A Short History of PCSK9
From Discovery to Clinical Trials

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Disclosures

Speaker/Consultant:

Pfizer
Regeneron
Merck
Roche
Eli Lilly
## Timeline: From Discovery to Clinical Trials

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Discovery: PCSK9 Mutations Cause Dominant ↑LDL

Pedigree of family HC92

Segregation of Chr 1p34.1–p32

Nat Genet, 2003 (Boileau)
PCSK9 Mutations Cause Dominant ↑ LDL

Nat Genet, 2003 (Boileau)
Proc Natl Acad Sci, 2003 (Seidah)
Overexpression of PCSK9 Eliminates LDLRs in Liver and Increases LDL-C

Proc Natl Acad Sci, 2004 (Breslow); J Biol Chem, 2004 (Horton); J Biol Chem, 2004 (Seidah)
Normal Function of PCSK9 to Promote Degradation of LDLRs

**Hypothesis**

- **Gain of Function (GOF) mutations** $\rightarrow$ ↑ LDL
- **Loss of Function (LOF) mutation** $\rightarrow$ ↓ LDL

*Proc Natl Acad Sci*, 2004 (Breslow); *J Biol Chem*, 2004 (Horton); *J Biol Chem*, 2004 (Seidah)
Do LOF Mutations in PCSK9 Lower LDL?

Dallas Heart Study
n = 3,557
50% African-American

African-Americans
2%

LDL-C

<5%

C679X

Y142X

SS Prodomain Catalytic domain C-terminal

N - C
LOF Mutations in PCSK9 Lower LDL-C

European-Americans
3%

African-Americans
2%

LDL↓: 21%
40%
Plasma Cholesterol Levels are Reduced in PCSK9 KO Mice

- Wild-type
- Pcsk9^+/−
- Pcsk9^−/−

Plasma Lipid Concentrations (mg/dl)

- Cholesterol
  - Wild-type: 100 ± 5
  - Pcsk9^+/−: 70 ± 5
  - Pcsk9^−/−: 40 ± 5

- Triglycerides
  - Wild-type: 150 ± 10
  - Pcsk9^+/−: 120 ± 10
  - Pcsk9^−/−: 90 ± 10
PCSK9 Mutations: A Tool to Test Role of LDL-Cholesterol in CHD Risk

CHD Deaths (per 1000 / 10 y)

Does Higher LDL-C Alone Explain Difference between Shanghai and MRFIT?
Mendelian Randomization

= No DNA sequence variant

= DNA sequence variant that alters LDL-C level

VS

Compare CHD in
LOF Mutations in PCSK9 Lower CHD

ARIC Study (NIH): Eric Boerwinkle

<table>
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<tr>
<th>Year</th>
<th>African-Americans</th>
<th>European-Americans</th>
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<tbody>
<tr>
<td>2003</td>
<td>28%</td>
<td>15%</td>
</tr>
<tr>
<td>2004</td>
<td></td>
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</tr>
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<td>2013</td>
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LDL: 28% lower 15% lower

CHD (over 15 y): 88% lower 46% lower

Lancet, 2007 (McPherson)
NEJM, 2008 (Katherisan)
LDL-Lowering and Reduction in CHD

Reduction in CHD

Reduction in LDL-C

-100%
-50%
-10%
0

PCSK9
Y142X
C679X

APOB
SORT1
LDLR
R46L

Statins - 5 y

Phenotype (LDL level)

Genotype(s)

Static
- single measurement

Integrated $\int_{0}^{80\ y}$
- chronic exposure

Cumulative LDL-C: mg/dL X years
PCSK9: From Genes to Public Health

**A little too little**

<table>
<thead>
<tr>
<th>Year</th>
<th>LDL Goal</th>
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<tr>
<td>'87</td>
<td>140</td>
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<tr>
<td>'93</td>
<td>110</td>
</tr>
<tr>
<td>'01</td>
<td>100</td>
</tr>
<tr>
<td>'04</td>
<td>100</td>
</tr>
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**A little too late**

**NCEP Guidelines**

- **Risk Reduction (%)**
- **Age at Onset of Rx (years)**
  - 30: 140
  - 40: 120
  - 50: 110
  - 60: 100
  - 70: 90

?
Healthy, college graduate & aerobic instructor (now 38 y)
Zimbabwe: 21 y woman pregnant woman. LDL=16 mg/dL

Hooper et al., Atherosclerosis 193:445
PCSK9: New Target for LDL-Lowering & CHD Prevention

Discovery

Target Validation

3 Years
PCSK9: Target for LDL-Lowering

- **Efficacy** ✓
- **Safety** ✓ (?)
- **Mechanism of action** ?

