Paul Ehrlich and PCSK9 inhibition: A Magic Bullet for CVD prevention?

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Cardiovascular Research Laboratories
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Endocrinology Rounds 19 Nov 2015
Disclosure J. Genest MD 2016

Advisory Board, Speaker’s Bureau, Consultant, Grants, Clinical Trials

- Merck *
- Pfizer
- Novartis *
- AMGEN *
- Cerenis *

- Sanofi/Regeneron *
- Lilly
- Valeant
- Aegerion *
- Ascati

Stock ownership: none;
Off label use: none
* Scientific Advisory

Relevant disclosure: JUPITER, IMPROVE-IT, CANTOS , CAPREE steering Committees; REVEAL , ACCELERATE, AMG145 , Lilly Clinical Trials.
Monoclonal Antibodies (mAbs) 101
Paul Ehrlich (1854-1915)

- Gram stain
- Arsphenamine (first Rx for Syphilis)
- Anti-serum against dyphteria
- Concept of “magische Kugel” – magic bullet

Nobel Prize 1908
Polyclonal Antibodies

B cell (humoral) immune responses are polyclonal

Polyclonal Abs:
• Recognize multiple epitopes on same antigen
• Usually high affinity

BUT
• Show major batch to batch variability
• Can include relatively non-specific antibodies
Monoclonal Antibodies (MAbs)

Monoclonal Abs:
• High specificity; detect only one epitope on antigen
• High homogeneity; once made, antibodies are constant, all batches identical
• Can be produced in unlimited quantities
Alirocumab Manufacturing – Production / Purification

Through a series of centrifugation, filtration membranes, and chromatographic steps, the antibody is purified to the desired quality for human use.
Antibody technology has evolved over past decades

**Fully Mouse**

1st generation

Highly Immunogenic

**Chimeric**

2nd generation

E.g. Abciximab

Chimeric, Still very immunogenic

**Humanized**

3rd generation

E.g. Bococizumab

Can be time-consuming to create

**“Fully” Human**

4th generation

E.g. Evolocumab and Alirocumab

Repeated dosing possible

Nomenclature: Prefix (Pharma) C (Cardiovascular) UMAB
Monoclonal antibodies in the clinic

Over 30 monoclonal antibodies approved for clinical use by European/US regulatory agencies in, for example:

- Asthma
- Autoimmune diseases
- Oncology
- Ophthalmic disorders

Approximately 235 monoclonal antibodies in active trials, for example:

- Alzheimer’s disease
- Cardiovascular disease
- Infectious disease
- Osteoporosis

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The biologics explosion

TNF Inhibitors
- Etanercept
- Adalimumab
- Infliximab
- Golimumab
- Certolizumab

Non-TNF Biologics
- Tocilizumab (IL-6)
- Rituximab (B cell CD20)
- Abatacept (B7-CTLA4Ig)
- Anakinra (IL-1)
- Canakinumab IL-1β)

Emerging non-TNF Biologics
- Sarilimab (IL6R)
- Olokizumab (IL6)
- Clazakizumab (IL6)
- Sirukumab (IL6)
- Secukinumab (IL-17)
- Brodalumab (IL-17R)

Emerging non-MAb Biologics
- Small molecules
  - Tofacitinib (JAK1/3)
- Biosimilars
PCSK9
PCSK9: A Canadian Discovery

Mutations in PCSK9 cause autosomal dominant hypercholesterolemia

Marianne Abifadel¹,², Mathilde Varret¹, Jean-Pierre Rabès¹,³, Delphine Allard¹, Khadija Ouguerram⁴, Martine Devillers¹, Corinne Cruaud⁵, Suzanne Benjannet⁶, Louise Wickham⁶, Danièle Erlich¹, Aurélie Derre¹, Ludovic Villéger¹, Michel Farnier⁷, Isabel Beucler⁸, Eric Bruckert⁹, Jean Chambaz¹⁰, Bernard Chanu¹¹, Jean-Michel Lecerf¹², Gerald Luc¹², Philippe Moulin¹³, Jean Weissenbach⁵, Annick Prat⁶, Michel Krempf⁴, Claudine Junien¹,³, Nabil G Seidah⁶ & Catherine Boileau¹,³

Nature Genetics 34, 154 - 156 (2003)

Dr. Nabil Seidah IRCM
Proprotein Convertase Subtilisin/Kexin Type 9

Evolutionary Conservation: Must be important

Bacillus amyloliquefaciens  saccharomyces cerevisiae  Homo sapiens
A: LDL-R pathway in absence of PCSK9

