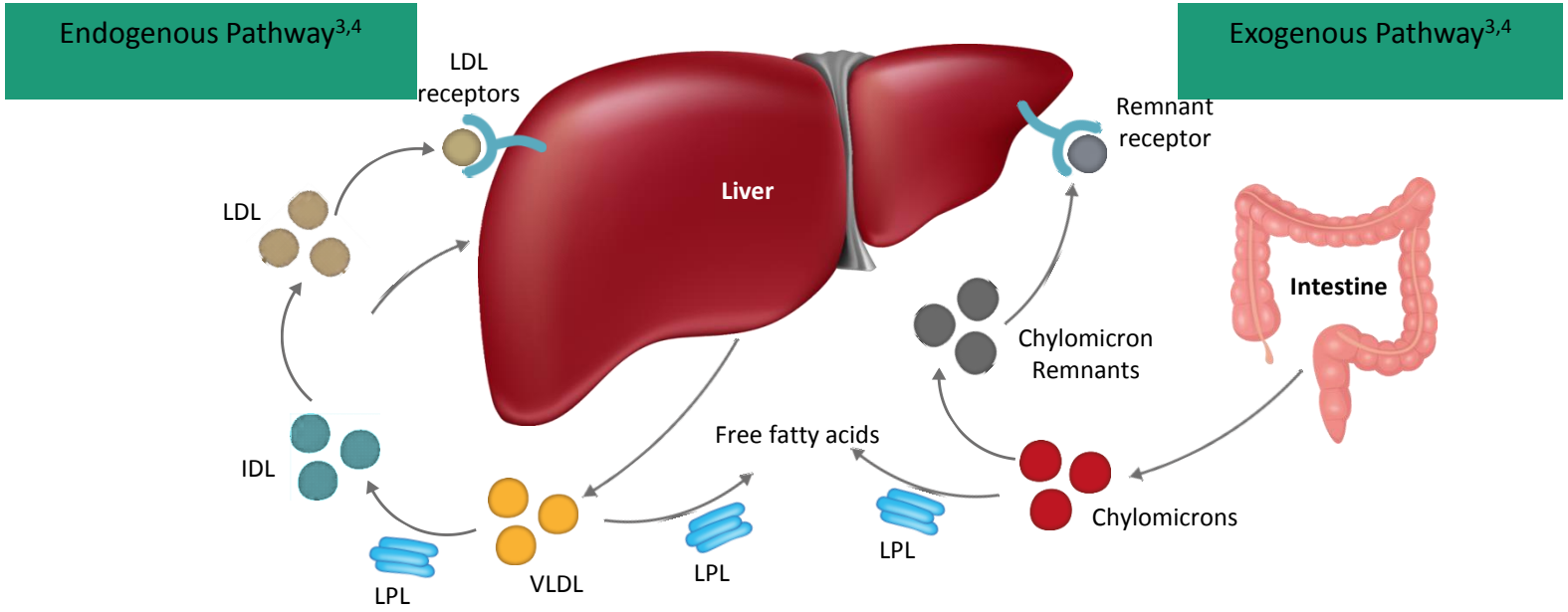


1. Konstantinov IE, et al. *Tex Heart Inst J.* 2006;33:417-423. 2. Framingham Heart Study. https://www.framinghamheartstudy.org/about-fhs/index.php. Accessed January 13, 2016. 3. Kannel WB, et al. *Ann Intern Med*. 1961;55:33-50. 4. Brown MS, Goldstein JL. Nobel lecture 1985. http://www.med.harvard.edu/md_phd/news/Brown%20and%20Goldstein%20Nobel%20Lecture.pdf. Accessed January 13, 2016. 5. Tobert JA. *Nat Rev Drug Discov*. 2003;2:517-526. 5. Nobelprize.org. The Nobel prize in physiology or medicine 1985. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1985/. Accessed January 13, 2016.

ExcelMedical CLINICAL TRIALS

LDL-C and LDL-P are the End Products of Endogenous Lipoprotein Metabolism[1]

- LDL receptors remove LDL from the circulatory system[1]
- LDL, to a minor degree, delivers cholesterol to peripheral tissues[2]

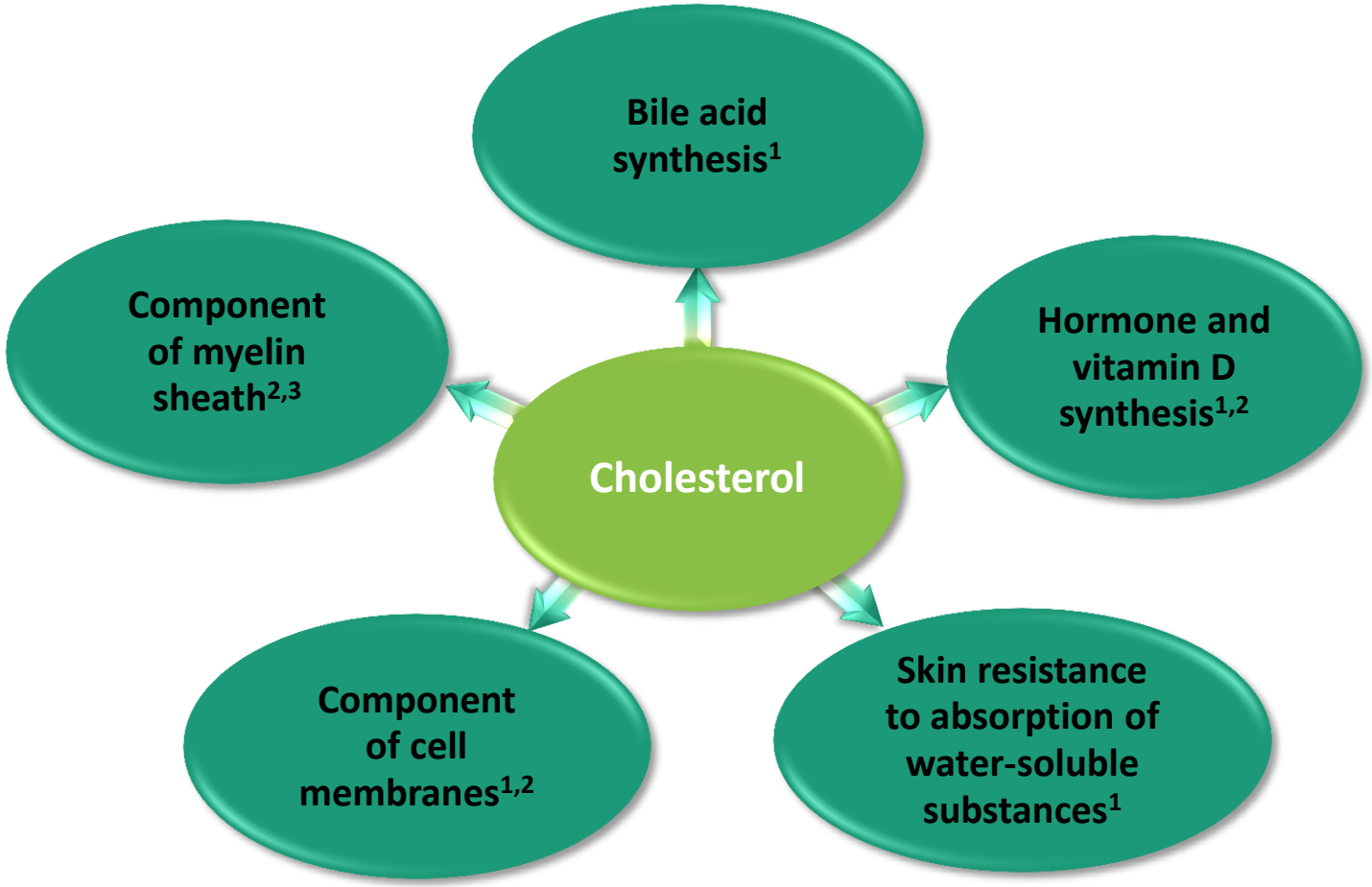


HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; LPL = lipoprotein lipase; VLDL = very low-density lipoprotein

1. Goldstein JL, Brown MS. *Arterioscler Thromb Vasc Biol*. 2009;29;431-438. 2. Rader DJ, et al. *J Clin Invest*. 2003;111:1795-1803.
3. Dietschy JM, Turley SD. *J Lipid Res.* 2004;45:1375-1397; 4. Mc Auley MT, et al. *BMC Syst Biol*. 2012;6:130.

ExcelMedical CLINICAL TRIALS

Cholesterol Plays a Role in Many Important Physiologic Functions

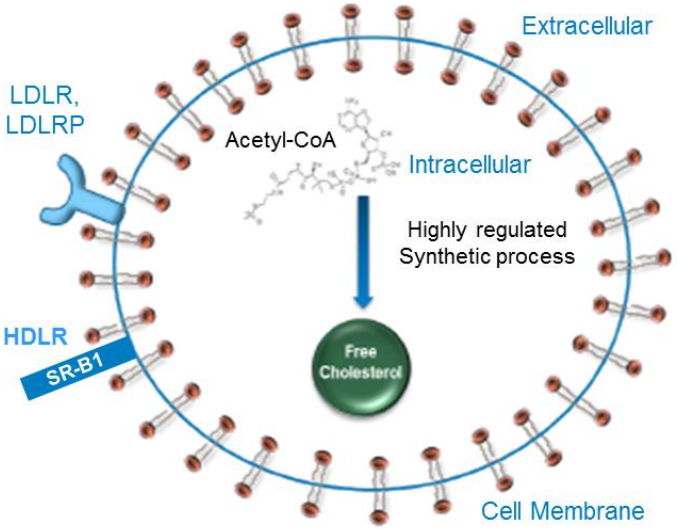


1. Hall JE, Guyton AC. In: *Guyton and Hall Textbook of Medical Physiology*. 12th ed. Philadelphia, PA: Saunders; 2011:819-830.
2. Goldstein JL, Brown MS. *Arterioscler Thromb Vasc Biol*. 2009;29:431-438. 3. Saher G, et al. *Nat Neurosci*. 2005;8:468-475.

ExcelMedical CLINICAL TRIALS

Cellular Acquisition of Cholesterol Can Be From Multiple Sources

- Cholesterol for cellular physiologic functions can be from intra and/or extracellular pathways[1–5]
  - Systemic distribution of cholesterol is important, but cells are not dependent on circulating plasma LDL-C[3]

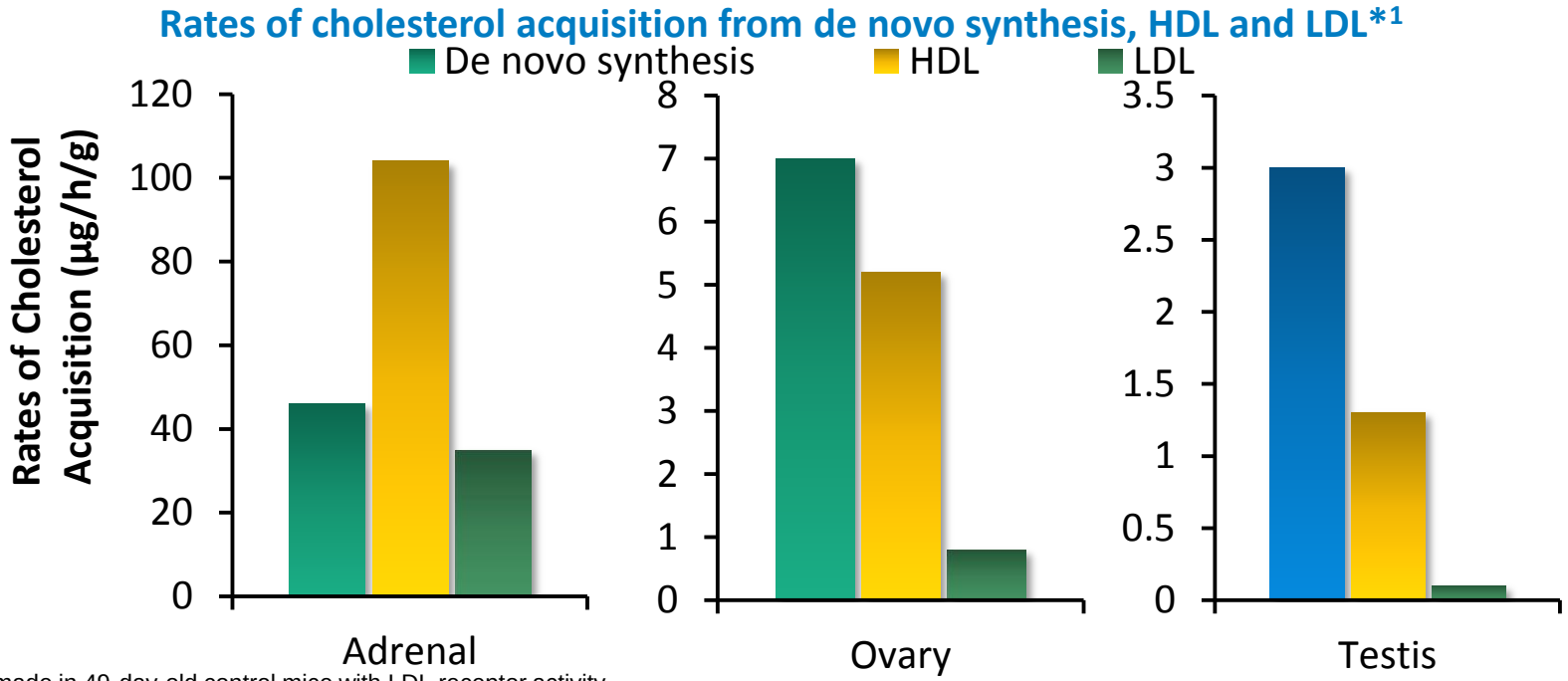


HDLR = high-density lipoprotein cholesterol receptor; LDL-C = low-density lipoprotein cholesterol; LDL-R = LDL receptor; LDLRP = LDLR protein; SR-B1 = scavenger receptor class B type 1.

1. Mc Auley MT, et al. *BMC Syst Biol.* 2012;6:130. 2. Xie C, et al. *J Lipid Res.* 2006;47:953-963. 3. Hu J, et al. *Nutr Metab (Lond).* 2010;7:47. 4. Orth M, Bellosta S. *Cholesterol.* 2012;2012:292598. 5. Dietschy JM, Turley SD. *J Lipid Res.* 2004;45:1375-1397.
Figure adapted from Dietschy 2004.

Animal Data Demonstrate Steroidogenic Tissues Acquire Cholesterol via HDL-C and De Novo Synthesis[1,2]

- Adrenal, ovarian, and testicular tissues can acquire cholesterol via LDL, HDL, and de novo synthesis
  - Predominant pathway is HDL and de novo synthesis[1,2]



*Data were calculated from measurements made in 49-day-old control mice with LDL receptor activity.

HDL = high-density lipoprotein; LDL = low-density lipoprotein.

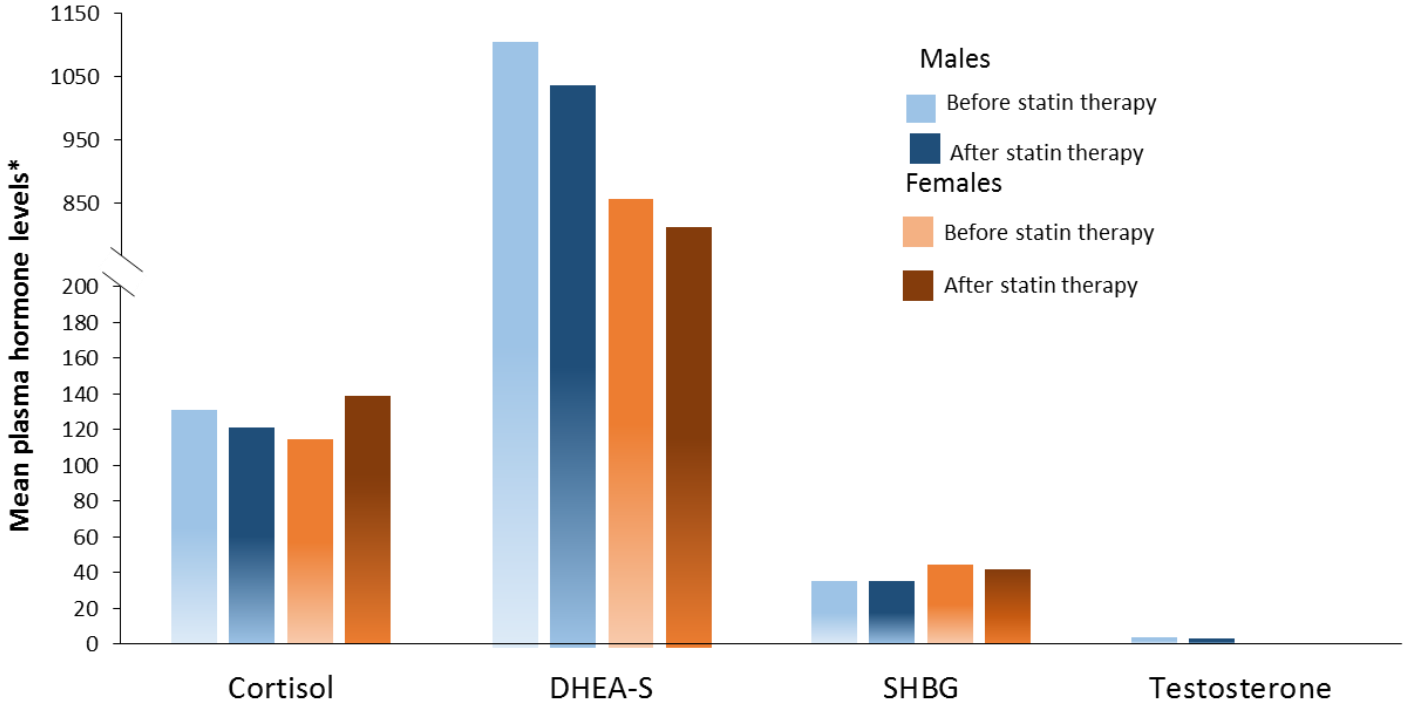
1. Xie C, et al. *J Lipid Res.* 2006;47:953-963. 2. Hu J, et al. *Nutr Metab (Lond).* 2010;7:47.

ExcelMedical CLINICAL TRIALS

Despite Reducing LDL-C, Statins Do Not Alter Gonadal or Adrenal Steroid Hormones in Humans

Plasma hormone levels before and 3 months after treatment with statin[1]

- Reduction in LDL-C with statins without changing steroid hormones has been consistently shown[1–3]



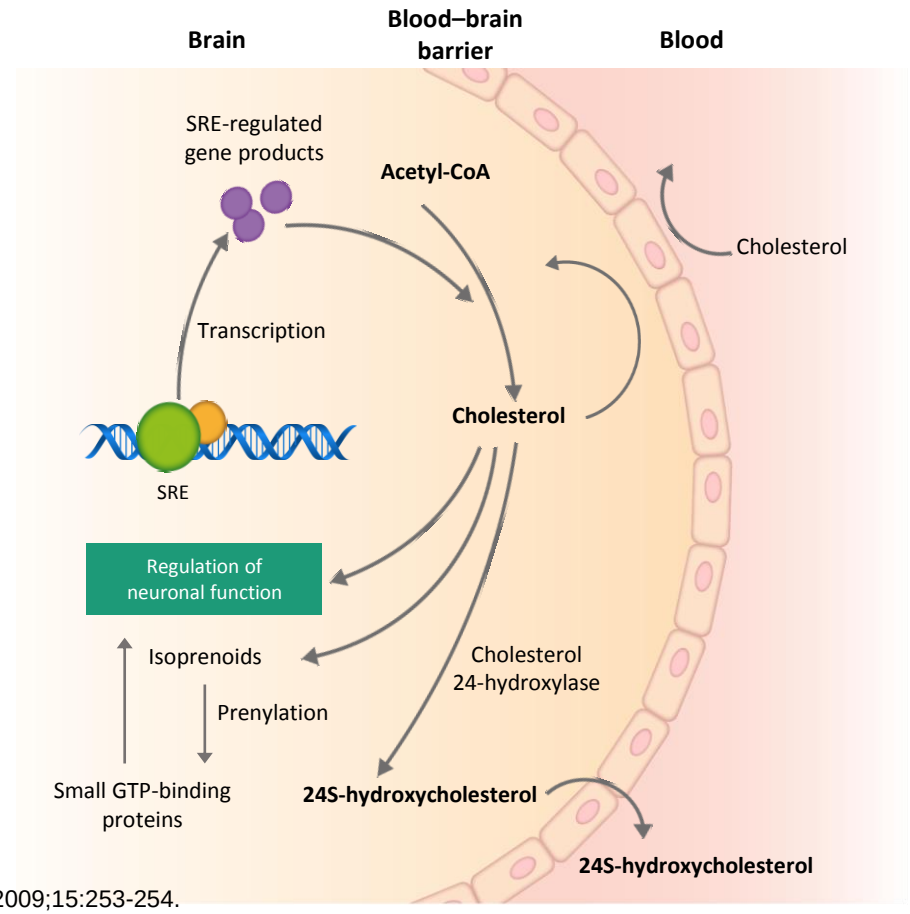
*ng/mL for cortisol, DHEA-S and testosterone and nmol/L for SHBG. Effect of statin on gonadal and adrenal hormones studied on 24 patients with type 2 diabetes, studied before and after a 3-month treatment with statin.

DHEA-S = dehydroepiandrosterone sulfate; SHBG = sex hormone binding globulin.

1. Santini SA, et al. *J Atheroscler Thromb*. 2003;10:160-164. 2. Sezer K, et al. *J Endocrinol Invest*. 2008;31:1075-1078.
3. Bohm M, et al. *Z Kardiol*. 2004;93:43–48.