**STATINS** ➔ **SREBP2** (transcription factor) ➔ **PCSK9** ➔ **LDLR** ➔ **LDL**
Parabiosis in Mice

PCSK9 acts extracellularly

PCSK9-Tg  WT

Immunoblot of liver proteins

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tg</th>
<th>WT</th>
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<tr>
<td>Parabiosis</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>LDLR</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>RAP</td>
<td>±</td>
<td>±</td>
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J Clin Invest, 2006 (Horton)
PCSK9 Promotes LDLR Degradation in Hepatocytes

- PCSK9

+ PCSK9

Normal
**PCSK9 Crystal Structure**

PCSK9

- C-terminal Domain
- Prodomain
- Catalytic Domain

*Nat Struc Mol Bio, 2007 (Pfizer); Structure, 2007 (Amgen); Proc Natl Acad Sci, 2007 (Novartis); Proc Natl Acad Sci, 2008 (Diesenhofer)*
PCSK9 & LDL Receptor

Adapted from Brown and Goldstein
Therapeutic Approaches to Inhibit PCSK9 Action

1. Inhibit enzymatic activity
   - Small molecule

2. Disrupt PCSK9:LDLR interaction
   - Anti-PCSK9 antibody
   - Peptides or small molecule

3. Inhibit PCSK9 synthesis
   - RNAi
   - Anti-sense RNA
Inhibiting PCSK9 Lowers LDL-C in Animals....

J Lipid Res, 2007
Proc Natl Acad Sci, 2008
Proc Natl Acad Sci, 2009
Effect of a Monoclonal Antibody to PCSK9 on LDL Cholesterol

Evan A. Stein, M.D., Ph.D., Scott Mellis, M.D., Ph.D.,
George D. Yancopoulos, M.D., Ph.D., Neil Stahl, Ph.D., Douglas Logan, M.D.,
William B. Smith, M.D., Eleanor Lisbon, M.D., M.P.H., Maria Gutierrez, M.D.,
Cheryle Webb, M.D., Richard Wu, Ph.D., Yunling Du, Ph.D.,
Therese Kranz, R.N., M.B.A., Evelyn Gasparino, B.S.,
and Gary D. Swergold, M.D., Ph.D.
Evolocumab (AMG 145) in Patients with Hypercholesterolemia (MENDEL-2)
Randomized, Double-blind, Placebo-controlled, Phase 3 Study

Alirocumab (REGN727) + Statin in Pt’s with Hypercholesterolemia
(Randomized, Double-blind, Placebo-controlled, Phase 2 Study)

siRNA to Silence PCSK9 (Phase I)

PCSK9: From Discovery to Clinical Trials in 7 Years

Discovery

Target Validation 3 Years

Target Validation

Phase I 4 Years

Outcomes

JCC Seminars: From Discovery to History in 7 Years

Phase 1
“Genetics of PCSK9”

Phase 2
“Mechanism of Action of PCSK9”

Phase 3
“A Short History Of PCSK9”
Human Genetics & Translation

• Selection of target
  - Lessons from lipoproteins

• Leveraging human genetic variation
  - Target validation
  - Target safety
  - ‘Goldilocks’ alleles (low frequency, large effect size)

• Genetics coupled to biological expertise
Is PCSK9 Paradigmatic or Exceptional?

Both.

Exceptional in the narrow sense (few relatively common alleles with large effects on disease).

Paradigmatic in the broad sense (more alleles, lower frequency, moderate effects on disease).
2009: Frameshift Mutations in ANGPTL3 and Lipoprotein Levels in the Dallas Heart Study
2010: Mutations in ANGPTL3 Cause Hypolipidemia*

*Musunuru et al. NEJM
Complex Diseases Are Not Necessarily So Complex

RX
- Diet
- Lipid-lowering meds

Age 50 y

Time

- Male sex
- Diabetes
- Smoking
- Hypertension
- HDL

↑ LDL X Time = Cumulative Exposure
FPLC of Plasma From $\textit{Angptl8}^{-/-}$ Mice
Reduced Fat Mass in *Angptl8*/* Mice

No significant differences (indirect calorimetry- 5 d)
- Food intake
- O₂ consumption
- RER
- Activity

* P < 0.05
** P < 0.01