**Diagram:**

- LDL-R
- Lysosome
- Endosome
- Degradation

**Text:**

Pre-PCSK9

PCSK9

APOB

LDL

Degradation
PCSK9 as a Target

Cohen JC, et al. NEJM 2006;354:1264
Patients with Genetically Lower LDL have Correspondingly Better CV Event Reduction

Greater effect than Pharmacologically Lower LDL-C—Possibly due to Lifetime Lower LDL levels

Genetically Lower LDL-C

Pharmacologically Lower LDL-C

PCSK9 Directly Binds to the LDLR

Kwon et al. 2008. PNAS. 105:1820
Clinical Data
Effect of the PCSK9 Inhibitor Evolocumab on Cardiovascular Outcomes

MS Sabatine, RP Giugliano, SD Wiviott, FJ Raal, CM Ballantyne, R Somaratne, J Legg, SM Wasserman, R Scott, MJ Koren, and EA Stein for the OSLER Investigators

American College of Cardiology – 64th Annual Scientific Session
Late-Breaking Clinical Trial
March 15, 2015
**LDL Cholesterol**

**Standard of care alone**

- 61% reduction (95% CI 59-63%), P<0.0001
- Absolute reduction: 73 mg/dL (95% CI 71-76%)

**Evolocumab plus standard of care**

<table>
<thead>
<tr>
<th>Time</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (Parent study)</td>
<td>4465</td>
</tr>
<tr>
<td>4 weeks (OSLER)</td>
<td>1258</td>
</tr>
<tr>
<td>12 weeks</td>
<td>4259</td>
</tr>
<tr>
<td>24 weeks</td>
<td>4204</td>
</tr>
<tr>
<td>36 weeks</td>
<td>1243</td>
</tr>
<tr>
<td>48 weeks</td>
<td>3727</td>
</tr>
</tbody>
</table>
Cardiovascular Outcomes

Composite Endpoint: Death, MI, UA → hosp, coronary revasc, stroke, TIA, or CHF → hosp

Cumulative Incidence (%)

Days since Randomization

Standard of care alone (N=1489)

HR 0.47
95% CI 0.28-0.78
P=0.003

Evolocumab plus standard of care (N=2976)

0.95%
2.18%
Efficacy and Safety of Alirocumab in Reducing Lipids and Cardiovascular Events

Jennifer G. Robinson, M.D., M.P.H., Michel Farnier, M.D., Ph.D., Michel Krempf, M.D., Jean Bergeron, M.D., Gérald Luc, M.D., Maurizio Averna, M.D., Erik S. Stroes, M.D., Ph.D., Gisle Langslet, M.D., Frederick J. Raal, M.D., Ph.D., Mahfouz El Shahawy, M.D., Michael J. Koren, M.D., Norman E. Lepor, M.D., Christelle Lorenzato, M.Sc., Robert Pordy, M.D., Umesh Chaudhari, M.D., and John J.P. Kastelein, M.D., Ph.D. for the ODYSSEY LONG TERM Investigators

March 15, 2015 | DOI: 10.1056/NEJMoa1501031

Comments open through March 22, 2015
ODYSSEY Long-Term: Alirocumab Plus Statin Achieved a 62% Reduction in LDL-C over Placebo+Statin at 24 weeks

ODYSSEY Long-Term: Reduction in the Rate of Cardiovascular Events- Post-hoc Analysis

Kaplan-Meier Estimates for Time to First Adjudicated Major CV Event*

Safety Analysis†
Cox model analysis
HR 0.46
95% CI: 0.26 to 0.82
P<0.01

- Alirocumab + max-tolerated statin ± other LLT (150 mg q2w)
- Placebo + max-tolerated statin ± other LLT

54% RRR

*Primary endpoint for the ODYSSEY OUTCOMES trial: CHD death, Non-fatal MI, Fatal and non-fatal ischemic stroke, Unstable angina requiring hospitalization. LLT, lipid-lowering therapy
†≥52 weeks for all patients continuing treatment, incl. 607 patients who completed W78 visit

Ratio of LDL Lowering to CV Event Reduction with PCSK9 Inhibitors Holds True to the “LDL Hypothesis”

Results of Bococizumab, A Monoclonal Antibody Against Proprotein Convertase Subtilisin/Kexin Type 9, from a Randomized, Placebo-Controlled, Dose-Ranging Study in Statin-Treated Subjects With Hypercholesterolemia