The Central Nervous System Synthesizes Cholesterol De Novo

- The central nervous system synthesizes cholesterol de novo[1,2]
- The blood–brain barrier prevents the uptake of systemic lipoprotein cholesterol[1,2]
- This segregation ensures that cholesterol metabolism within the brain is isolated from changes in the circulating lipid levels[2]



1. Björkhem I, Meaney S. *Arterioscler Thromb Vasc Biol.* 2004;24:806-815. 2. Katsuno M, et al. *Nat Med*. 2009;15:253-254.
Figure adapted from Katsuno M et al. 2009.

ExcelMedical CLINICAL TRIALS

Our Evolving Understanding of LDL and Cholesterol Biology

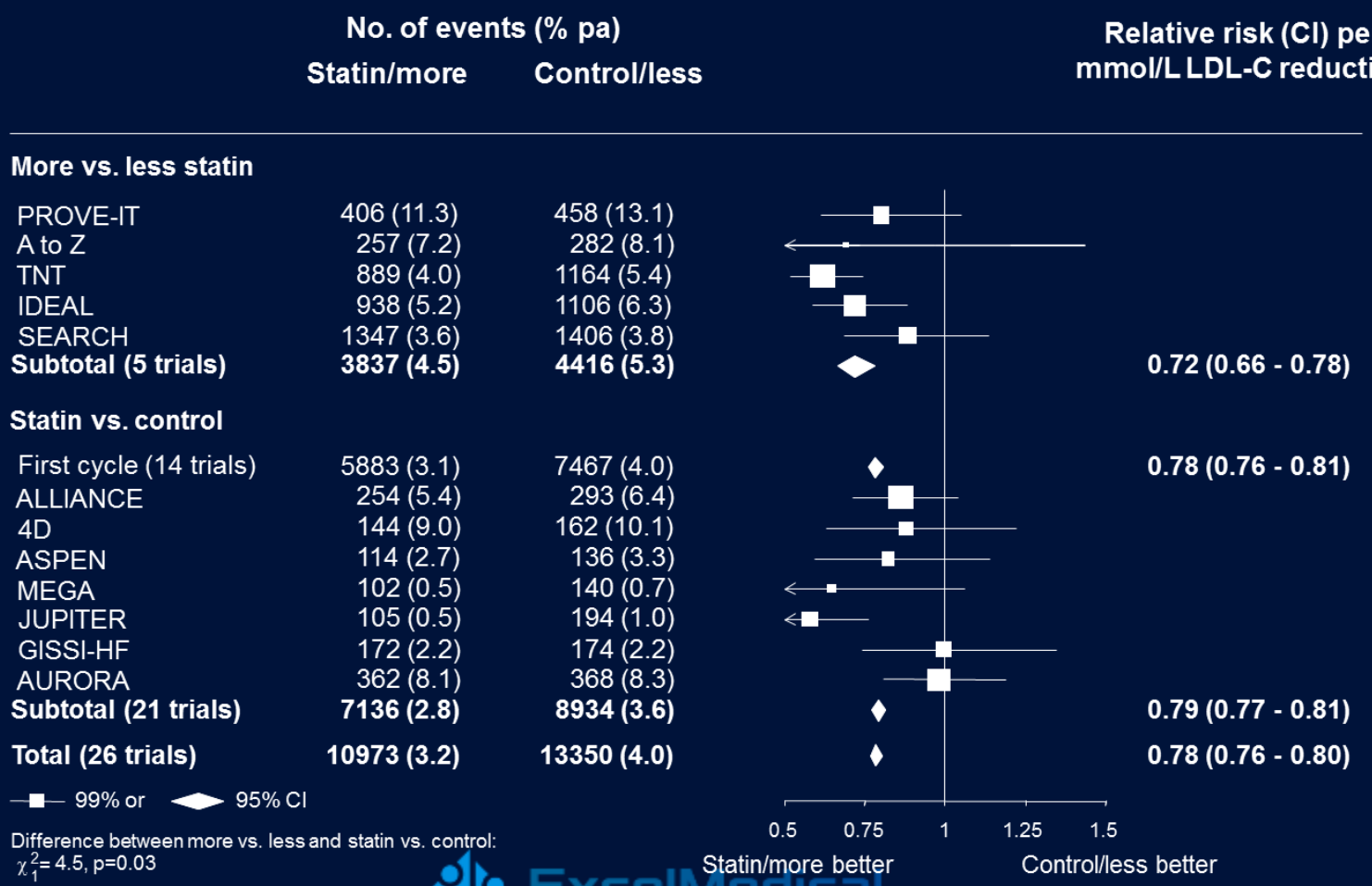
- Knowledge of cholesterol's role and trafficking mechanisms has dramatically evolved over the last century
- Cholesterol plays a role in various vital physiologic functions
- Cellular cholesterol is minimally dependent on extracellular acquisition; and not at all in the brain
- LDL is an insignificant source of cholesterol for steroid hormone synthesis
- Conflating LDL and cholesterol is a common and dangerous error: LDL ≠ Cholesterol
- In modern humans, LDL carries cholesterol destined for excretion

ExcelMedical CLINICAL TRIALS

RCTs prove that lower LDL is better:
Statin effectiveness

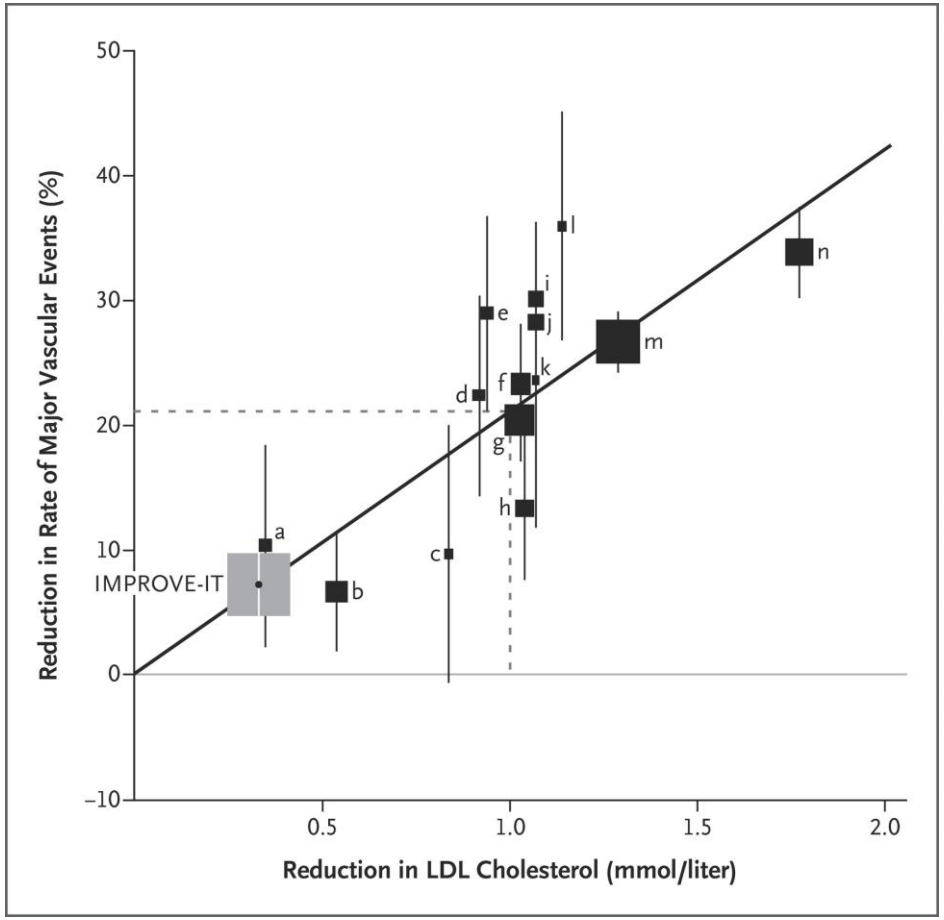
ExcelMedical
CLINICAL TRIALS

Proportional effects on major vascular events per mmol/L LDL-C reduction



Lancet 2010; **376**: 1670–81.

IMPROVE-IT: EZ, Another piece of the puzzle



Cannon CP et al. N Engl J Med 2015;372:2387-2397

ExcelMedical CLINICAL TRIALS

Statin and EZ trials show that LDL is causally related to vascular disease

But can we further prove causation?

ExcelMedical
CLINICAL TRIALS

Declaring Causation: the need for accuracy

- “Cardiology has a long history of finding a marker of a bad outcome and treating that marker of that bad outcome as if it were the *cause* of the bad outcome.” In other words, conflating association with causation.
  - David Brown, MD

ExcelMedical CLINICAL TRIALS

Association doesn’t equal causation

- Grey hair is associated with ASCVD.
- Therefore, grey hair causes ASCVD.
- No: Older individuals have more grey hair and also a higher incidence of ASCVD events. This is an association, but not causal.

Reverse Causation, a common error in interpretation

- Low cholesterol is associated with cancer.
- Low cholesterol causes cancer.
- No: Cancer causes low cholesterol.
- This is reverse causation.

Mendelian Randomization (MR) Studies: Their Rationale

- Genotypes are unmodified by disease, limiting reverse causation.
- Genotypes are assigned randomly at meiosis, limiting confounding. (Mendel's Law of Independent Assortment)

MR Requirements

1. The genetic variant affects the biomarker only. It does not impact other phenotypes.
2. The genetic variant associates with the disease in question in the same direction as the biomarker.
3. Exogenous (environmental) factors must be randomly distributed.

3 Components of a Mendelian Randomization Study

- “Instrument”: Gene variant that exclusively affects biomarker of interest; doesn’t affect other phenotypes (no confounding)
- “Theoretically predicted risk estimate”: based on amount of biomarker change, can accurately predict effect of variant on disease
- “Observed Risk Estimate”: Test variant for relationship with disease: Does the predicted estimate match the observed effect?

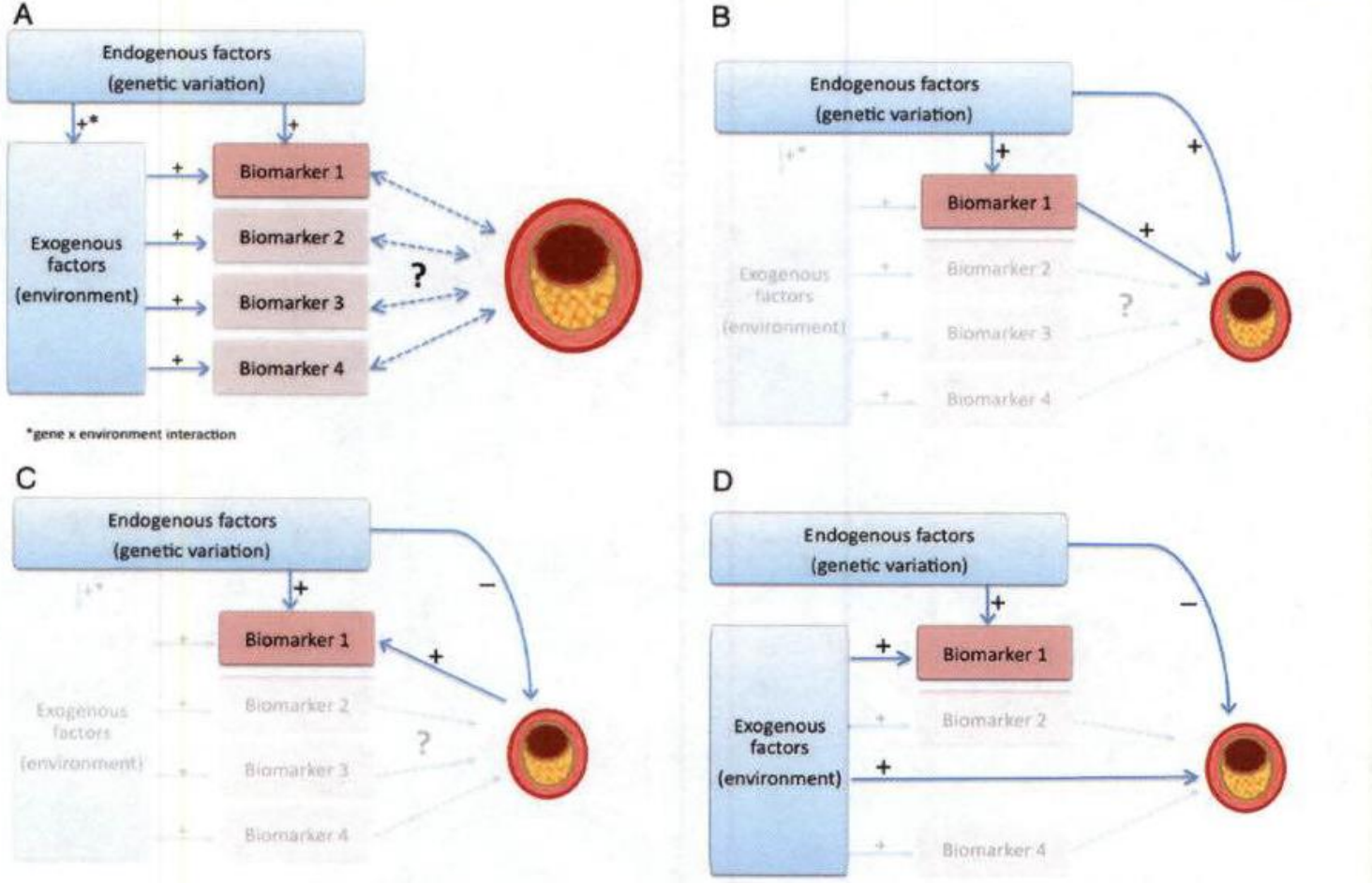


Figure 1 Conceptual background for Mendelian randomization studies: (*A*) Biomarkers 1–4 are associated with coronary artery disease but causality is unclear. Genetic variants and environmental factors affect the levels of these biomarkers. (*B*) Here a genetic variant not only associates statistically significant with the biomarker (+), but also with the complex disease. As a DNA variant has no immediate effect on disease manifestation, it can be expected that its effect on the biomarker acts as an indispensible intermediate step. Thus, the biomarker is causally involved in the disease process. (*C*) Here the genetic variant shows a sizable effect on the biomarker (+) but no association with coronary artery disease. Thus, it can be assumed that an equivalent variability of the biomarker has likewise no effect on disease risk; the biomarker is not causally involved in disease manifestation. (*D*) In this case exogenous factors influence the biomarker as well as coronary artery disease risk. Even if the genetic variant associates with the biomarker, its causal involvement in coronary artery disease cannot be assumed, since the single nucleotide polymorphism does not associate with coronary artery disease risk.

European Heart Journal
doi:10.1093/eurheartj/ehu208

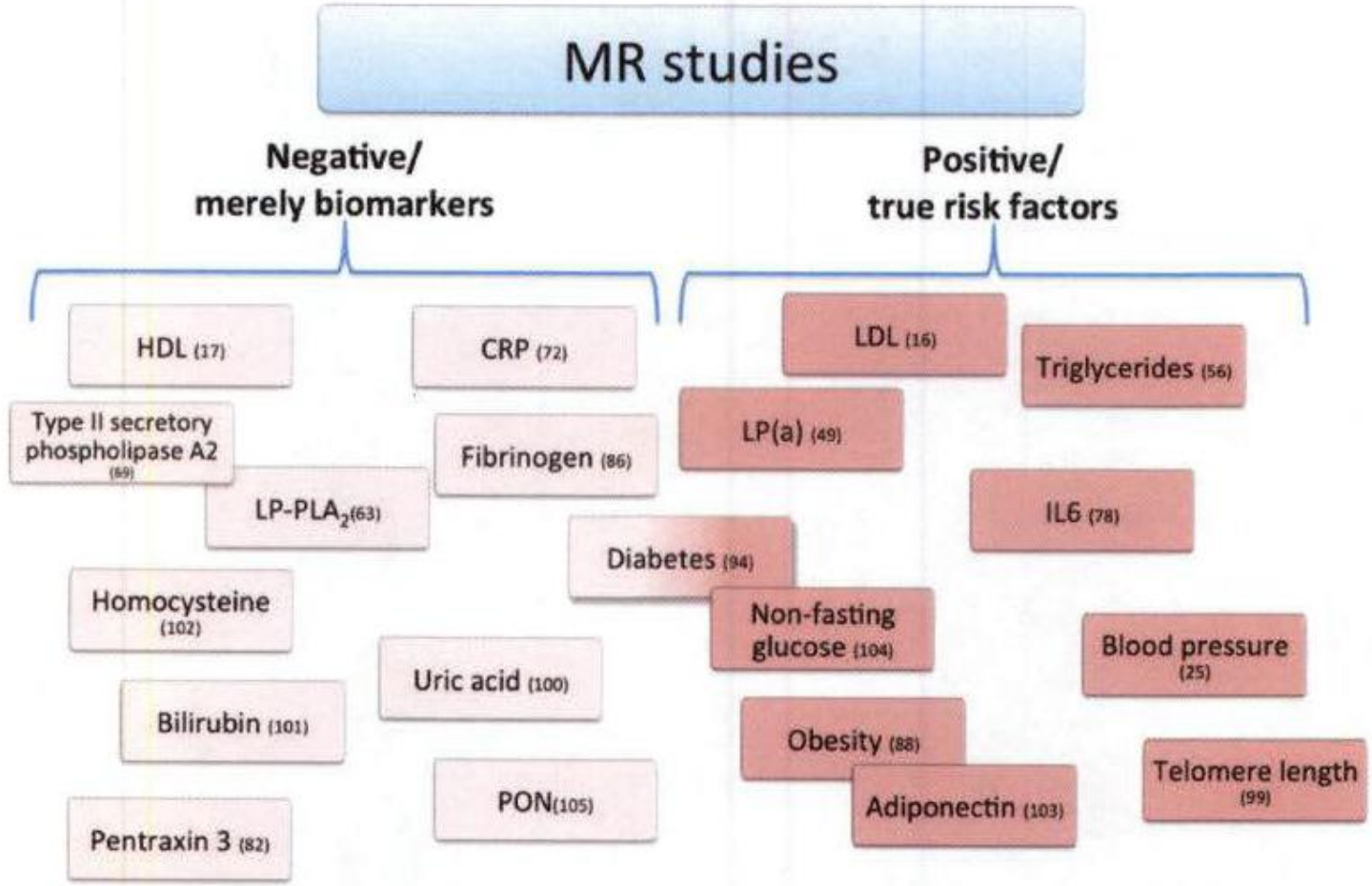
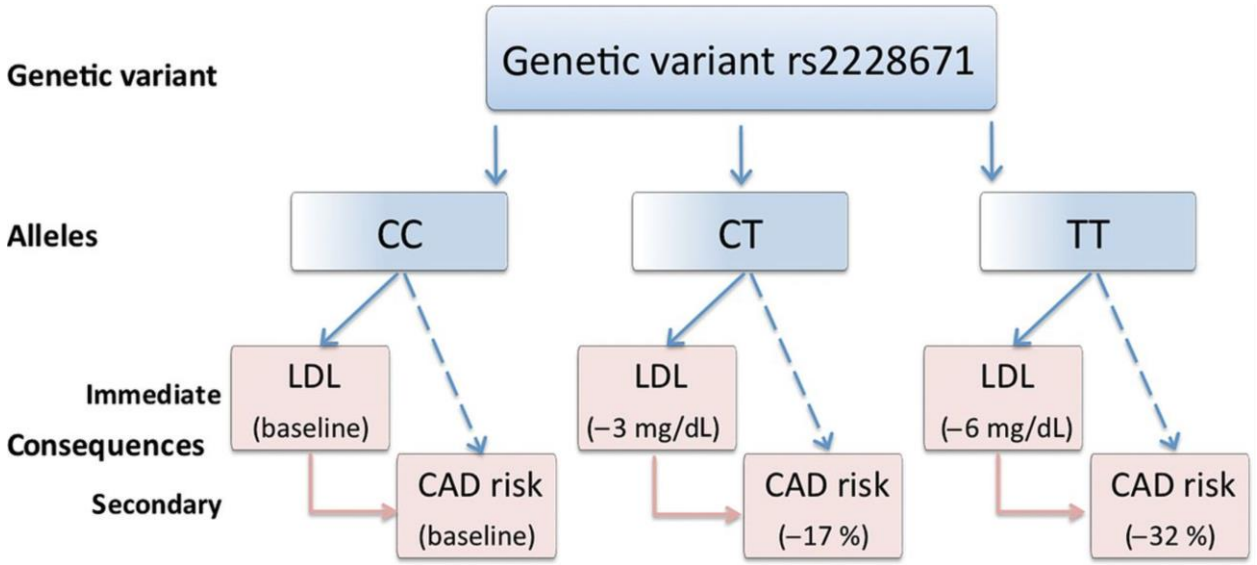


Figure 4 Brief overview about candidates tested in Mendelian randomization settings. While many biomarkers suggested a causal role in coronary artery disease in Mendelian randomization studies, others disappointed by negative results. The effect of diabetes mellitus single nucleotide polymorphisms was by far smaller than expected and barely significant. Numbers refer to the references in which the Mendelian randomization data have been reported. [100–105]

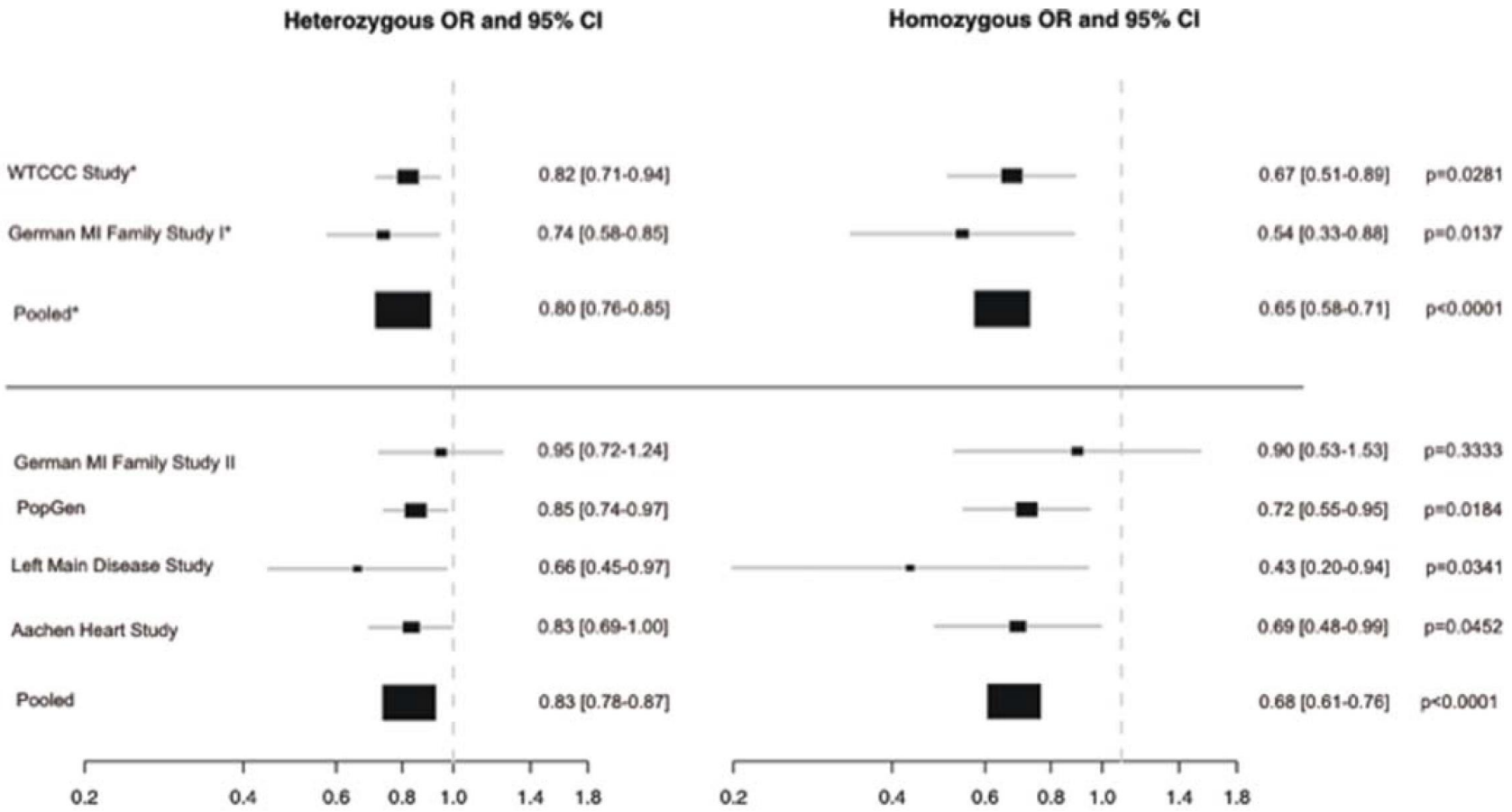
European Heart Journal
doi:10.1093/eurheartj/ehu208

SNP that lowers LDL, A MR Study



From: Mendelian randomization studies in coronary artery disease
Eur Heart J. 2014;35(29):1917-1924. doi:10.1093/eurheartj/ehu208

Association of rs2228671 with risk of CAD in six case-control studies



Linsel-Nitschke P et al. PLoS. 2008; 3: e2986.

ExcelMedical CLINICAL TRIALS

“LDL Hypothesis” is no longer “Hypothetical”; it is Reality

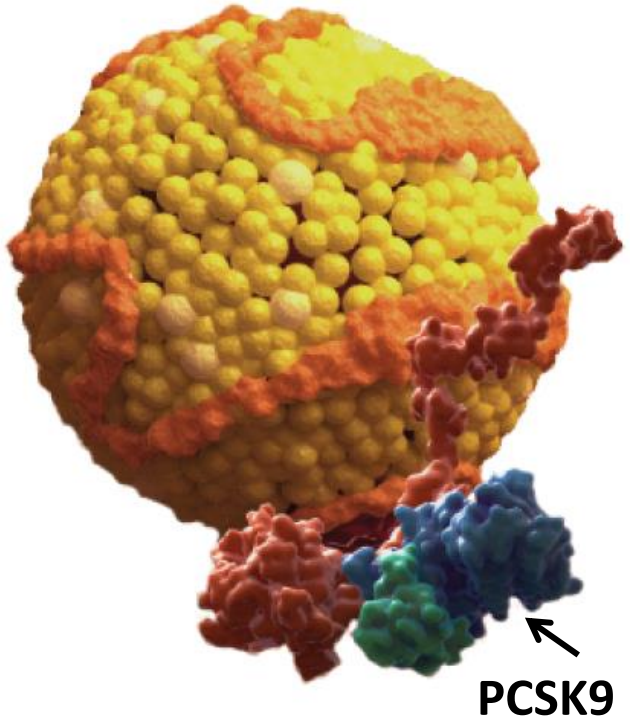
- LDL biology and pathophysiology
- Epidemiology
- Statin RCTs
- IMPROVE-IT
- FOURIER (To be discussed)
- Mendelian Randomization Studies

Enter PCSK9

PCSK9: Major modulator in LDL-C Metabolism

ExcelMedical
CLINICAL TRIALS

PCSK9 is a regulator of LDL metabolism

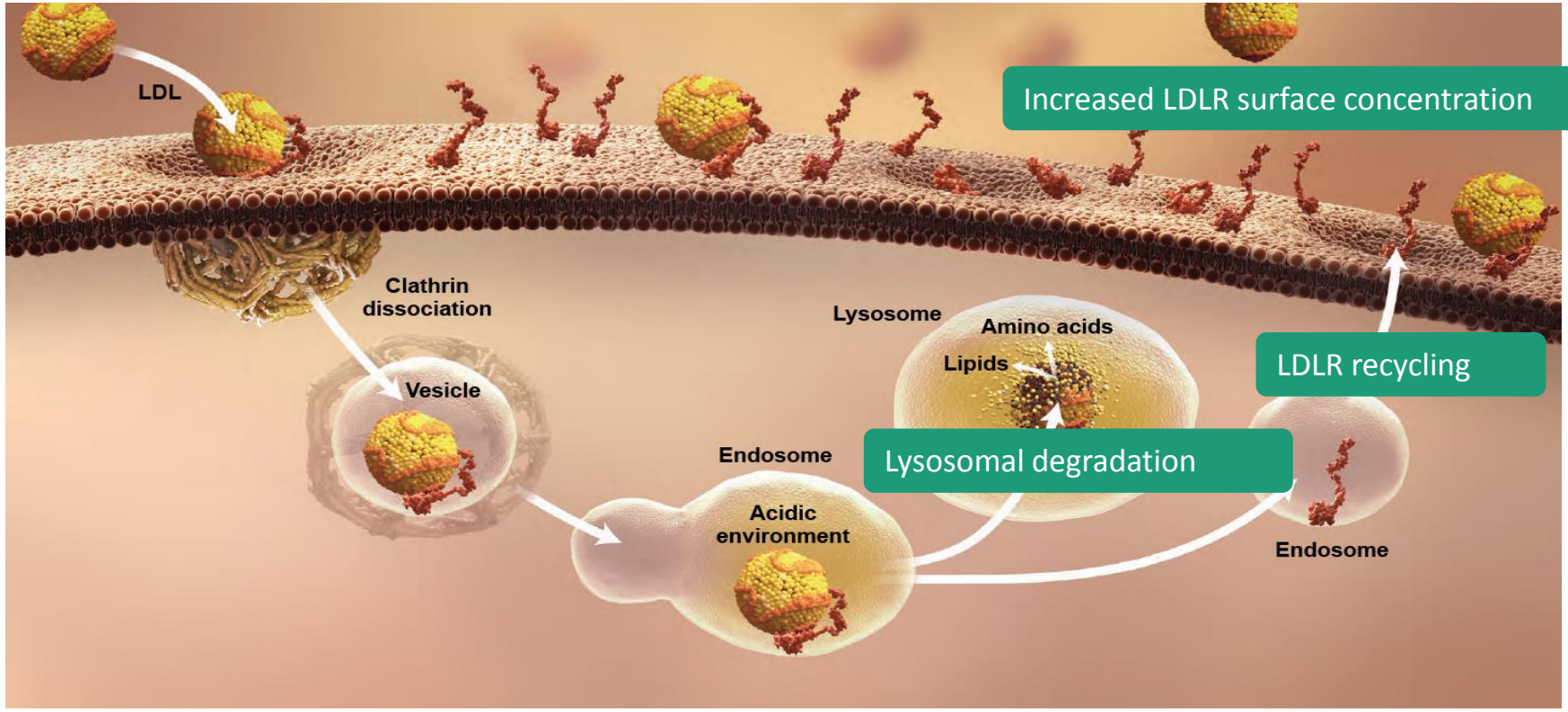


PCSK9

- **Proprotein convertase subtilisin/kexin type 9[1]**
- **Secreted by liver into plasma[1]**
- **Binds LDL receptor on surface of hepatocyte[1,2]**
- **Targets LDL receptor for degradation[1,2]**

1. Seidah NG, et al. *Circ Res.* 2014;114:1022-1036. 2. Steinberg D, Witztum JL. *Proc Natl Acad Sci U S A.* 2009;106:9546-9547.

LDL particles are cleared from the plasma by binding to LDLR receptors and being internalized by the hepatocyte[1-3]

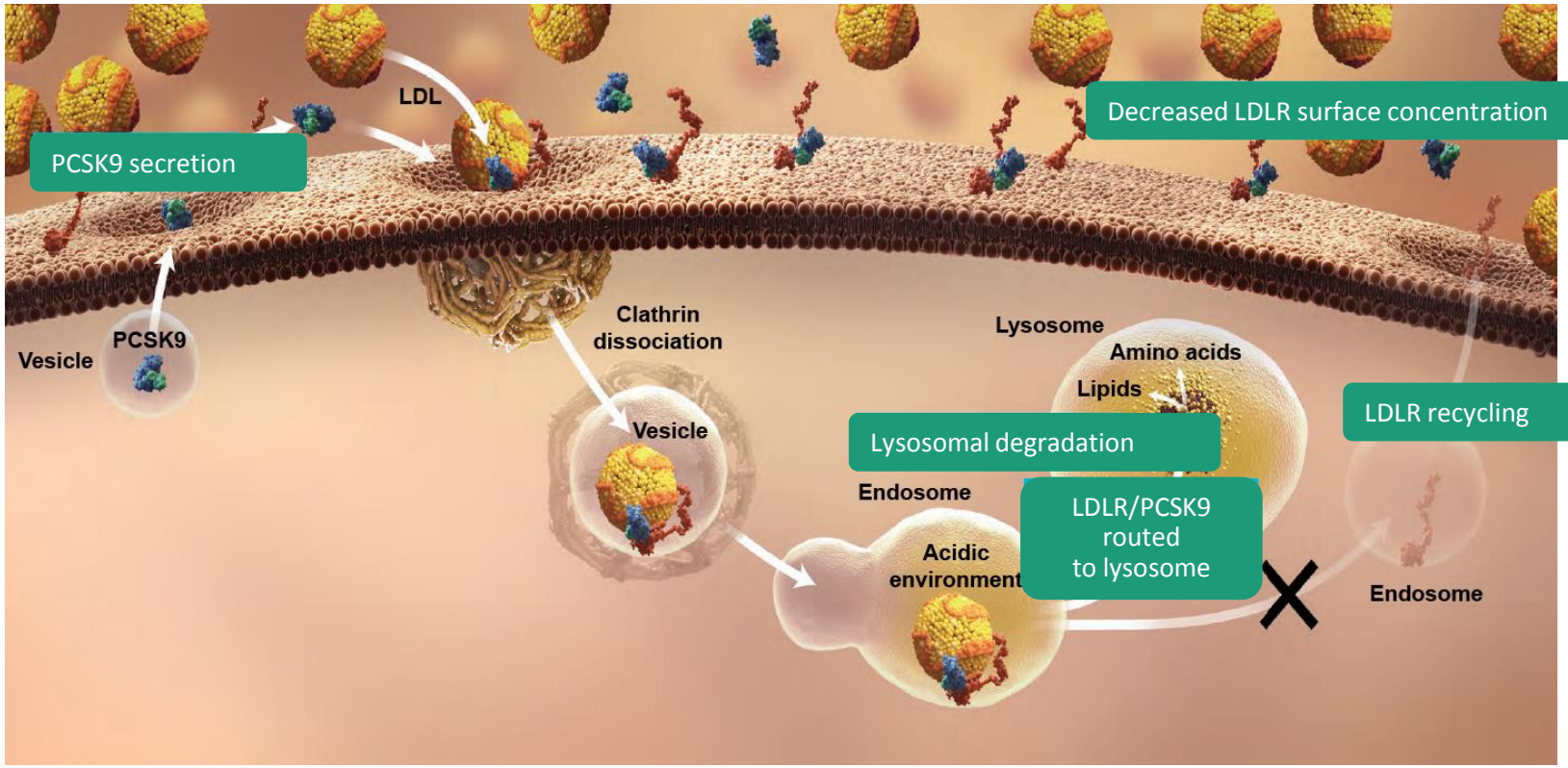


Recycled LDL receptors continue to clear plasma LDL

1. Brown MS, Goldstein JL. *Proc Natl Acad Sci U S A.* 1979;76:3330-3337. 2. Brown MS, Goldstein JL. *Science*. 1986;232:34-47. 3. Steinberg D, Witztum JL. *Proc Natl Acad Sci U S A.* 2009;106:9546-9547.

ExcelMedical CLINICAL TRIALS

PCSK9 binds to the LDL receptor, targeting it for degradation[1-3]



Fewer LDL receptors on hepatocyte surface result in increased plasma LDL-C

1. Abifadel M, et al. *Hum Mutat.* 2009;30:520-529. 2. Seidah NG, et al. *Circ Res.* 2014;114:1022-1036. 3. Steinberg D, Witztum JL. *Proc Natl Acad Sci U S A.* 2009;106:9546-9547.

The NEW ENGLAND JOURNAL of MEDICINE

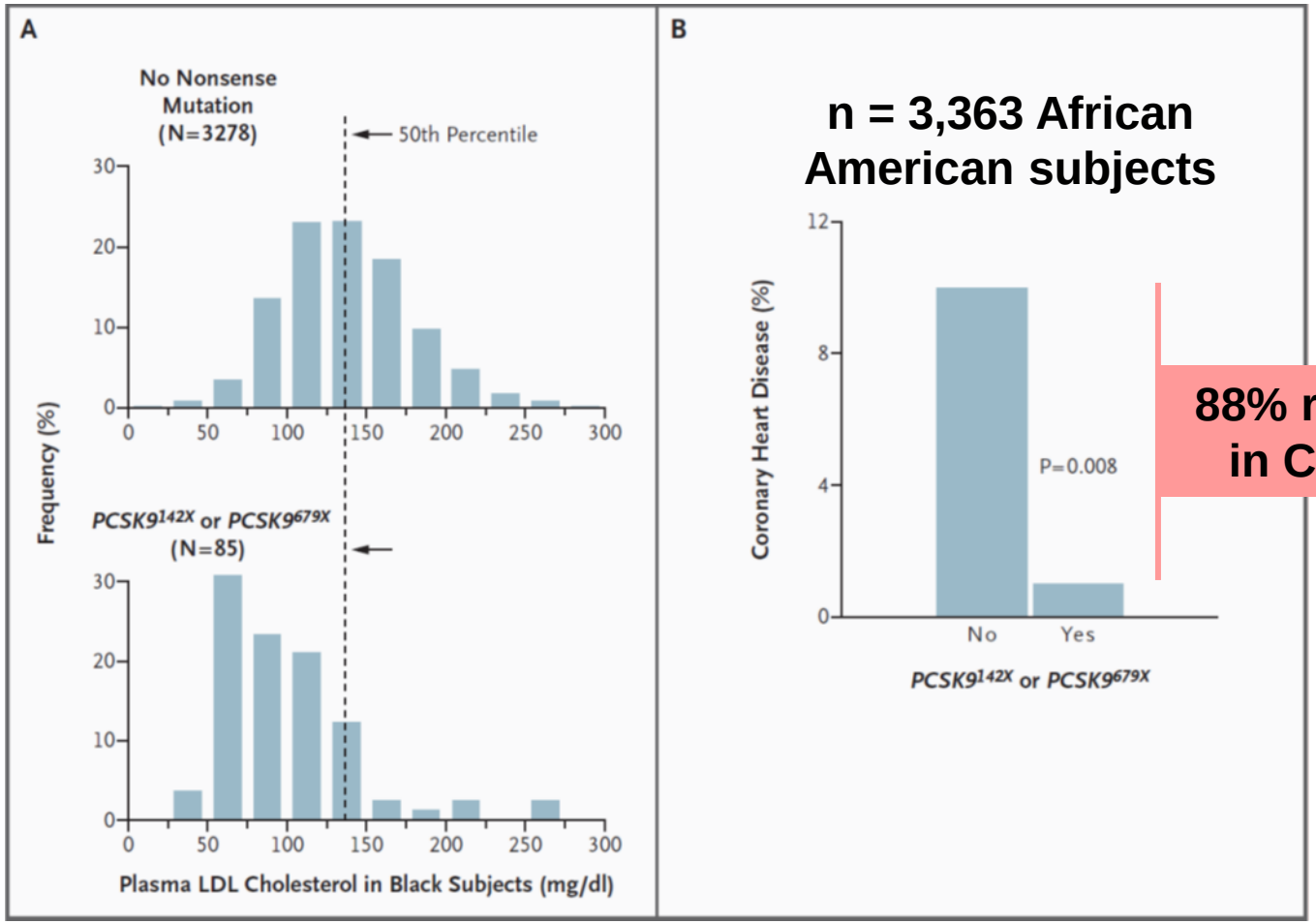
ORIGINAL ARTICLE

Sequence Variations in *PCSK9*, Low LDL, and Protection against Coronary Heart Disease

Jonathan C. Cohen, Ph.D., Eric Boerwinkle, Ph.D., Thomas H. Mosley, Jr., Ph.D., and Helen H. Hobbs, M.D.

Compared the incidence of CHD (MI, fatal CHD, or coronary revascularization) over a 15-year interval in the ARIC study according to the presence or absence of sequence variants in the PCSK9 gene that are associated with reduced plasma levels of LDL cholesterol.

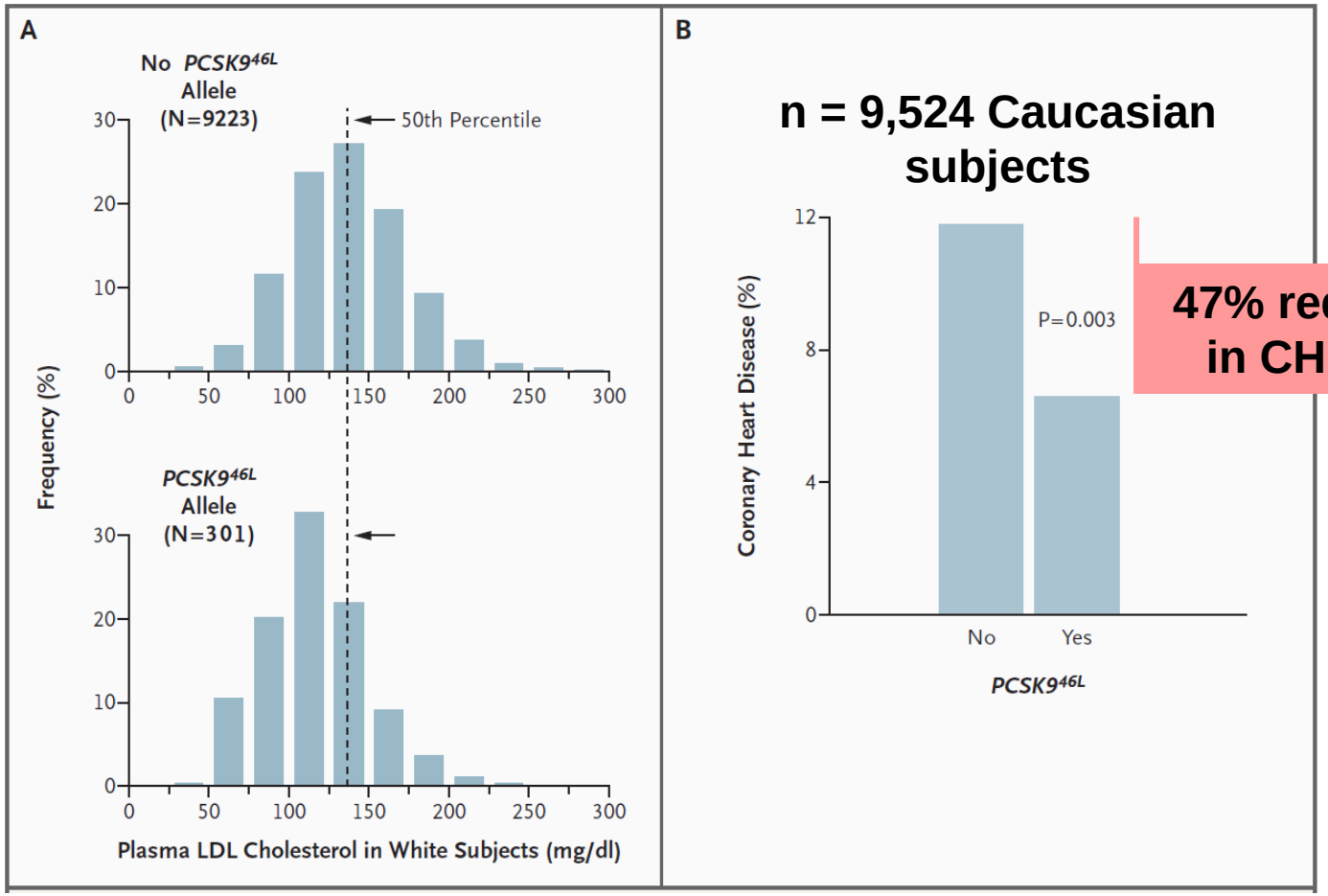
Plasma LDL cholesterol levels and incidence of CHD in African Americans



Cohen JC et al. *N Engl J Med*. 2006; 354: 1264-1272.