Christie M. Ballantyne, MD<sup>a,b,∗</sup>, Joel Neutel, MD<sup>c</sup>, Anne Cropp, PharmD<sup>d</sup>, William Duggan, PhD<sup>e</sup>, Ellen Q. Wang, PhD<sup>f</sup>, David Plowchalk, PhD<sup>g</sup>, Kevin Sweeney, PhD<sup>g</sup>, Nitin Kaila, PhD<sup>g</sup>, John Vincent, MD, PhD<sup>h</sup>, and Harold Bays, MD<sup>i</sup>

Am J Cardiol 2015;115:1212e1221)
Results of Bococizumab, A Monoclonal Antibody Against Proprotein Convertase Subtilisin/Kexin Type 9, from a Randomized, Placebo-Controlled, Dose-Ranging Study in Statin-Treated Subjects With Hypercholesterolemia

The American Journal of Cardiology, Volume 115, Issue 9, 2015, 1212–1221
Figure 4. Mean percentage change from baseline in LDL-C. Change over time is shown for the (A) Q14 days and (B) Q28 days placebo and bococizumab dose groups.

Ballantyne CM. The American Journal of Cardiology, 2015;115:1212–1221
### Bococizumab: AE

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (n = 49)</th>
<th>Bococizumab (mg)</th>
<th>Placebo (n = 51)</th>
<th>Bococizumab (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AEs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>84% [29%]</td>
<td>74% [24%]</td>
<td>84% [31%]</td>
<td>82% [37%]</td>
</tr>
<tr>
<td><strong>Serious AEs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14% [0%]</td>
<td>8% [0%]</td>
<td>4% [0%]</td>
<td>8% [2%]</td>
</tr>
<tr>
<td><strong>Discontinuation of treatment due to AE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2% [0%]</td>
<td>2% [0%]</td>
<td>4% [0%]</td>
<td>10% [8%]</td>
</tr>
<tr>
<td><strong>Most frequent AEs (≥10%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>12% [0%]</td>
<td>16% [0%]</td>
<td>14% [0%]</td>
<td>10% [0%]</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>14% [0%]</td>
<td>8% [0%]</td>
<td>10% [0%]</td>
<td>6% [2%]†</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>8% [0%]</td>
<td>6% [0%]</td>
<td>8% [4%]</td>
<td>10% [2%]</td>
</tr>
</tbody>
</table>
FDA approves Praluent to treat certain patients with high cholesterol
Praluent is approved for use in addition to diet and maximally tolerated statin therapy in adult patients with heterozygous familial hypercholesterolemia (HeFH) or patients with clinical atherosclerotic cardiovascular disease such as heart attacks or strokes, who require additional lowering of LDL cholesterol.

Repatha is approved for use in addition to diet and maximally-tolerated statin therapy in adult patients with heterozygous familial hypercholesterolemia (HeFH), homozygous familial hypercholesterolemia (HoFH), or clinical atherosclerotic cardiovascular disease, such as heart attacks or strokes, who require additional lowering of LDL cholesterol.
Anti-drug Antibodies (ADA)
Anti-drug antibodies (ADA): the challenge

**Immunogenicity:**
- The potential for an antigen to induce an immune response
- Immunogenicity against therapeutic proteins that are not in the normal human repertoire is a normal immune response.
- Reaction to neo-antigens
  - Proteins are non-human
  - Fusion proteins create new epitopes
  - Unusual glycosylation
The detection of anti-drug binding antibody formation is highly dependent on the sensitivity and specificity of the assay. Comparison of the incidence of antibodies to evolocumab with incidence of antibodies to other products may be misleading.

<table>
<thead>
<tr>
<th>Evolocumab</th>
<th>Alirocumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Overall incidence of anti-evolocumab binding antibodies after at least one dose of evolocumab was 0.3% (13 of 4915)</td>
<td>• Observed in 4.8% of patients following alirocumab treatment vs. 0.6% of patients in control group</td>
</tr>
<tr>
<td>• Responses were of low-titer, most were transient</td>
<td>• Most responses were of low-titer, non-neutralizing, and/or transient</td>
</tr>
<tr>
<td>• No neutralizing antibodies have been detected</td>
<td>• Neutralizing antibodies were reported in 1.2% of patients treated with alirocumab</td>
</tr>
<tr>
<td>• No impact of binding antibodies on safety, pharmacokinetics, or pharmacodynamics</td>
<td></td>
</tr>
</tbody>
</table>
PCSK9 RNAi

Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled study.