ExcelMedical CLINICAL TRIALS

Plasma LDL cholesterol levels and incidence of CHD in Caucasians



Cohen JC et al. *N Engl J Med*. 2006; 354: 1264-1272.

ExcelMedical CLINICAL TRIALS

Loss-of-Function Mutations in PCSK9 Are Associated With Decreased LDL-C

PCSK9 Variant	Population	LDL-C
R46L	ARIC[1], DHS[2]	↓ 15%[1]
Y142X or C679X	ARIC[1], DHS[2]	↓ 28%–40%[1]
R46L	CGPS[3]	↓ 11%[3]

- Heterozygous LOF mutations found in 1% to 3% of representative populations[1,3]
- Associated with
  - Lower serum LDL-C[1]
- PCSK9 null individual identified (compound heterozygote for two inactivating mutations)
  - No detectable circulating PCSK9 with strikingly low LDL-C (14 mg/dL)[4]

LOF = loss of function
ARIC = Atherosclerosis Risk in Communities (N ~ 4,000); DHS = Dallas Heart Study (N = 3,553);
CGPS = Copenhagen General Population Study (N = 26,013)

1. Cohen JC, et al. *N Engl J Med*. 2006;354:1264-1272. **2.** Cohen J, et al. *Nat Genet*. 2005;37:161-165
3. Benn M, et al. *J Am Coll Cardiol*. 2010;55:2833-2842. **4.** Zhao Z, et al. *Am Journal of Hum Gen*. 2006;79:514-534.

Gain-of-Function Mutations in PCSK9 Cause Familial Hypercholesterolemia*†

PCSK9 Variant	Population	Clinical/Biochemical Characteristics
D374Y[1]	British, Norwegian families, 1 Utah family	Tendon xanthomas, severe hypercholesterolemia
S127R[1]	French, South African, Norwegian families	Tendon xanthomas
R218S[2]	French families	Tendon xanthomas, arcus corneae

- Associated with:
  - High serum LDL-C[1]
  - In vitro testing in many identified mutations shows decreased levels of LDLRs[3]

*Autosomal Dominant Hypercholesterolemia

1. Abifadel M, et al. *Hum Gen*. 2009;30:520-529. **2.** Lopez D. *Biochem Biophys Acta*. 2008;1781:184-191.
3. Cameron J, et al. *Hum Mol Genet*. 2006;15:1551-1558.

†*For a full list of ADH mutations, please refer to Abifadel reference.*

Genetic Variants Establish PCSK9 as a Modulator of LDL-C

- Increased plasma levels of TC and LDL-C[1]
- FH-associated physical abnormalities[1]

Increasing PCSK9 (GOF)

- Fewer LDL receptors[1,2]
- Higher LDL-C[1,2]

Plasma LDL-C

Decreasing PCSK9 (LOF)

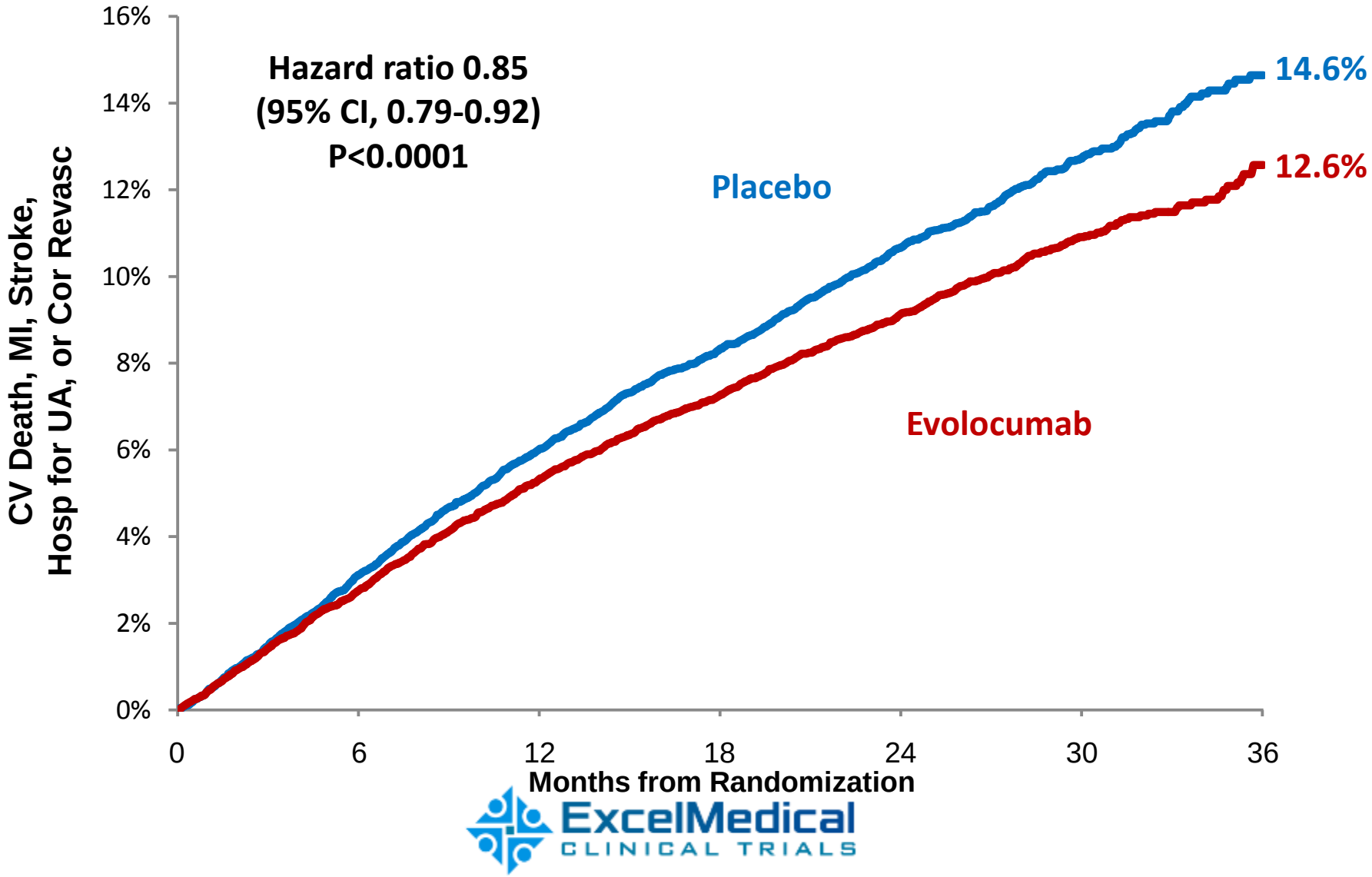
- More LDL receptors[1,2]
- Lower LDL-C[1,2]

- Reduced plasma levels of TC and LDL-C[1,3]

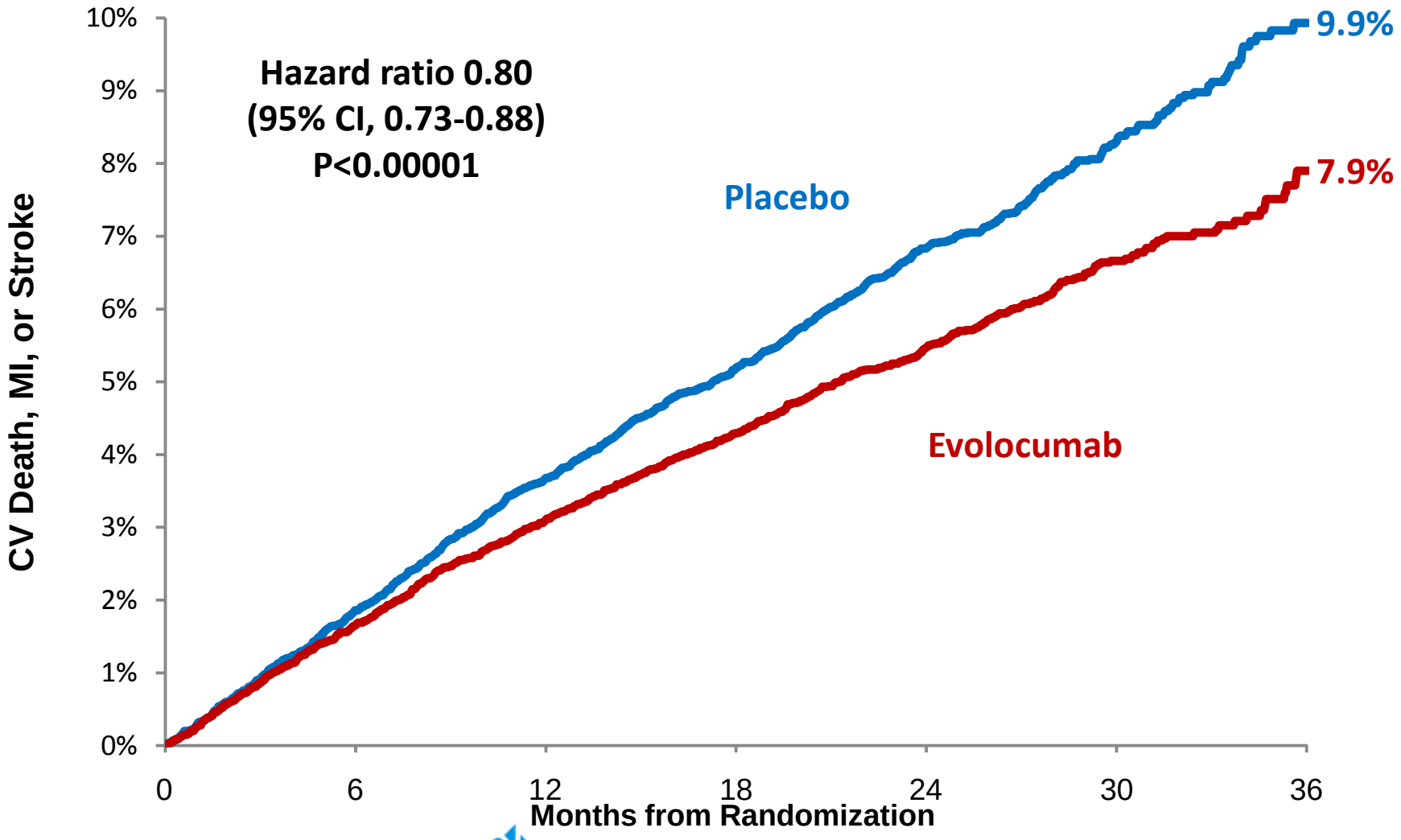
FH = familial hypercholesterolemia; GOF = gain of function; LDL-C = low-density lipoprotein cholesterol; LOF = loss of function; TC = total cholesterol.

1. Abifadel M, et al. *Hum Mutat*. 2009;30:520-529. 2. Seidah NG, et al. *Circ Res.* 2014;114:1022-1036. 3. Benn M, et al. *J Am Coll Cardiol.* 2010;55:2833:2842.

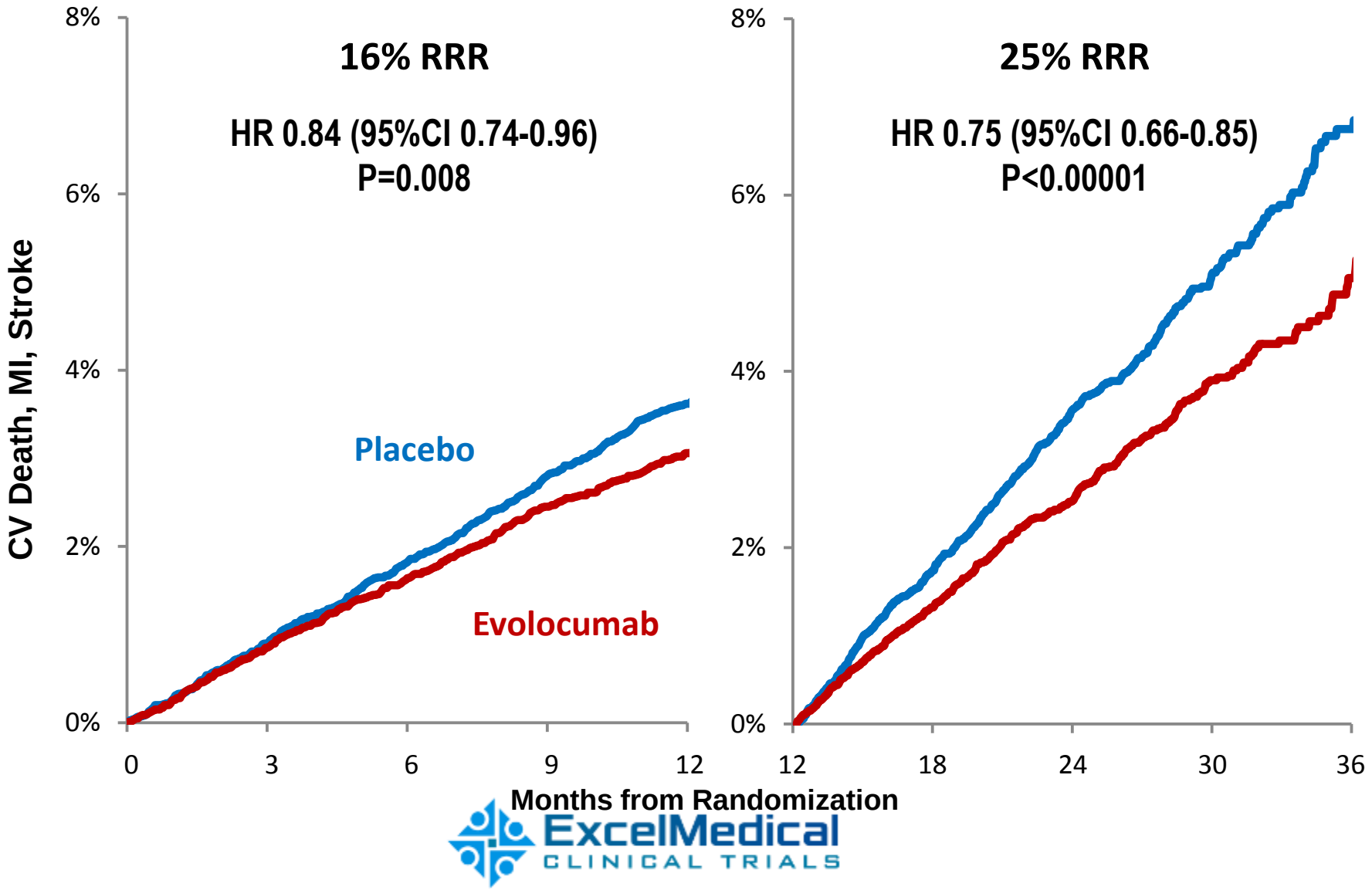
FOURIER Primary Endpoint



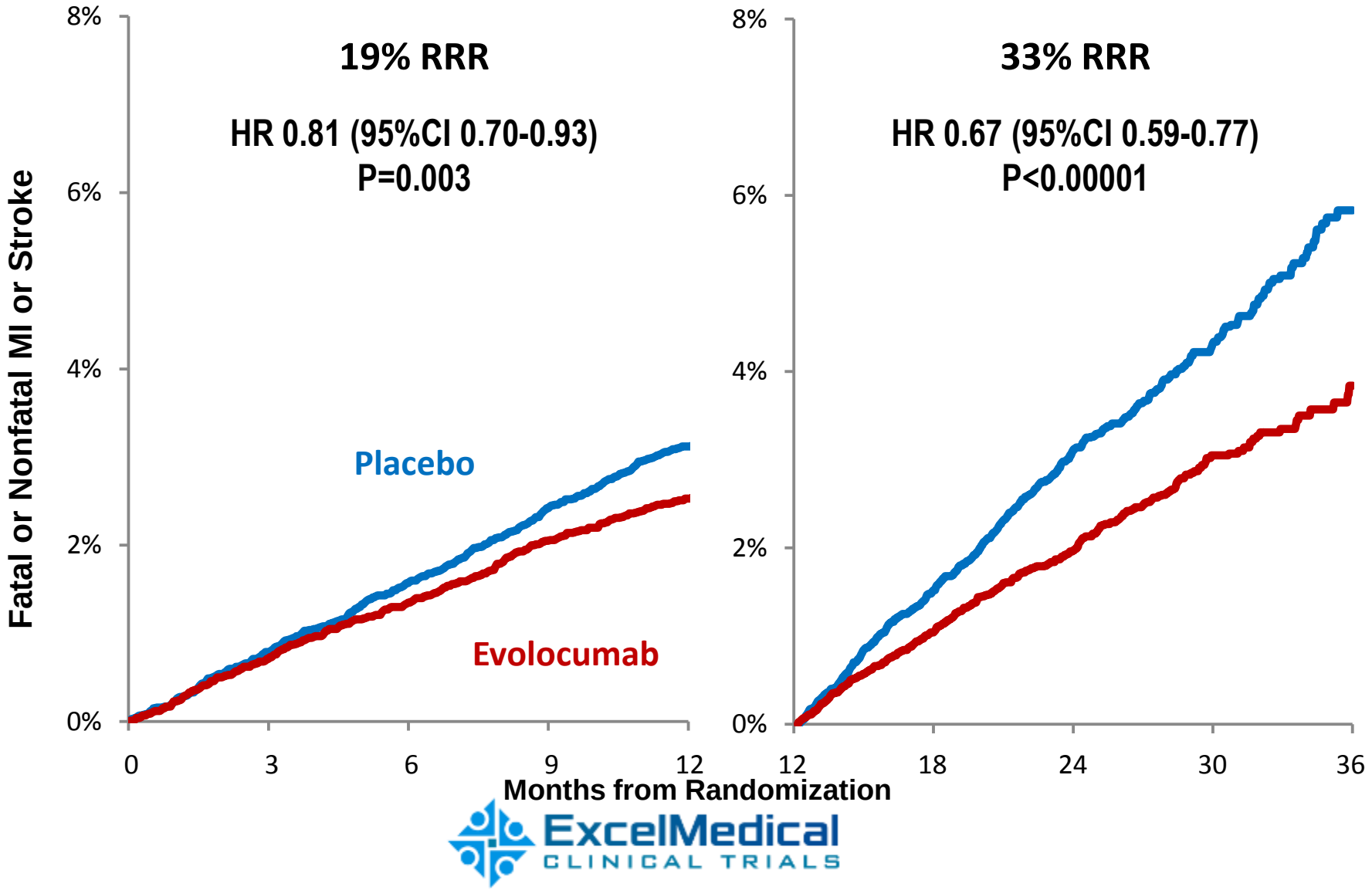
Key Secondary Endpoint



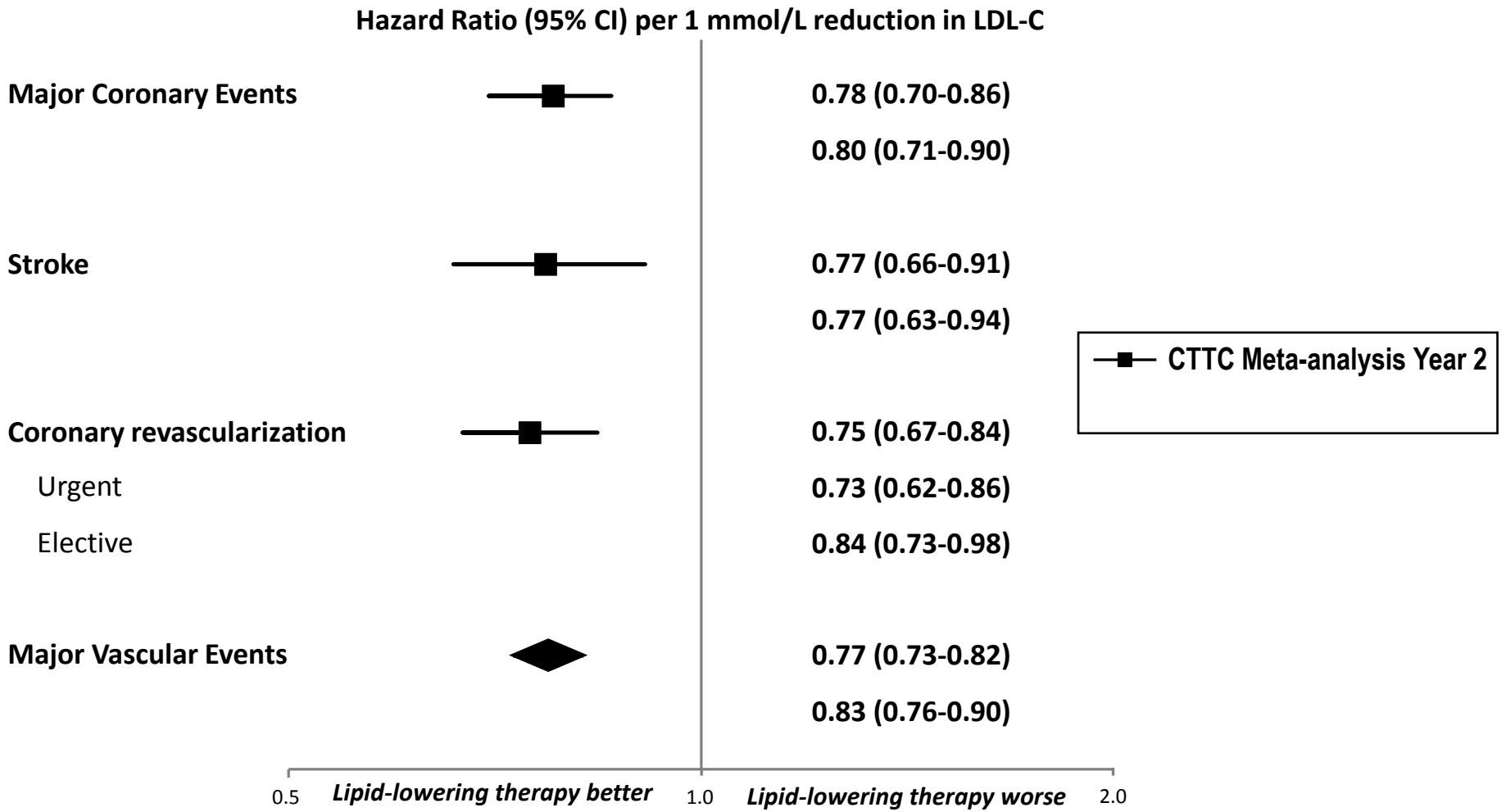
Landmark Analysis



Fatal or Nonfatal MI or Stroke

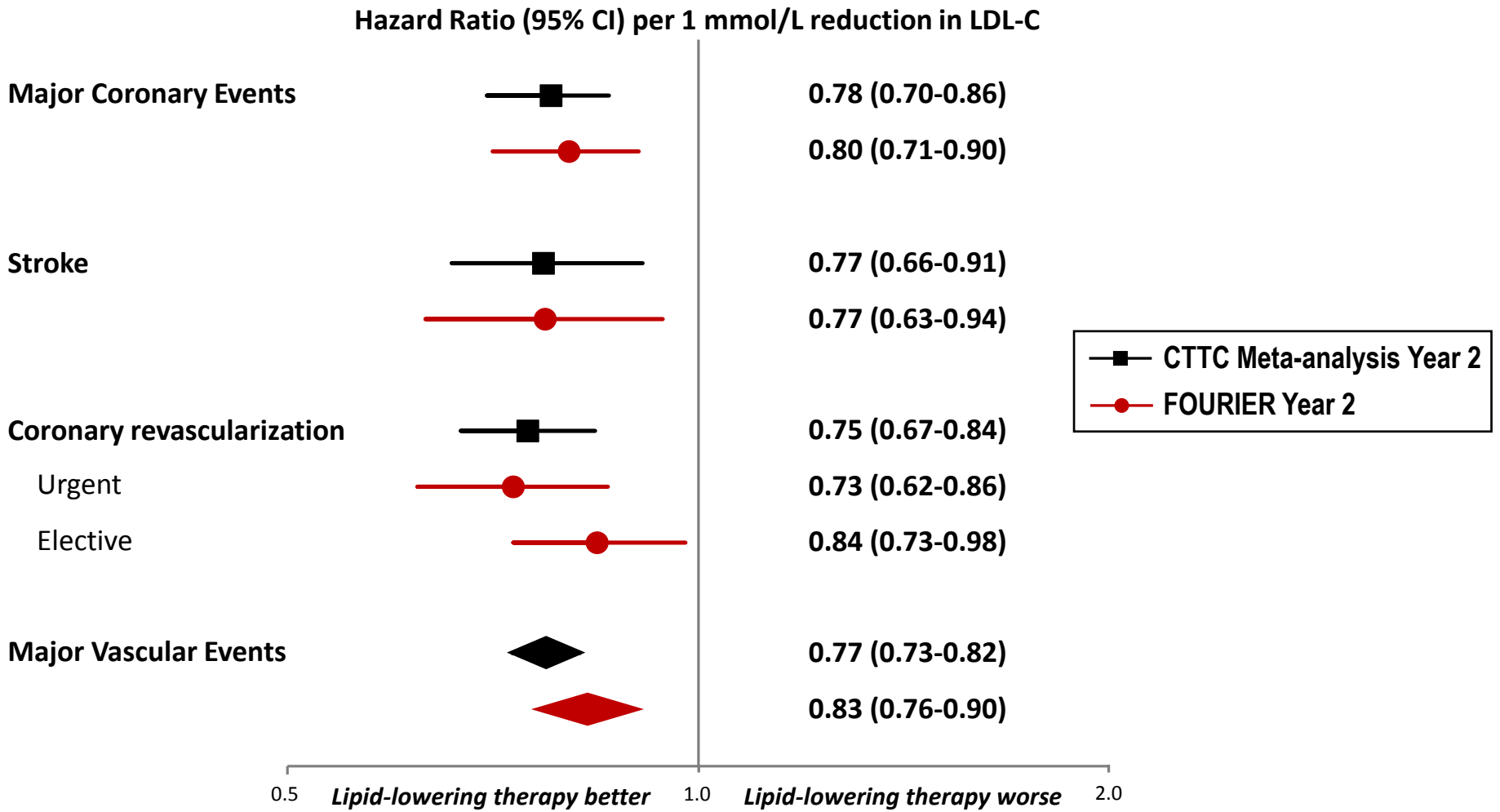


Comparison to Cholesterol Treatment Trialists Collaboration (CTTC)



CTTC data from *Lancet* 2010;376:1670-81

Comparison to Cholesterol Treatment Trialists Collaboration (CTTC)



CTTC data from *Lancet* 2010;376:1670-81

Safety

	Evolocumab (N=13,769)	Placebo (N=13,756)
Adverse events (%)		
Any	77.4	77.4
Serious	24.8	24.7
Allergic reaction	3.1	2.9
Injection-site reaction	2.1	1.6
Treatment-related and led to d/c of study drug	1.6	1.5
Muscle-related	5.0	4.8
Diabetes (new-onset)	8.1	7.7
Neurocognitive	1.6	1.5
Laboratory results (%)		
Binding Ab	0.3	n/a
Neutralizing Ab	*none*	n/a

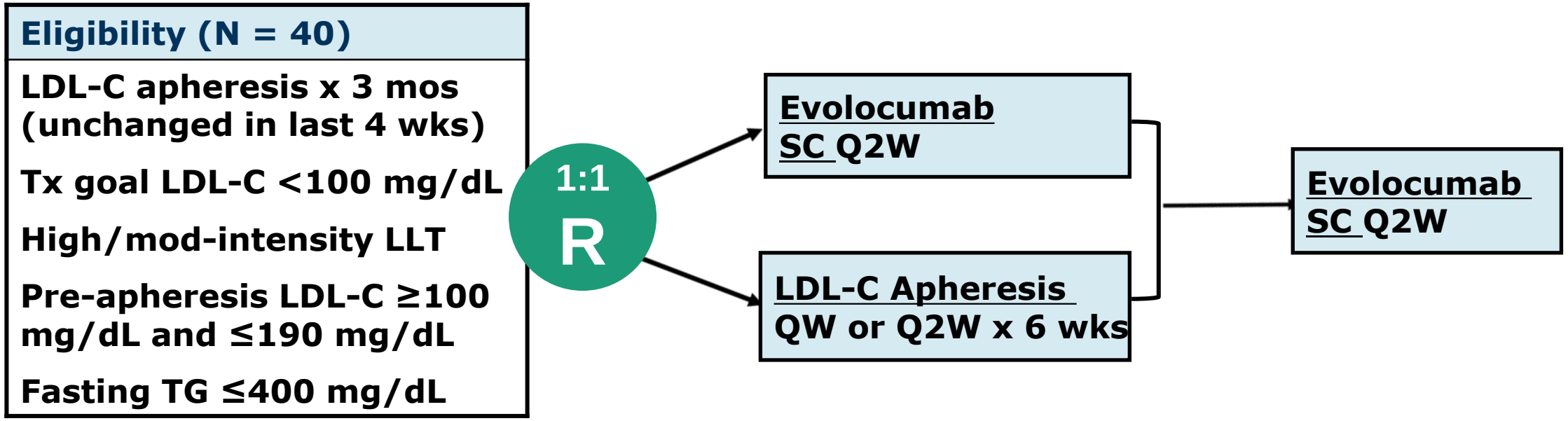
New-onset diabetes assessed in patients without diabetes at baseline; adjudicated by CEC

PCSK9 Summary

- PCSK9 regulates hepatic surface expression of LDLR and, in turn, systemic LDL-C levels
- Mutations in PCSK9 (both GOF and LOF) influence LDL-C levels as well as ASCVD risk
- PCSK9 is an excellent therapeutic target for LDL reduction
- Two PCSK9i were FDA approved the summer of 2015
  - But our prescriptions for this novel therapy were being consistently denied… And, now we know that evolocumab significantly reduced MI and CVA in FOURIER. But, denials persist!

ExcelMedical CLINICAL TRIALS

Amgen 20140316 Study Design



Primary endpoint:
Apheresis avoidance at end of randomized therapy (wks5-6)

Secondary endpoints:
Percent changes from baseline in LDL-C, non-LDL-C and CHOL/HDL-C

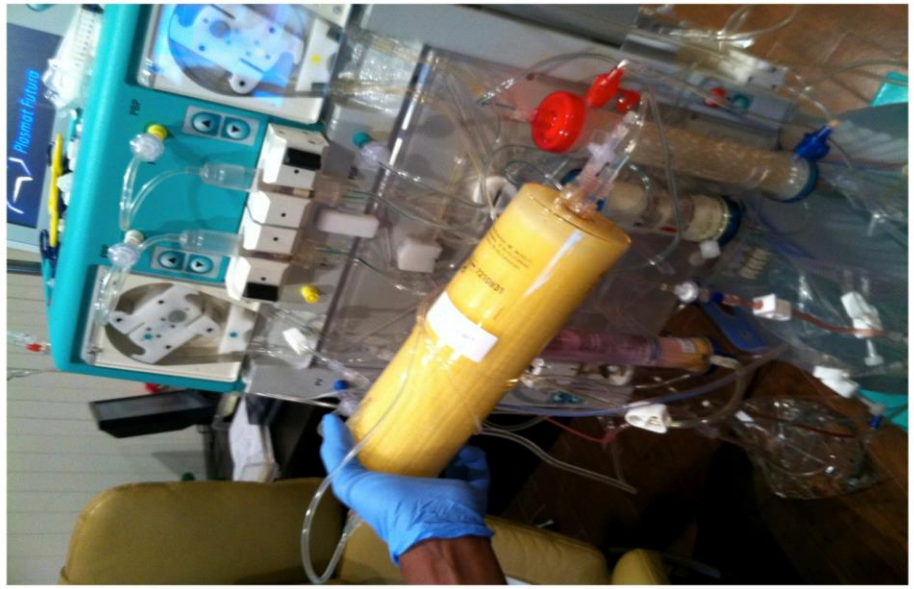
Clinicaltrials.gov (NCT02585895)

ExcelMedical CLINICAL TRIALS

Lipoprotein Apheresis Effective and “Comfortable”

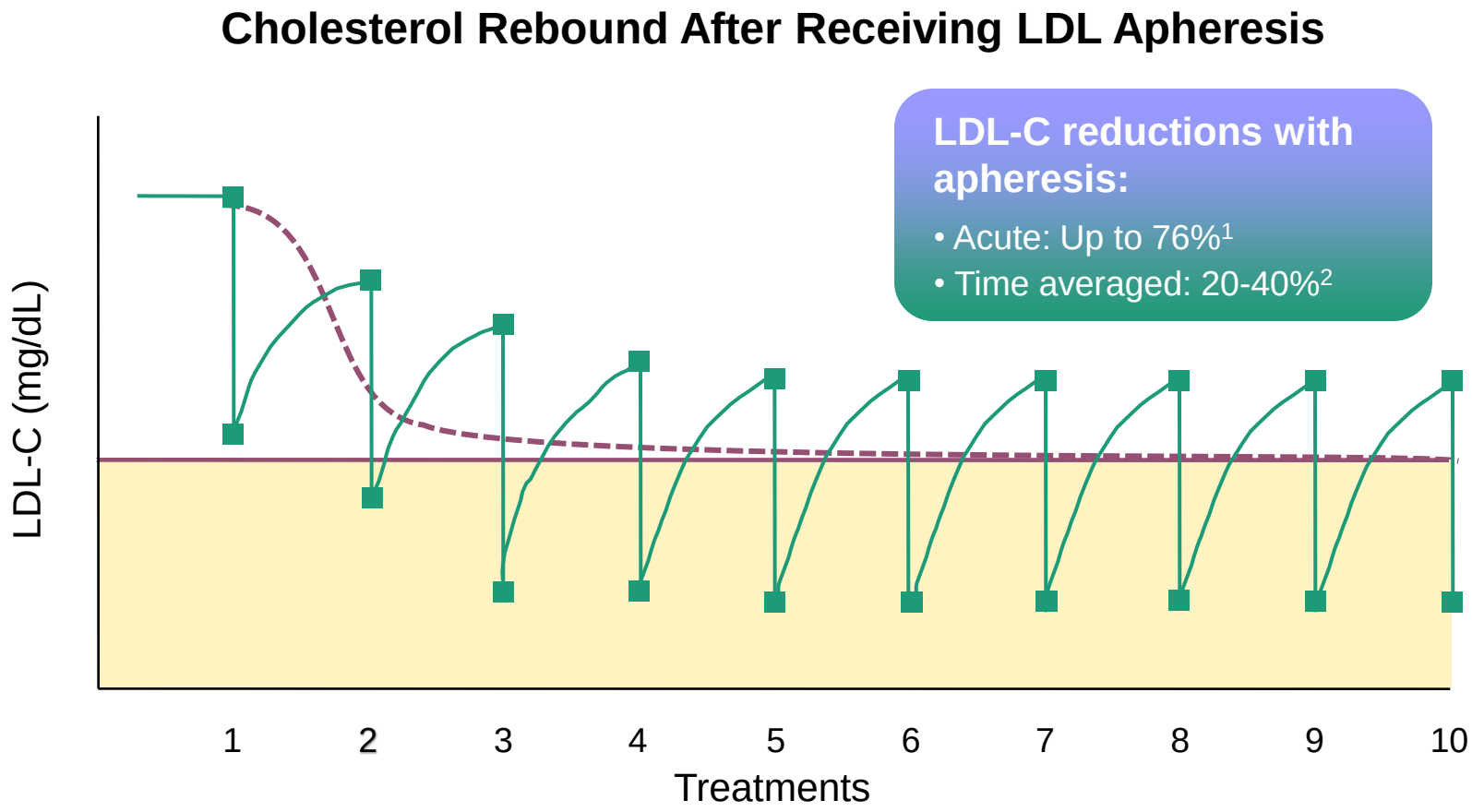
ExcelMedical CLINICAL TRIALS

End of Treatment: White Filter Becomes Yellow



ExcelMedical
CLINICAL TRIALS

LDL-Apheresis Demonstrated 72% Risk Reduction



Curr Opin Lipidol. 2010.

1. Gordon et al. *Am J of Card*. 1998;81:407-11.
2. Ito et al. *J Clin Lipidol.* 2011;5(3 Suppl):S38-S45.

ExcelMedical CLINICAL TRIALS

Clinical Studies
1002-038-048 Bempedoic Acid

- LDL lowering with and without CVD
- LDL lowering in statin intolerance
- LDL lowering in different LLT combinations

ExcelMedical
CLINICAL TRIALS

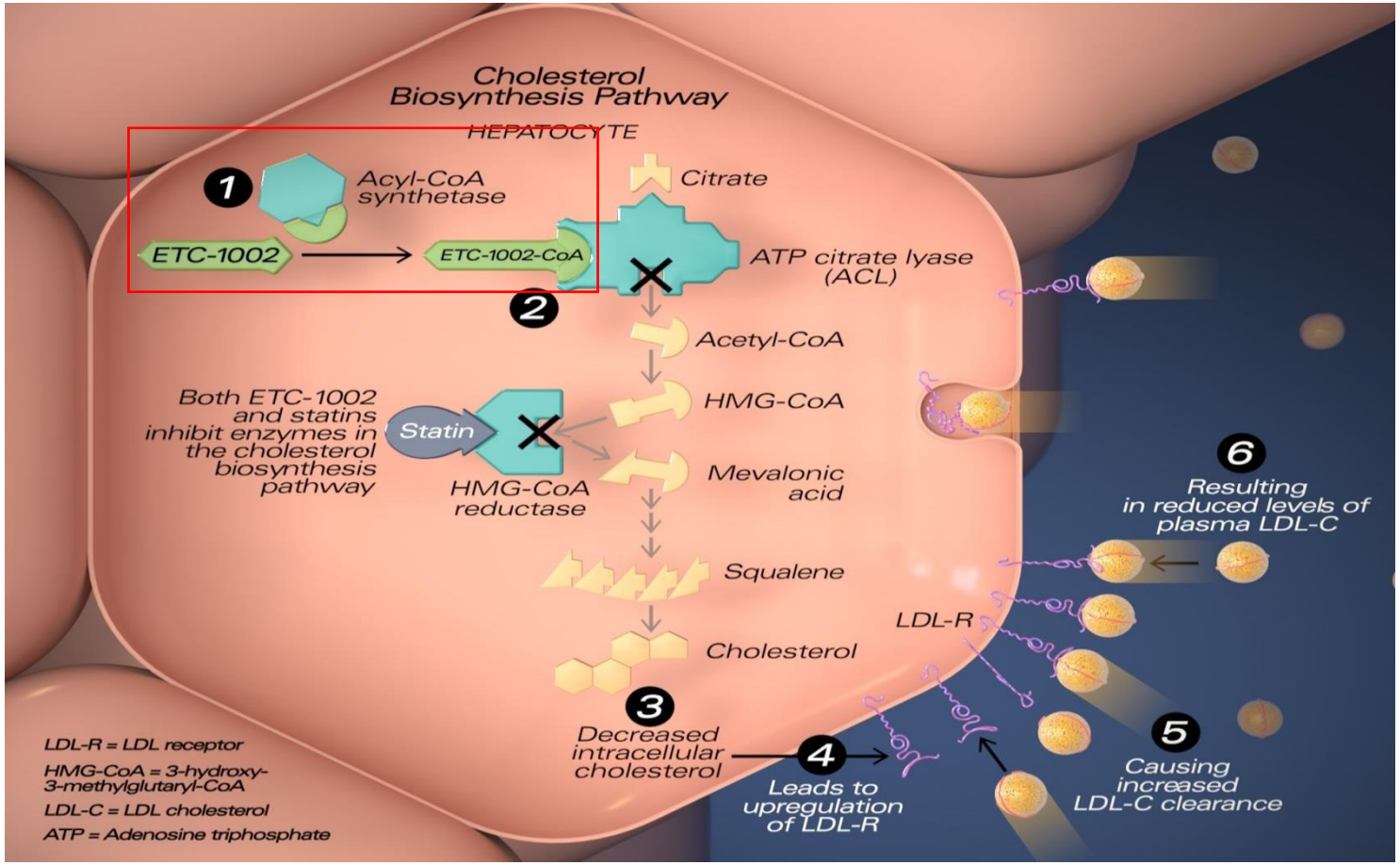
Bempedoic acid: Lowering LDL-C

- **Developing a differentiated oral therapy for treatment of patients with elevated LDL-cholesterol (LDL-C)**
  - Works “like a statin”; but with reduced potential for muscle-related side effects
  - Targeting statin intolerance in patients with elevated LDL-C, a high unmet medical need
- **ETC-1002 (bempedoic acid) lowers elevated LDL-C**
  - Up to 30% as monotherapy and 50% in combination with ezetimibe
  - Incremental 24% LDL-C lowering when added to stable statin therapy
- **Lowers high-sensitivity C-reactive protein (hsCRP) up to 44%**
- **Appears safe and well tolerated**
- **Continuing progress on Phase 3 global development plans**
  - Robust Phase 1 & 2 program completed in >1,000 patients
  - Phase 3 global development plans to be formalized and communicated Q2 2016

ExcelMedical CLINICAL TRIALS

Mechanism of action

BEMPEDOIC ACID REDUCES LDL-C VIA INHIBITION OF ATP-CITRATE LYASE (ACL)



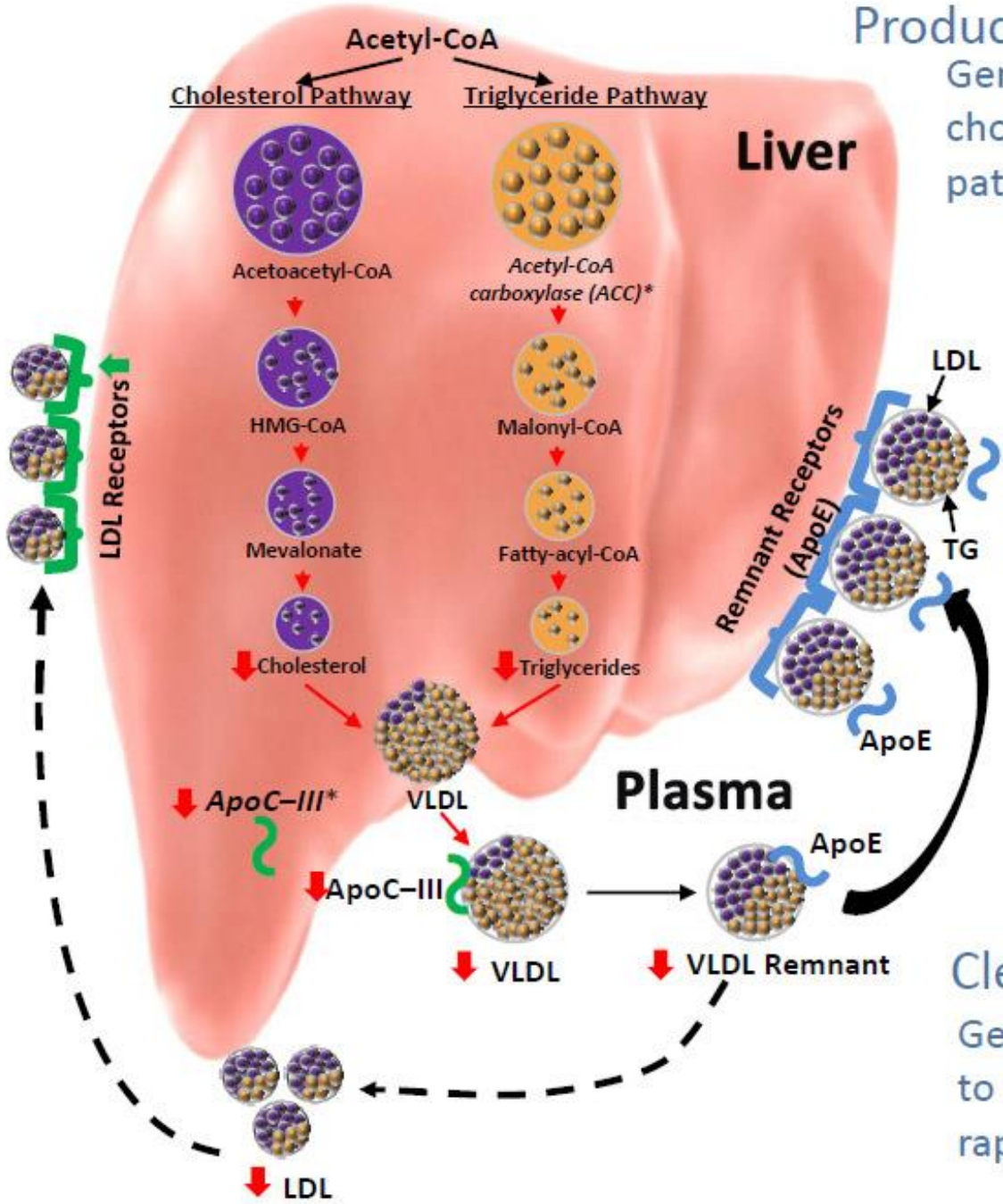
Bempedoic acid is converted to ETC-1002-CoA in the liver which directly inhibits ACL, reduces cholesterol synthesis, and up-regulates LDL receptor activity