Lancet 383;9911, 60–68
PCSK9 mAb Clinical Indications

Familial Hypercholesterolemia

ASCVD (Atherosclerotic Cardiovascular Disease) not at goal despite maximally tolerated lipid-lowering therapy

Statin intolerant
PCS K9 mAb: Whom, When?

Numbers (Guess)

20-30 HoFH

FH + CAD

FH Not @ Goal

CAD* Not @ Goal

Hi Risk Not @ Goal

CAD* Approx 1.5 M CDN

10,000

10,000

250,000

20-30 HoFH
PCSK9 and Diabetes
Background: Statin Treatment and Increased Risk of T2DM

### Table: Placebo-controlled or standard care-controlled

<table>
<thead>
<tr>
<th>Statin treatment</th>
<th>Placebo-controlled or standard care-controlled</th>
<th>OR (95% CI)</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>Non-case</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSSS</td>
<td>198</td>
<td>1.03 (0.84-1.27)</td>
<td>5.51</td>
</tr>
<tr>
<td>WOSCOPS</td>
<td>75</td>
<td>0.79 (0.58-1.08)</td>
<td>2.77</td>
</tr>
<tr>
<td>AFCAPS TexCAPS</td>
<td>72</td>
<td>0.98 (0.71-1.36)</td>
<td>2.47</td>
</tr>
<tr>
<td>LIPID</td>
<td>126</td>
<td>0.91 (0.71-1.17)</td>
<td>4.14</td>
</tr>
<tr>
<td>GISSI Prevenzione</td>
<td>96</td>
<td>0.89 (0.67-1.19)</td>
<td>3.19</td>
</tr>
<tr>
<td>LIPS</td>
<td>17</td>
<td>1.27 (0.62-2.59)</td>
<td>0.55</td>
</tr>
<tr>
<td>HPS</td>
<td>335</td>
<td>1.15 (0.98-1.35)</td>
<td>8.32</td>
</tr>
<tr>
<td>ALLHAT-LLT</td>
<td>238</td>
<td>1.15 (0.95-1.40)</td>
<td>6.27</td>
</tr>
<tr>
<td>PROSPER</td>
<td>165</td>
<td>1.32 (1.04-1.68)</td>
<td>4.38</td>
</tr>
<tr>
<td>ASCOT-LLA</td>
<td>154</td>
<td>1.14 (0.90-1.44)</td>
<td>4.46</td>
</tr>
<tr>
<td>SPARCL</td>
<td>166</td>
<td>1.48 (1.16-1.89)</td>
<td>4.12</td>
</tr>
<tr>
<td>MEGA</td>
<td>172</td>
<td>1.07 (0.86-1.34)</td>
<td>5.02</td>
</tr>
<tr>
<td>CORONA</td>
<td>100</td>
<td>1.14 (0.85-1.53)</td>
<td>3.01</td>
</tr>
<tr>
<td>JUPITER</td>
<td>270</td>
<td>1.26 (1.05-1.51)</td>
<td>6.88</td>
</tr>
<tr>
<td>GISSI-HF</td>
<td>225</td>
<td>1.10 (0.90-1.34)</td>
<td>5.86</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>2409</strong></td>
<td><strong>1.11 (1.03-1.20)</strong></td>
<td><strong>66.98</strong></td>
</tr>
</tbody>
</table>

\( (I^2=29.6\%; p=0.134) \)

### Table: Intensive vs moderate dose

<table>
<thead>
<tr>
<th>Statin treatment</th>
<th>Intensive vs moderate dose</th>
<th>OR (95% CI)</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>Non-case</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROVE-IT TIMI22</td>
<td>101</td>
<td>1.01 (0.76-1.34)</td>
<td>3.18</td>
</tr>
<tr>
<td>A to Z</td>
<td>65</td>
<td>1.37 (0.94-2.01)</td>
<td>1.87</td>
</tr>
<tr>
<td>TNT</td>
<td>418</td>
<td>1.19 (1.02-1.38)</td>
<td>9.25</td>
</tr>
<tr>
<td>IDEAL</td>
<td>240</td>
<td>1.15 (0.95-1.40)</td>
<td>6.33</td>
</tr>
<tr>
<td>SEARCH</td>
<td>625</td>
<td>1.07 (0.95-1.21)</td>
<td>12.39</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>1449</strong></td>
<td><strong>1.12 (1.04-1.22)</strong></td>
<td><strong>33.02</strong></td>
</tr>
</tbody>
</table>