ExcelMedical CLINICAL TRIALS

Clinical Studies Gemcabene

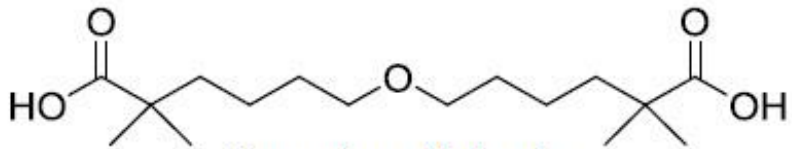
- LDL Lowering
- TG Lowering

ExcelMedical CLINICAL TRIALS



Production Mechanism:

Gemcabene reduces production of cholesterol and triglycerides pathways inside the liver



Gemcabene Molecule

- **Plasma half life of 32 to 41 hours**
- **Liver is target organ**
- **Gemcabene is the active compound**
- **Renal elimination**

**Potential molecular targets in the liver (ApoC-III, ACC)*

Clearance Mechanism:

Gemcabene clears VLDL efficiently due to a reduction in ApoC-III followed by rapid uptake by the remnant receptor

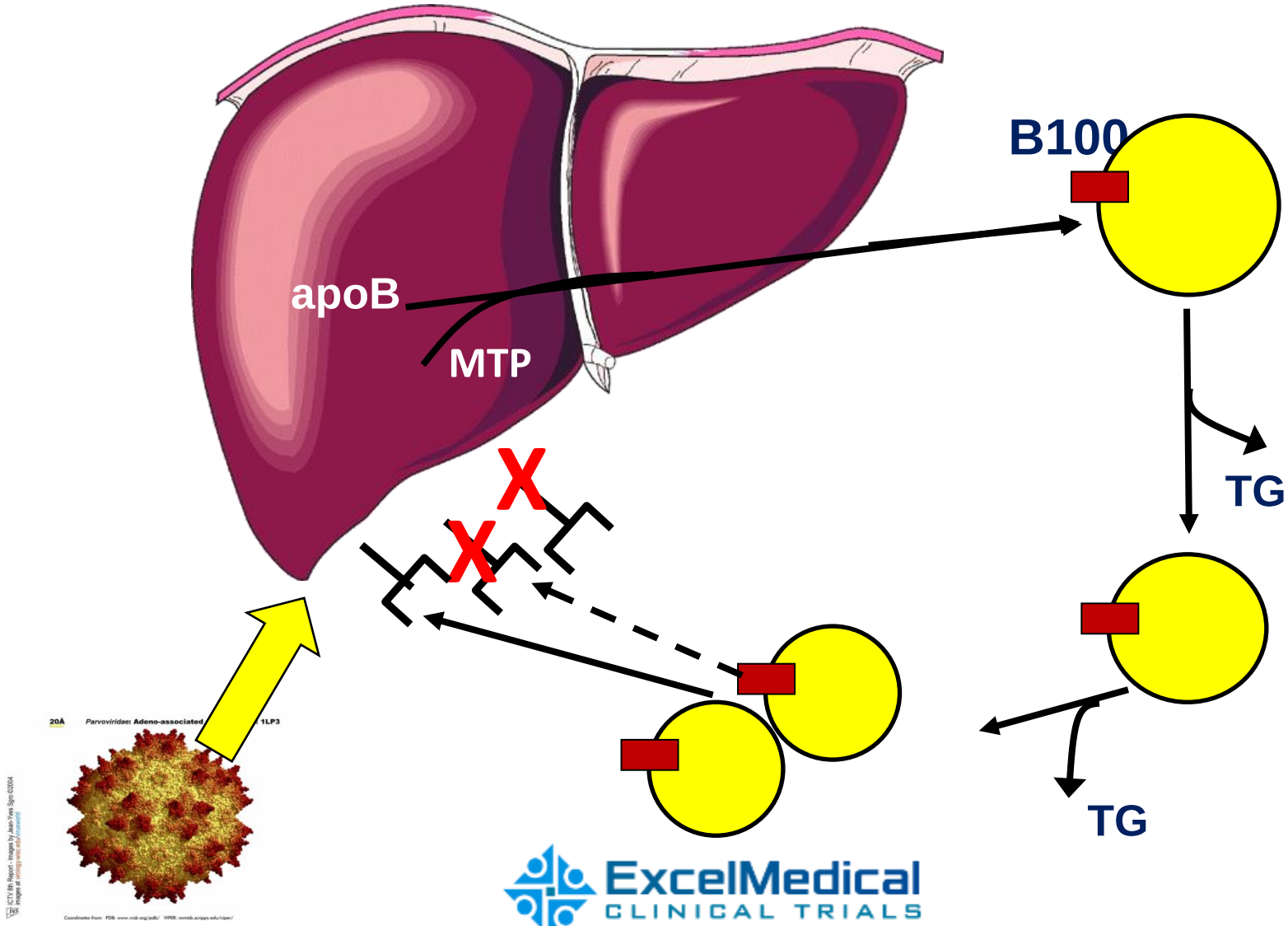
Clinical Studies
UPENN FHGT002

AAV8-mediated low density lipoprotein receptor (LDLR) gene replacement in subjects with homozygous familial hypercholesterolemia (HoFH)

- Molecularly defined LDLR mutations in both alleles and clinical presentation consistent with HoFH
- No cardiovascular event or cardiovascular intervention within 12 weeks of enrollment
- A baseline serum AAV8 neutralizing antibody titer ≤ 1:10

ExcelMedical
CLINICAL TRIALS

Is AAV8-LDLR administration safe?
Does it reduce LDL-C levels in HoFH?



Clinical Studies
Hyperlipoproteinemia(a) CS6

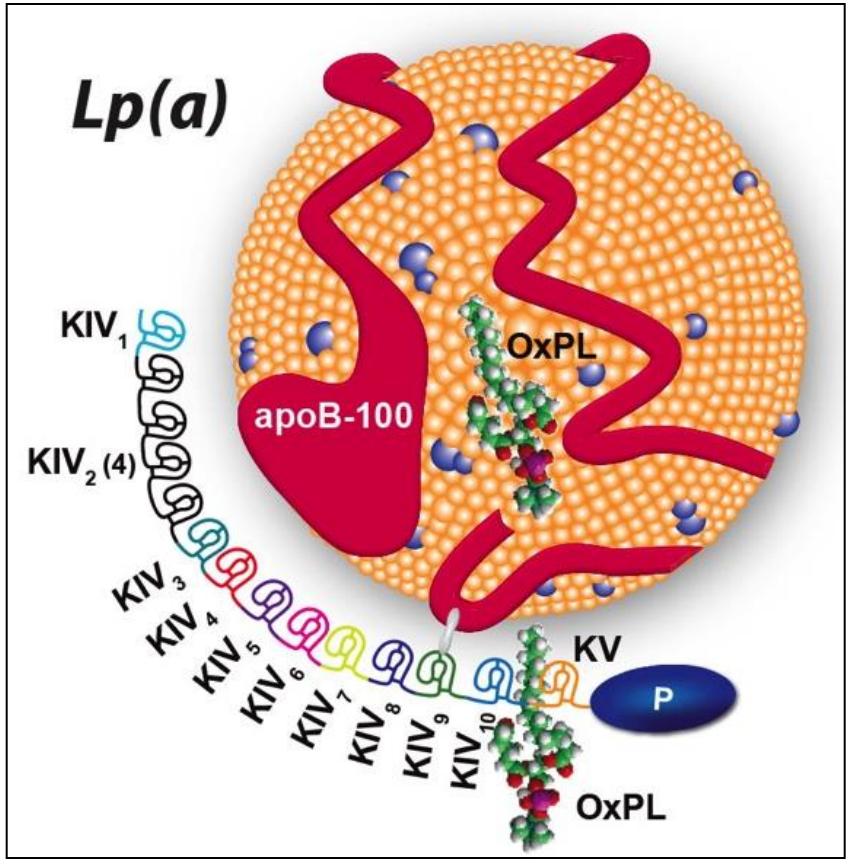
A randomized, double-blind, placebo-controlled, dose-ranging phase 2 study of ISIS 681257 administered subcutaneously to patients with hyperlipoproteinemia(a) and established cardiovascular disease (CVD)

- **Lp(a) ≥ 60 mg/dL + CVD (documented CAD, stroke or PAD)**
- **Exclusions: Warfarin, direct thrombin inhibitors, factor Xa inhibitors (DOAC and NOACs)**

ExcelMedical CLINICAL TRIALS

Structure, Metabolism and Pathophysiology of Lp(a)

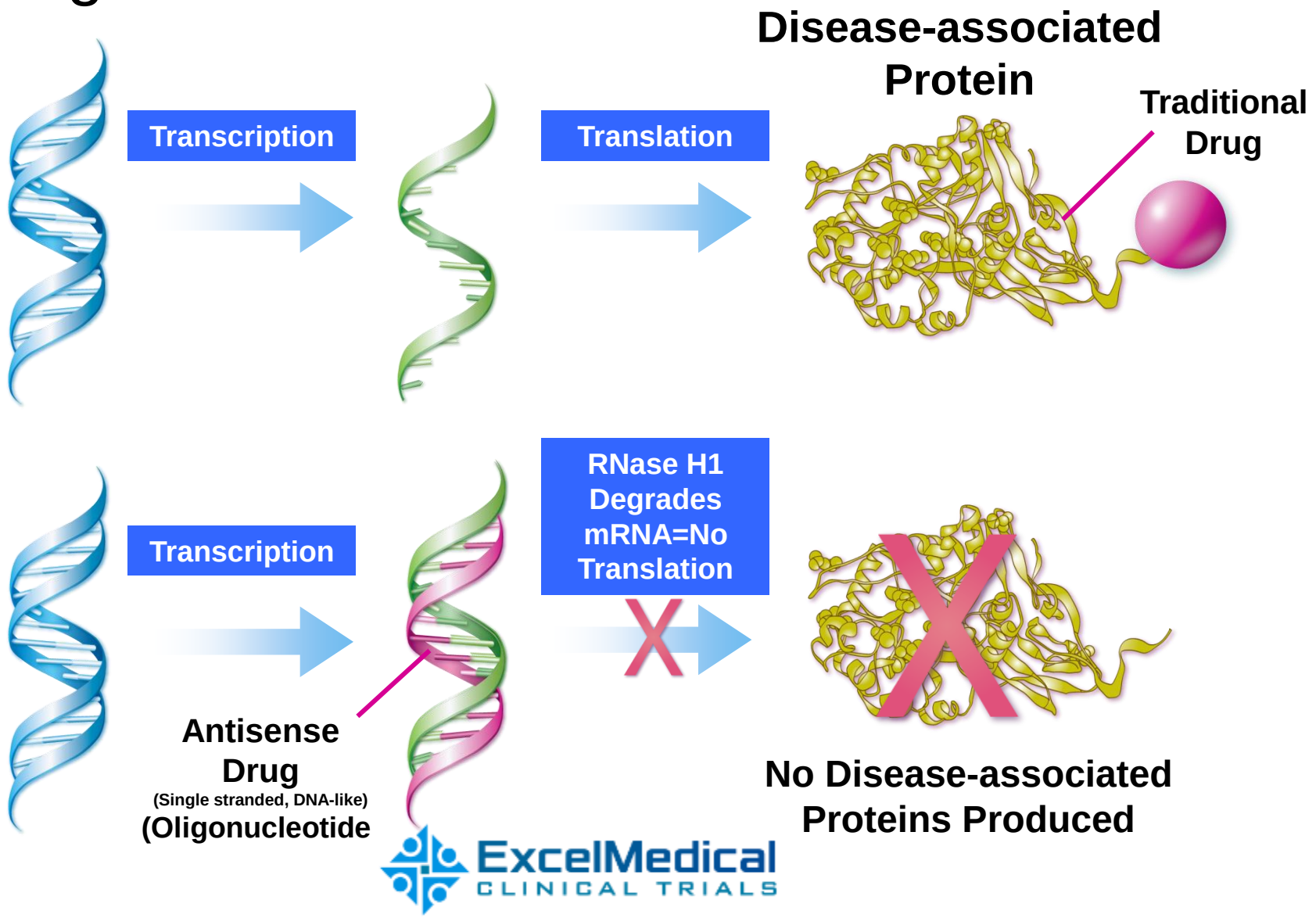
- Lp(a) MR studies prove ASCVD Causation
- Lp(a) is composed of apo(a) covalently bound to apoB-100 of LDL
- Plasma Lp(a) levels are >90% genetically determined.
- Apo(a) is produced in hepatocytes and is the primary determinant of plasma Lp(a) levels. Clearance of Lp(a) plays a secondary role.
- Currently, there is no effective therapy to potently and specifically lower Lp(a) levels
- Lp(a) is pro-atherogenic via three major components
  - 1—Its cholesterol content
  - 2– Its apo(a) content which may make it pro-thrombotic
  - 3—Its content of OxPL



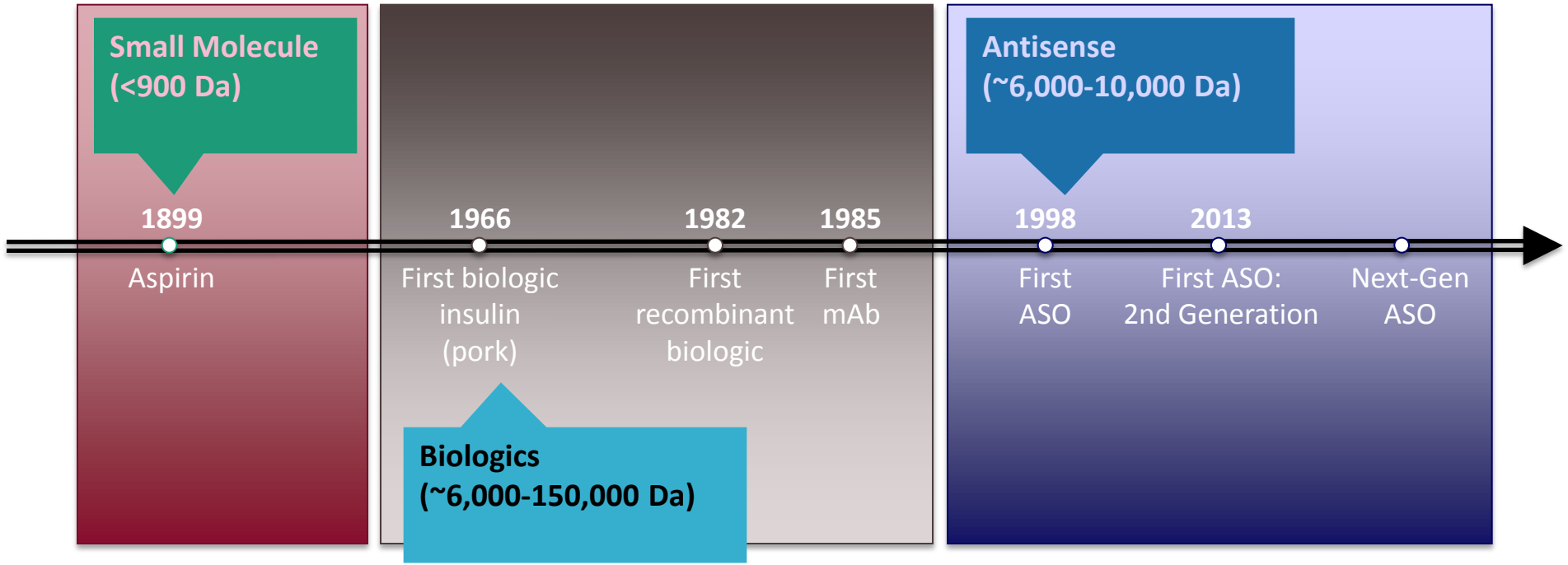
Bergmark et al JLR 2008, Leibundgut et al *JACC* 2012 and JLR 2013, Rao *ATVB* 2015, JLR cover figure 2016

ExcelMedical CLINICAL TRIALS

Antisense Drugs Prevent the Translation of a Specific Targeted Protein



Antisense – The 3rd Wave of Pharmacology



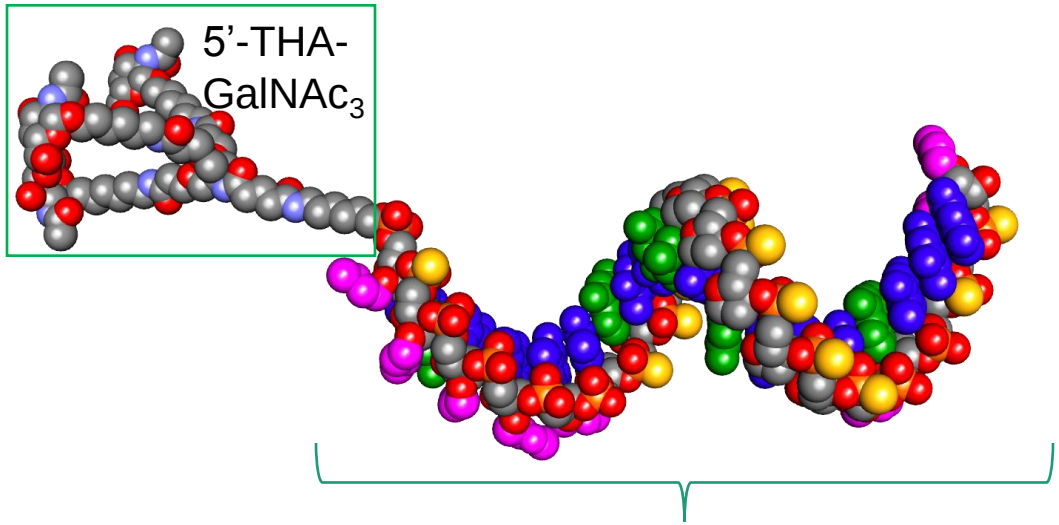
McCamish M, et al. *The RPM Report.* July/August 2013.US FDA. Press Release. January 29, 2013.
U.S. Food and Drug Administration. Drugs at FDA. Drug Approval Package. NDA: 20-961. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/98/20961_Vitravene.cfm. April 10, 2002. Accessed October 5, 2015.

ExcelMedical CLINICAL TRIALS

Differentiating Pharmacologic Properties of ASOs

- Highly Specific: reduced potential for off-target binding
  - ASOs hybridize specifically to their target mRNA through Watson-Crick base-pair interactions without affecting other mRNAs
- No known drug−drug interactions: metabolized without CYP450 enzymes
  - 90% of drugs are metabolized by CYP450 enzymes, mostly in the liver
  - ASOs are not metabolized via the CYP450 pathway, but rather by endonucleases and exonucleases and are excreted in the urine
- Intact blood brain barriers and placental barriers are impermeable to ASOs

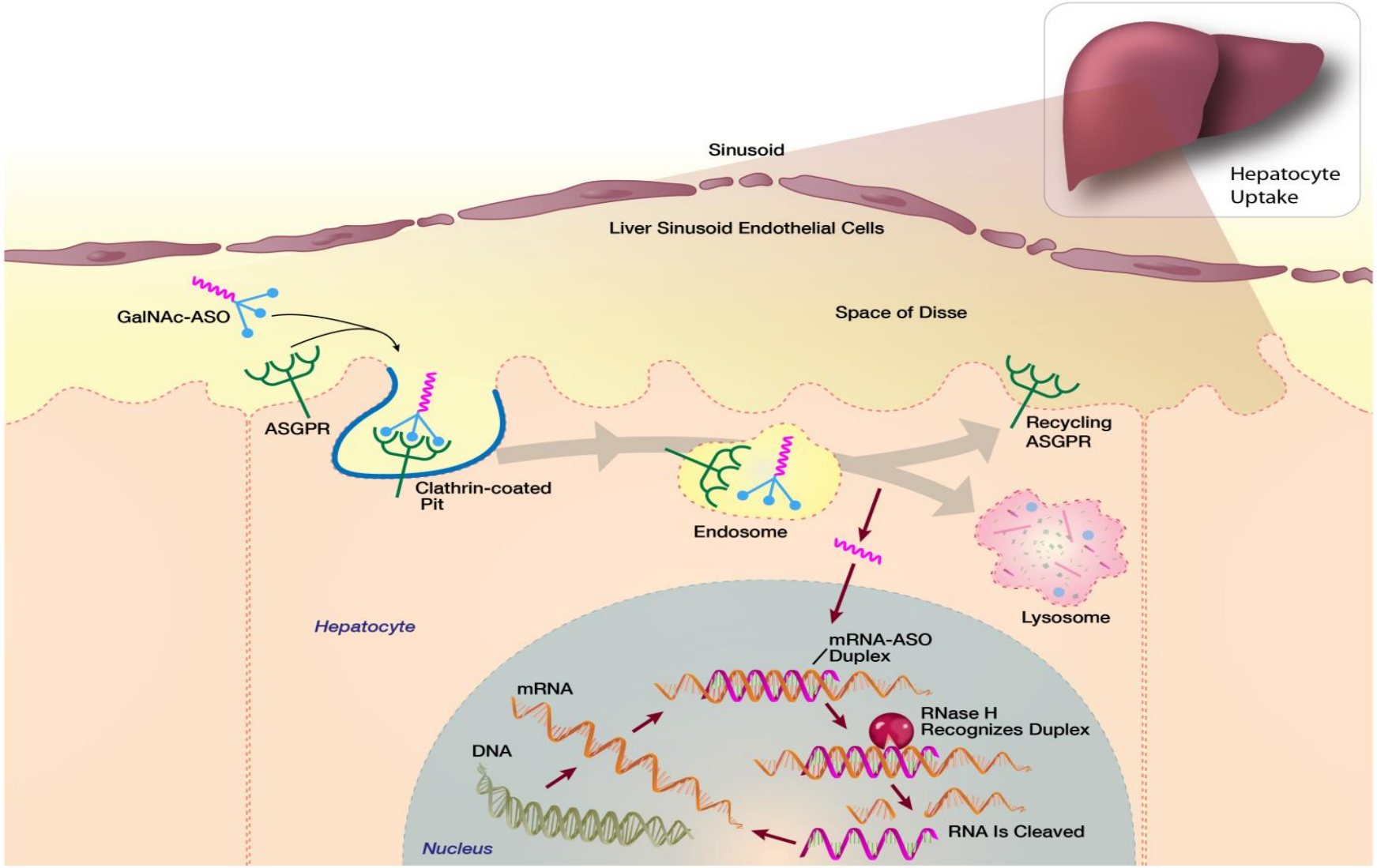
Significant Advantages of $GalNAc_3$ Conjugate of ISIS 681257



- $ASO\text{-}GalNAc_3$ conjugate approach results in enhanced ASO delivery to hepatocytes versus non-parenchymal cells.
- $GalNAc_3$-ASO conjugate is rapidly cleaved to liberate the free ASO.
- Results in equal potency at approximately $1/15^{th}$ of the dose compared to the unconjugated ASOs.
- Greatly reduced dose results in proportionally reduced class effects.
- Flexible dosing - weekly, monthly, quarterly

ExcelMedical
CLINICAL TRIALS

Mechanism of Action of $GalNAc_3$-Conjugated Antisense Oligonucleotides



Prakash et al Nucl Acid Res 2014

ExcelMedical CLINICAL TRIALS

Clinical Studies
TG Lowering

- Strength
- CS-7

ExcelMedical
CLINICAL TRIALS

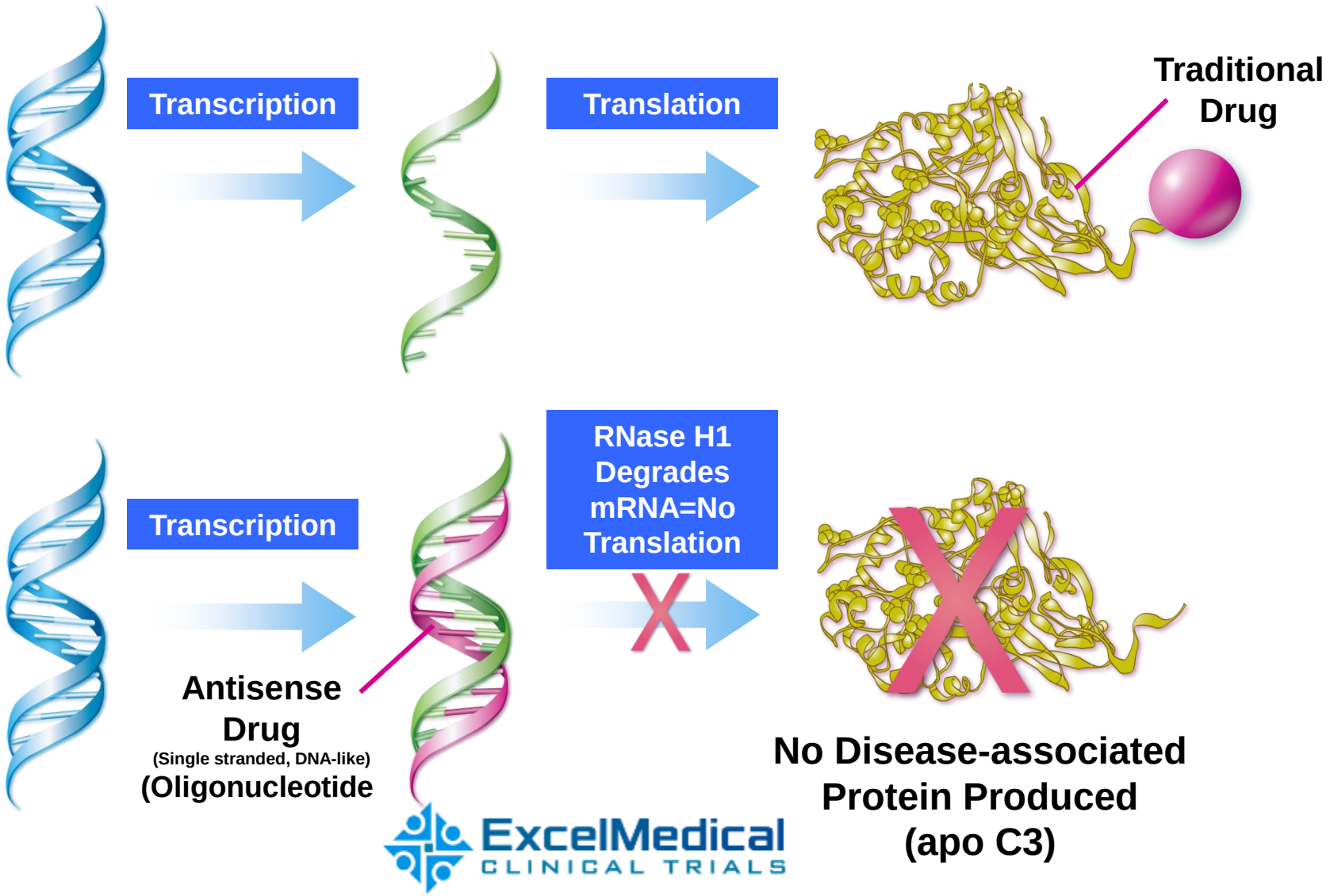
Clinical Studies
CS-7 APPROACH

An open-label extension study of volanesorsen administered subcutaneously to patients with familial chylomicronemia syndrome (FCS)

- FCS defined by lactescent serum or documentation of fasting TG ≥ 880 mg/dL
- Confirmed for loss of function mutations in Type 1 causing genes (such as LPL, apoC-II, GPIHBP1, or LMF1)
- Fasting TG ≥ 750 mg/dl at screening

ExcelMedical
CLINICAL TRIALS

Volanesorsen is an Antisense Drug that Prevents the Translation of apo C3



What's in a Name?
Familial Chylomicronemia Syndrome

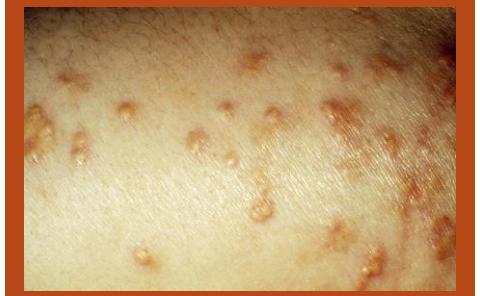
- *Chylomicronemia syndrome*
- *Chylomicronemia, familial*
- *Familial chylomicronemia*
- *Hyperchylomicronemia familial*
- ***Hyperlipemia idiopathic Burger-Grutz type***
- *Hyperlipoproteinemia Type I*
- *Lipase D deficiency*
- ***Lipoprotein lipase deficiency (LPLD)***
- *Burger-Grutz syndrome*
- *Endogenous hypertriglyceridemia*
- *Familial fat-induced hypertriglyceridemia*
- ***Familial hyperchylomicronemia***
- *Familial LPL deficiency*
- ***Hyperlipidemia Type I (Fredrickson)***
- *Hyperlipoproteinemia Type IA*
- *Lipase D deficiency*

US Department of Health & Human Services. National Center for Advancing Translational Services. Genetic and Rare Diseases Information Center. https://rarediseases.info.nih.gov/gard/6414/hyperlipoproteinemia-type-1/synonyms. Accessed December 11, 2015; Genetics Home Reference. Familial lipoprotein lipase deficiency. http://ghr.nlm.nih.gov/condition/familial-lipoprotein-lipase-deficiency. December 7, 2015. Accessed December 11, 2015;; Brahm AJ Hegele RA. Chylomicronaemia—current diagnosis and future therapies. *Nat Rev Endocrinol.* 2015;11:352-362. doi:10.1038/nrendo.2015.26

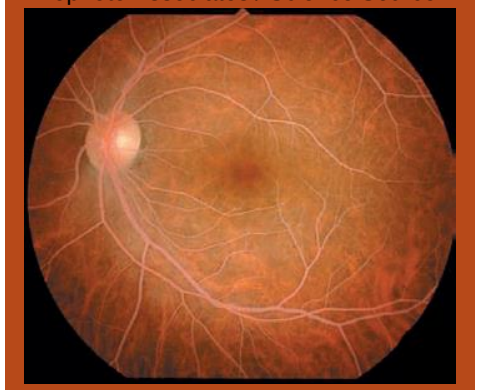
ExcelMedical CLINICAL TRIALS

What is FCS?

- **Background:**
  - Rare autosomal recessive disorder
  - Severely elevated levels of plasma TGs, generally unresponsive to lipid-lowering therapies[1-3]
- **Clinical expression/risk:**
  - Signs and symptoms:
    - Plasma lactescence and viscosity
    - Lipemia retinalis
    - Abdominal pain
    - Hepatosplenomegaly
    - Eruptive xanthomas
  - Pancreatic insufficiency
  - Recurrent acute pancreatitis/Chronic pancreatitis



Biophoto Associates / Science Source



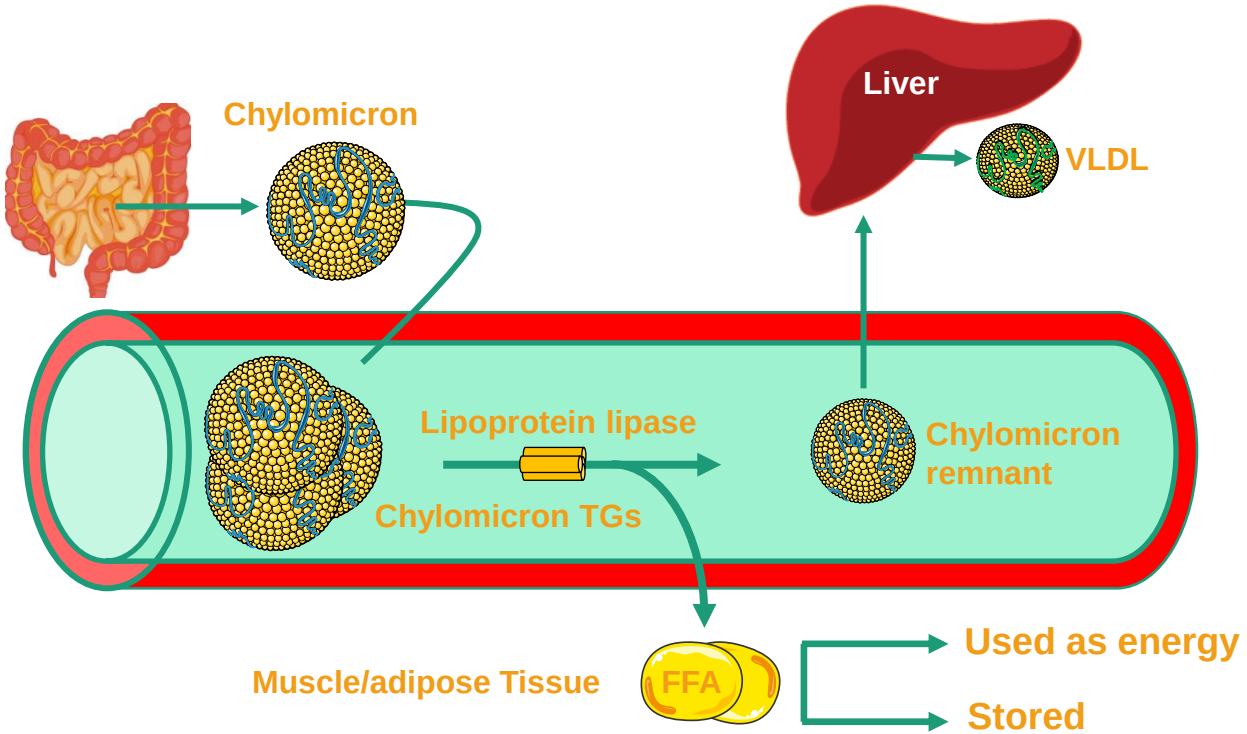
1. Brahm AJ, Hegele RA. Chylomicronaemia—current diagnosis and future therapies. *Nat Rev Endocrinol.* 2015;11:352-362. doi:10.1038/nrendo.2015.26. 2. Toth PP, Grabner M, Ramey N, Higuchi K. Clinical and economic outcomes in a real-world population of patients with elevated triglyceride levels. *Atherosclerosis.* 2014;237(2):790-797. doi:10.1016/
3. Brunzell JD. Familial lipoprotein lipase deficiency. In: Pagon RA, Adam MP, Ardinger HH, et al, eds. GeneReviews. Seattle, WA: University of Washington; 1999. http://www.ncbi.nlm.nih.gov/books/NBK1308/?report=printable. Updated 2014. Accessed May 4, 2016;

ExcelMedical CLINICAL TRIALS

Familial Chylomicronemia Syndrome (FCS): Pathophysiology

Chylomicrons in healthy individual

- Large lipoprotein particles that transport TGs derived from dietary fat[1,2]
- Appear in circulation shortly after a meal; cleared after a few hours[3]
- TGs broken down by LPL to FFAs, which are used by various tissues[2]



Adapted from Braham, *Nat Rev Endocrinol*, 2015.

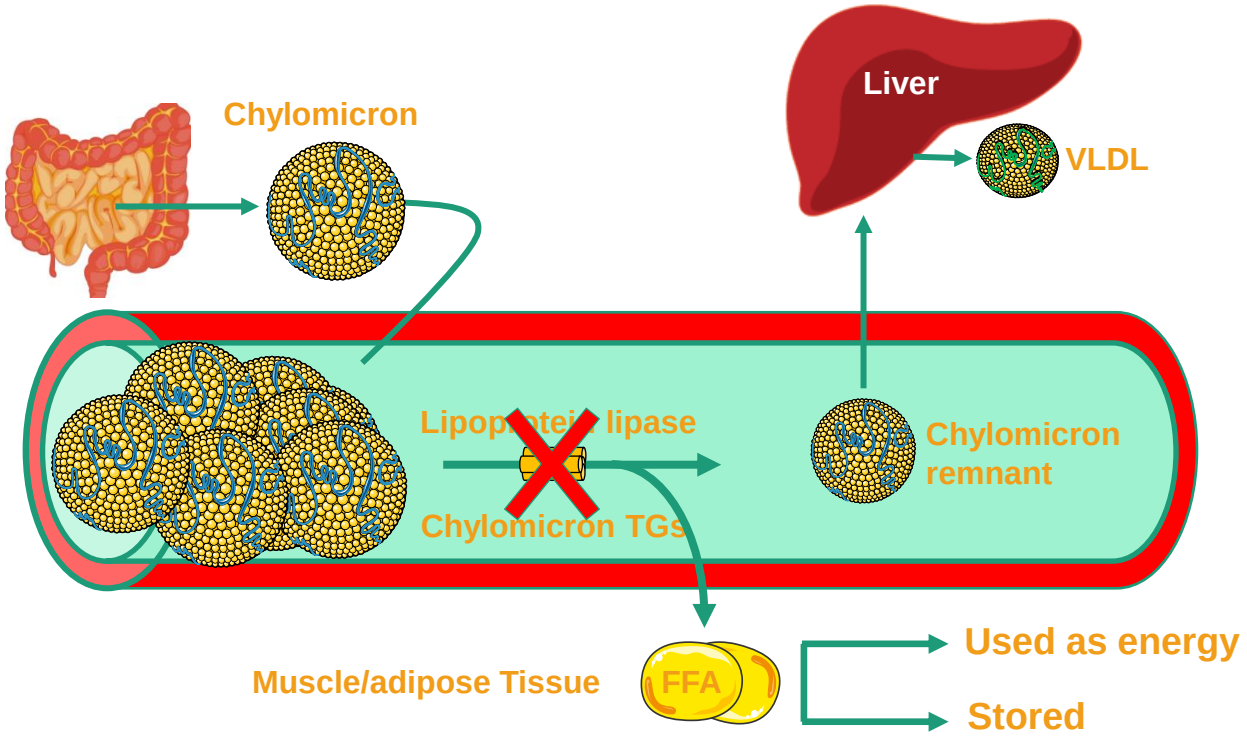
Abbreviations: FFA, free fatty acid; TG, triglyceride; VLDL, very low-density lipoprotein.

1. Brunzell JD, Bierman EL. Chylomicronemia syndrome: interaction of genetic and acquired hypertriglyceridemia. *Med Clin North Am.* 1982;66(2):455-468;
2. Gaudet D, Brisson D, Tremblay K, et al. Targeting APOC3 in the familial chylomicronemia syndrome. *N Engl J Med.* 2014;371(23):2200-2206. doi:10.1056/NEJMoa1400284; 3. Hall JE, Guyton AC. Lipid metabolism. In: *Guyton and Hall Textbook of Medical Physiology*. 13th edition. Philadelphia, PA: Elsevier; 2016. 863.

ExcelMedical CLINICAL TRIALS

FCS pathophysiology

- Chylomicronemia: pathological persistence of chylomicrons in plasma following a fasting period of 10 to 14 hours[1,2]
- In FCS, chylomicronemia is caused by inherited defects in chylomicron processing[2]



Adapted from Braham, *Nat Rev Endocrinol,* 2015.

Abbreviations: FFA, free fatty acid; TG, triglyceride; VLDL, very low-density lipoprotein.

1. Brunzell JD, Bierman EL. Chylomicronemia syndrome: interaction of genetic and acquired hypertriglyceridemia. *Med Clin North Am.* 1982;66(2):455-468;
2. Brahm AJ, Hegele RA. Chylomicronaemia—current diagnosis and future therapies. *Nat Rev Endocrinol.* 2015;11:352-362. doi:10.1038/nrendo.2015.26.

ExcelMedical CLINICAL TRIALS

Genetics: Known mutations responsible for FCS

Gene	Gene product function	Molecular features	% of Monogenic mutations
LPL	Hydrolysis of TGs and peripheral uptake of FFA	Severely reduced or absent LPL enzyme activity	95%
APOC2	Required cofactor of LPL	Absent or nonfunctional ApoC-II	2.0%
GPIHBP1	Stabilizes the binding of chylomicrons near LPL	Absent or defective GPI-HBP1	2.0%
APOA5	Enhancer of LPL activity	Absent or defective apoA-V	0.6%
LMF1	Chaperone molecule required for proper LPL folding	Absent or defective LMF1	0.4%

Adapted from Brahm, Nat Rev Endocrinol, 2015.

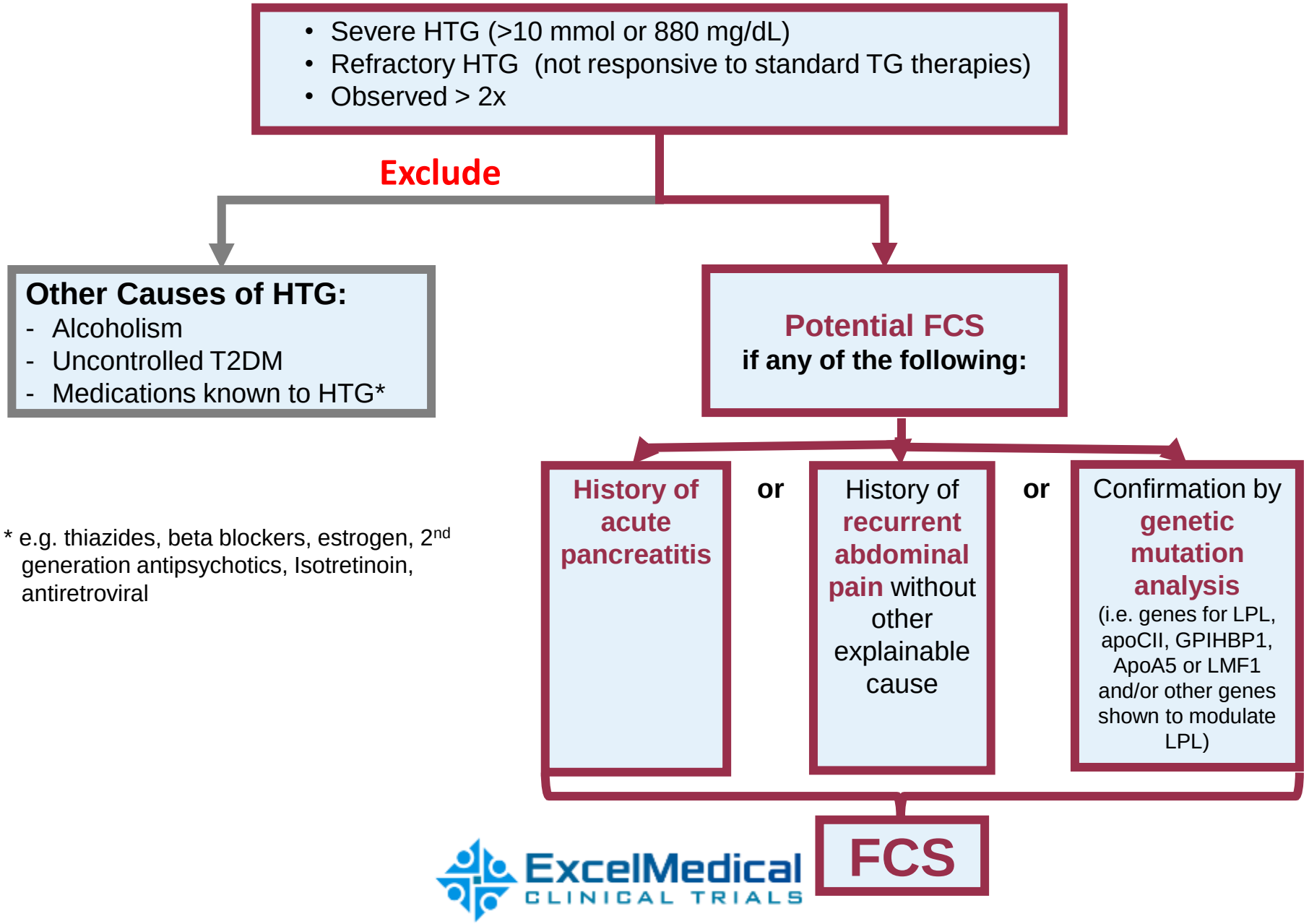
Abbreviations: FFA, free fatty acid; LPL, lipoprotein lipase; TG, triglyceride.