\( (I^2=0.0\%; p=0.598) \)

### Overall

<table>
<thead>
<tr>
<th>Case</th>
<th>Non-case</th>
<th>OR (95% CI)</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3858</strong></td>
<td><strong>60700</strong></td>
<td><strong>1.12 (1.06-1.18)</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

\( (I^2=16.2\%; p=0.253) \)

CI, confidence interval; OR, odds ratio; T2DM, type 2 diabetes mellitus.
Case = developed T2DM. Non-case = did not develop T2DM.
Efficacy and Safety of Alirocumab: Pooled Analyses of 1051 Individuals with Diabetes Mellitus from Five Placebo-Controlled Phase 3 Studies of at least 52 weeks’ duration

Henry N Ginsberg¹, Michel Farnier², Jennifer G Robinson³, Christopher P Cannon⁴, Naveed Sattar⁵, Marie T Baccara-Dinet⁶, Christelle Lorenzato⁷, Maja Bujas-Bobanovic⁸, Michael J Louie⁹, Helen M Colhoun¹⁰

¹Columbia University, New York, NY; ²Point Medical, Dijon, France; ³University of Iowa, Iowa City, IA; ⁴Harvard Clinical Res Inst, Boston, MA; ⁵University of Glasgow, Glasgow, UK; ⁶Sanofi, Montpellier, France; ⁷Sanofi, Chilly-Mazarin, France; ⁸Sanofi, Paris, France; ⁹Regeneron Pharmaceuticals, Tarrytown, NY; ¹⁰University of Dundee, Dundee, UK.

This study was funded by Sanofi and Regeneron Pharmaceuticals, Inc.
Mean Fasting Plasma Glucose Over Time (Safety Population)

41 mg Q2W increased to 150 mg Q2W at W12 if LDL-C levels at Week 8 were ≥70 mg/dL (1.81 mmol/L).

FPG, fasting plasma glucose.
Mean HbA₁c Over Time (Safety Population)

- **Alirocumab with DM**
- **Placebo with DM**
- **Alirocumab without DM**
- **Placebo without DM**

**Alirocumab 75/150 mg Q2W †**

- Week 0: 6.5%
- Week 12: 7.0%
- Week 24: 7.5%
- Week 52: 8.0%

**Alirocumab 150 mg Q2W**

- Week 0: 6.5%
- Week 12: 7.0%
- Week 24: 7.5%
- Week 52: 8.0%

† 75 mg Q2W increased to 150 mg Q2W at Week 12 if LDL-C levels at Week 8 were ≥70 mg/dL (1.81 mmol/L).

HbA1c, glycated hemoglobin.
Evaluation of the One-Year Efficacy, Safety and Glycaemic Effects of Evolocumab (AMG 145) in 4,802 Subjects With, at High Risk for, or at Low Risk for, Diabetes Mellitus

Naveed Sattar,1 David Preiss,1 Dirk Blom,2 C. Stephen Djedjos,3 Mary Elliott,4 Andrea Pellacani,3 Scott M Wasserman,3 Michael Koren,5 Rury Holman6

1BHF Cardiovascular Research Centre, University of Glasgow, UK; 2Division of Lipidology, Department of Medicine, University of Cape Town, South Africa; 3Amgen Inc., Thousand Oaks, CA, USA; 4Amgen Limited, Cambridge, UK; 5Jacksonville Center for Clinical Research, Jacksonville, FL, USA; 6Diabetes Trials Unit, OCDEM, University of Oxford, UK

European Association for the Study of Diabetes
Stockholm, Sweden
17 September, 2015 – Session OP 27
Results: Median Fasting Plasma Glucose Over One Year*

*48 weeks of open-label treatment
Error bars represent SE of the median
SoC, standard of care; T2DM, type 2 diabetes mellitus
Results: Median HbA$_{1c}$ Over One Year*

*48 weeks of open-label treatment
Error bars represent SE of the median
HbA$_{1c}$, glycated haemoglobin; SoC, standard of care; T2DM, type 2 diabetes mellitus
Conclusion

• In patients with T2DM, or people at high or low risk of T2DM, one year of treatment with evolocumab or alirocumab:
  – markedly reduced LDL-C in all groups
  – showed encouraging safety
  – showed no measurable effect on glycaemic parameters including new-onset T2DM vs SoC alone

• Clinical trials are ongoing to examine the effects of evolocumab on patients with T2DM, and on the incidence of new-onset T2DM