1. Brahm AJ, Hegele RA. Chylomicronaemia—current diagnosis and future therapies. *Nat Rev Endocrinol.* 2015;11:352-362. doi:10.1038/nrendo.2015.26.

2. Rodrigues R, Artieda M, Tejedor D, et al. Pathogenic classification of LPL gene variants reported to be associated with LPL deficiency. *J Clin Lipidol.* 2016;10(2):394-409. doi:10.1016/j.jacl.2015.12.015.

ExcelMedical CLINICAL TRIALS

Familial Chylomicronemia Syndrome – Clinical/Differential Diagnosis



* e.g. thiazides, beta blockers, estrogen, 2nd generation antipsychotics, Isotretinoin, antiretroviral

ExcelMedical CLINICAL TRIALS

FCS Management Goals

Reduce plasma triglyceride levels

Avoid abdominal pain and recurrent acute pancreatitis

Alleviate signs and symptoms/physical manifestations of FCS[1,2,3]

Reduce risk of long term consequences[1,4,5]

1. The physician's guide to lipoprotein lipase deficiency (LPLD). National Organization for Rare Disorders Physician Guides website. http://nordphysicianguides.org/wp-content/uploads/2015/04/NORD_Physician%E2%80%99s-Guide-to-Lipoprotein-Lipase-Deficiency.pdf. Published 2015. Accessed May 5, 2016; 2. Brunzell JD. Familial lipoprotein lipase deficiency. In: Pagon RA, Adam MP, Ardinger HH, et al, eds. *GeneReviews.* Seattle, WA: University of Washington; 1999. http://www.ncbi.nlm. nih.gov/books/NBK1308/?report=printable. Updated 2014. Accessed May 4, 2016; 3. Afari ME, Shafqat H, Shafi M, Marmoush FY, Roberts MB, Minami T. Hypertriglyceridemia-induced pancreatitis: a decade of experience in a community-based teaching hospital. *R I Med J* (2013). 2015;98(12):40-43. 4. Brahm AJ, Hegele RA. Chylomicronaemia—current diagnosis and future therapies. *Nat Rev Endocrinol.* 2015;11:352-362. doi:10.1038/nrendo.2015.26; 5. Jacobson TA, Ito MK, Maki KC, et al. National lipid association recommendations for patient-centered management of dyslipidemia: part 1—full report. *J Clin Lipidol.* 2015;9(2):129-169. doi:10.1016/j.jacl.2015.02.003;

ExcelMedical CLINICAL TRIALS

There are no FDA approved drugs indicated to lower triglycerides in FCS patients

Fibrates[1,2]	Fish oils[3,4]	Niacin[5]	Statins[6,7]
Increase lipolysis and elimination of TG-rich particles **by activating lipoprotein lipase** Decrease VLDL-C, increase HDL-C	Increased β-oxidation of DGAT **Increase plasma lipoprotein lipase activity** Decrease hepatic TG synthesis	Inhibits release of free fatty acids from adipose tissue **Increases lipoprotein lipase activity** Decreases hepatic synthesis of VLDL, LDL	Block cholesterol synthesis Increase number of hepatic LDL receptors to enhance uptake and catabolism of LDL Inhibit hepatic synthesis of VLDL and LDL

FCS patients are generally unresponsive to lipid-lowering therapies[8]

*Proposed; exact mechanisms may not be fully delineated.

Abbreviations: DGAT, diacylglycerol acyltransferase; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; TG, triglyceride; VLDL, very low-density lipoprotein; VLDL-C, very low-density lipoprotein cholesterol.

1. TRICOR [package insert]. North Chicago, IL: AbbVie Inc; 2016; 2. LOPID [package insert]. New York, NY: Parke-Davis; 2010; 3. VASCEPA [package insert]. Bedminster, NJ: Amarin Pharma Inc; 2015; 4. LOVAZA [package insert]. Research Triangle Park, NC: GlaxoSmithKline, 2014; 5. NIASPAN [package insert]. North Chicago, IL: AbbVie Inc; 2015; 6. CRESTOR [package insert]. Wilmington, DE: AstraZeneca Pharmaceuticals LP; 2015. 7. LIPITOR [package insert]. New York, NY: Parke-Davis; 2012; 8. Toth PP, Grabner M, Ramey N, Higuchi K. Clinical and economic outcomes in a real-world population of patients with elevated triglyceride levels. *Atherosclerosis.* 2014;237(2):790-797. doi:10.1016/j.atherosclerosis.2014.09.029. 9. Brunzell JD. Familial lipoprotein lipase deficiency. In: Pagon RA, Adam MP, Ardinger HH, et al, eds. *GeneReviews.* Seattle, WA: University of Washington, 1999. http://www.ncbi.nlm.nih.gov/books/NBK1308/?report=printable. Updated 2014. Accessed May 4, 2016.

ExcelMedical CLINICAL TRIALS

Management of FCS: Expert Dietary Guidance

Strict Lifelong Dietary Restriction

Sugar Restriction

Extreme low-fat diet (≤15% of energy)

Complete Avoidance of Alcohol

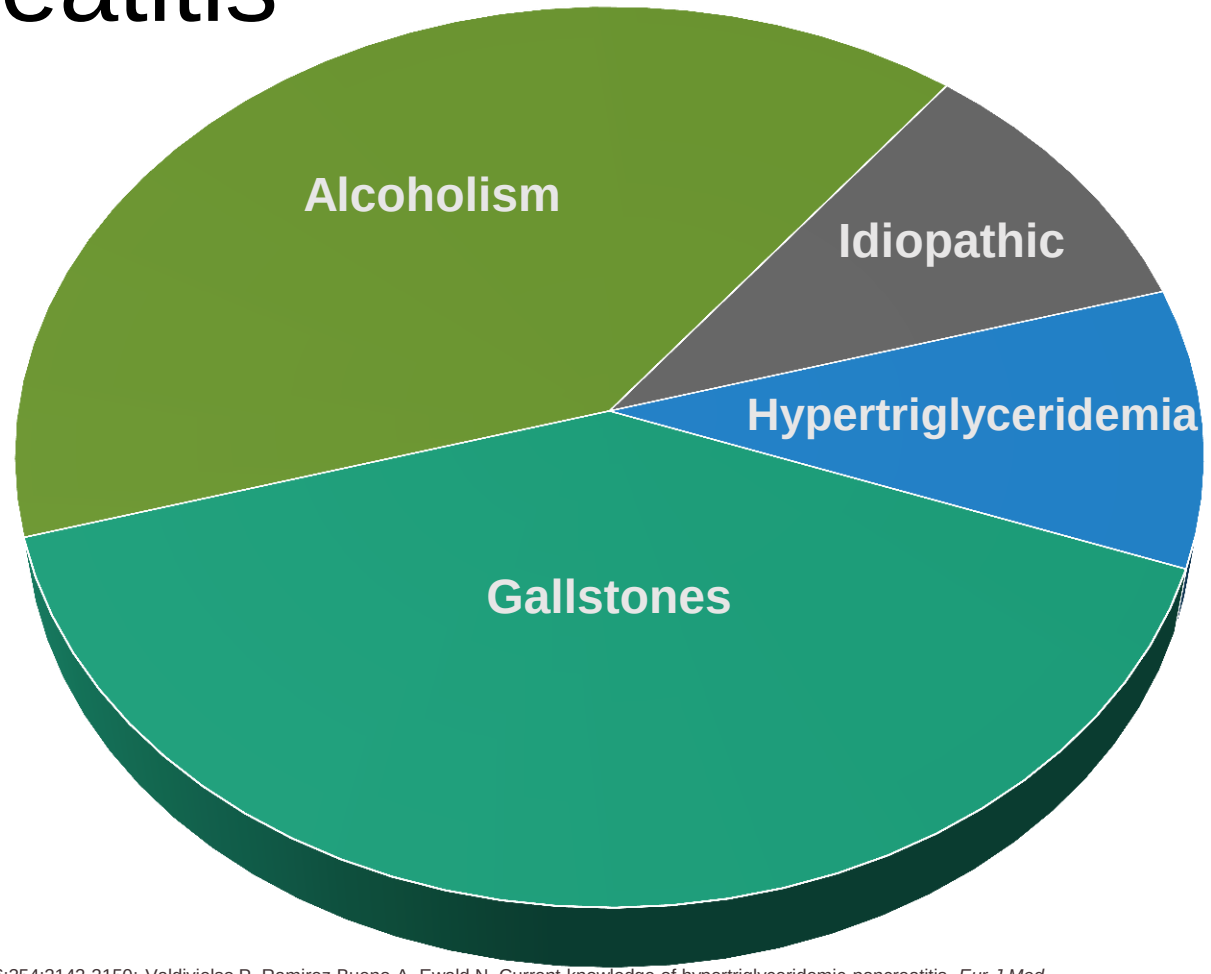
Although extremely difficult to follow, these mainstays of therapy can improve clinical manifestations[1,2,3,4-6]

Reduce risk of hepatosplenomegaly
Reduce abdominal pain
Reduce risk of xanthomas
Reduce risk of pancreatitis[1,2,3,4,5]

1. Brahm AJ, Hegele RA. Chylomicronaemia—current diagnosis and future therapies. *Nat Rev Endocrinol.* 2015;11:352-362. doi:10.1038/nrendo.2015.26; 2. Jacobson TA, Ito MK, Maki KC, et al. National lipid association recommendations for patient-centered management of dyslipidemia: part 1—full report. *J Clin Lipidol.* 2015;9(2):129-169. doi:10.1016/j.jacl.2015.02.003; 3. The physician's guide to lipoprotein lipase deficiency (LPLD). National Organization for Rare Disorders Physician Guides website. http://nordphysicianguides.org/wp-content/uploads/2015/04/NORD_Physician%E2%80%99s-Guide-to-Lipoprotein-Lipase-Deficiency.pdf. Published 2015. Accessed May 5, 2016; 4. Rahalkar AR, Hegele RA. Monogenic pediatric dyslipidemias: classification, genetics and clinical spectrum. *Mol Genet Metab.* 2008;93(3):282-294. doi:10.1016/j.ymgme.2007.10.007; 5. Pouwels ED, Blom DJ, Firth JC, Henderson HE, Marais AD. Severe hypertriglyceridaemia as a result of familial chylomicronaemia: the Cape Town experience. *S Afr Med J.* 2008;98(2):105-108. doi:10.7196/SAMJ.424; 6. Berglund L, Brunzell JD, Goldberg AC, et al. Evaluation and treatment of hypertriglyceridemia: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* 2012;97(9):2969-2989. doi:10.1210/jc.2011-3213

ExcelMedical CLINICAL TRIALS

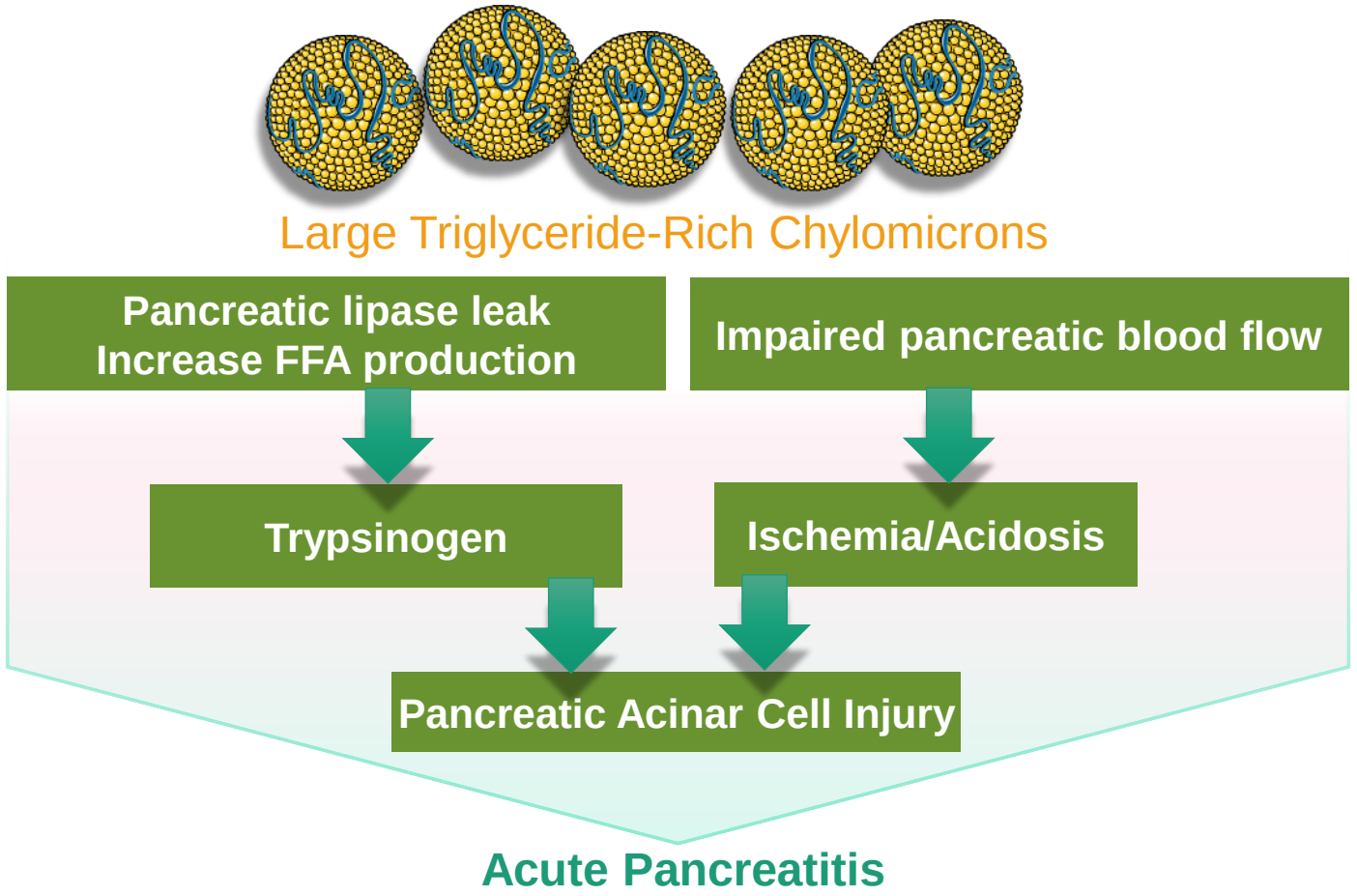
Hypertriglyceridemia is likely the third leading cause of acute pancreatitis



Whitcomb DC. Clinical practice. Acute pancreatitis. *NEJM*. 2006;354:2142-2150; Valdivielso P, Ramirez-Bueno A, Ewald N. Current knowledge of hypertriglyceridemic pancreatitis. *Eur J Med.* 2014;25:689-694; Ranson JH. Etiology and prognostic factors in human acute pancreatitis: a review. *Am J Gastroenterol.* 1982;77:633-638; Fortson MR, Freedman SN, Webster PD. 3rd Clinical assessment of hyperlipidemic pancreatitis. *Am J Gastroenterol.* 1995;90:2134-2139.

ExcelMedical CLINICAL TRIALS

Pathophysiologic Mechanism of Acute Pancreatitis in FCS Patients



Gan SI, et al. *World J Gastroenterol.* 2006;12:7197-7202; Valdivielso P, Ramirez-Bueno A, Ewald N. Current knowledge of hypertriglyceridemic pancreatitis. *Eur J Med.* 2014;25:689-694; Khokar AS, Seidner DL. The pathophysiology of pancreatitis. *Nutr Clin Pract.* 2004;19:5-15.

ExcelMedical CLINICAL TRIALS

Cardiovascular Inflammation Reduction Trial (CIRT)

A randomized, double-blind, placebo-controlled, event-driven trial of weekly low-dose methotrexate (LDM) in the prevention of cardiovascular events among stable coronary artery disease patients with Type 2 Diabetes or Metabolic Syndrome

- History of MI or multi-vessel CAD
- DM II or Metabolic Syndrome
- NIH Trial

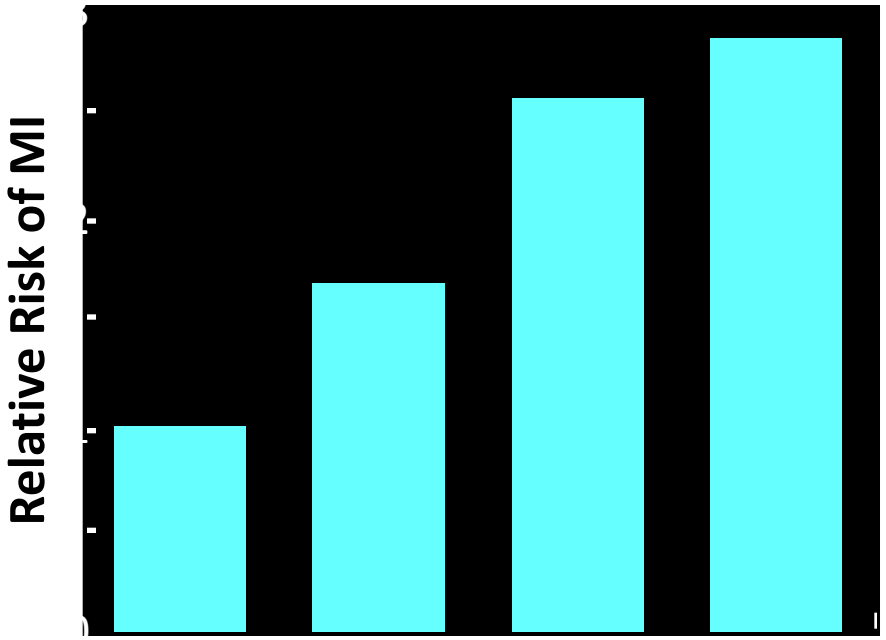
ExcelMedical CLINICAL TRIALS

Cardiovascular Inflammation Reduction Trial (CIRT)

hsCRP, a clinical biomarker of inflammation, is commonly used as a method to predict cardiovascular risk

Risk of future heart attack

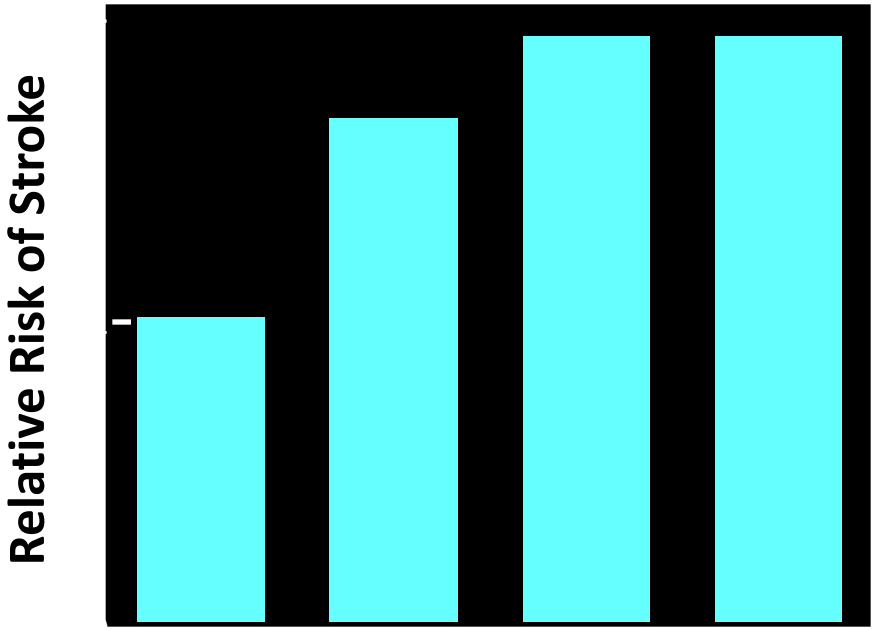
***P* Trend <0.001**



Quartile of hsCRP

Risk of future stroke

***P* Trend <0.01**



Quartile of hsCRP

Ridker et al, N Engl J Med 1997;336:973–979.

ExcelMedical CLINICAL TRIALS

CANTOS

- **Primary Goal**
- To determine whether long-term treatment with canakinumab (50 mg, 150 mg or 300 mg subcutaneous every three months) as compared to placebo will reduce rates of recurrent cardiovascular events among stable post-myocardial infarction patients who remain at elevated vascular risk as gauged by increased levels of hsCRP (≥2mg/L) despite usual care, including statin therapy.
- **Positive Trial**: Results in Barcelona 8/17

ExcelMedical CLINICAL TRIALS

Clinical Studies
1245.121 HFrEF & 1245.110 HFpEF

Phase III randomized, double-blind study to evaluate efficacy and safety of once daily empagliflozin 10 mg compared to placebo, in patients with heart failure.

ExcelMedical
CLINICAL TRIALS

Clinical Trials: The path to cure

- Once Cause and Effect have been proved, therapeutics should be optimized
- LDL
- TG
- Lp(a)
- Inflammation
- People want to participate in clinical trials

ExcelMedical
CLINICAL TRIALS