Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

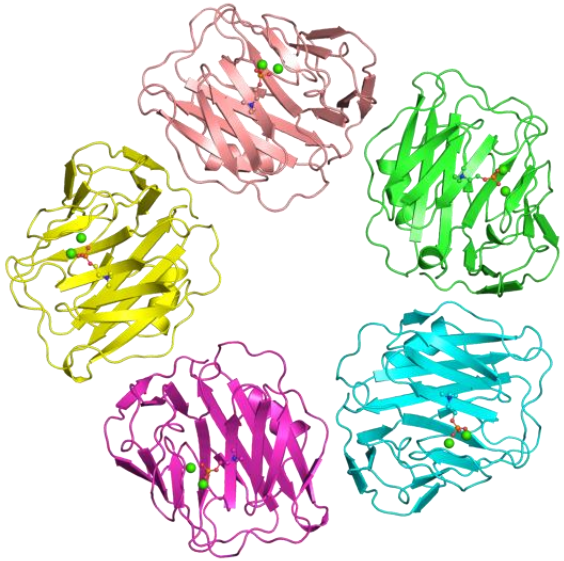
MEDIZINISCHE UNIVERSITÄT WIEN

CRP and Atheroscerlosis, the JUPITER-Study and Rosuvastatin

A story of confusion and commercial interest

Background
CRP

- Pentamer protein, synthesized by the liver
- Part of acute-phase reaction
- Binds strongly to bacterial LPS
- Activates complement reaction and makrophage binding



http://www.ebi.ac.uk/pdbe-srv/view/images/entry/1b09600.png
European Bioinformatics institute

Background

Atherosclerosis

- **Arteriosclerosis** = pathological process that leads to thickening, hardening and loss of elasticity of vessel walls
- **Atherosclerosis** = narrowing arteries from building up a plaque (cholesterol, fatty acids, cell waste products) by chronic inflammation in medium to large vessels

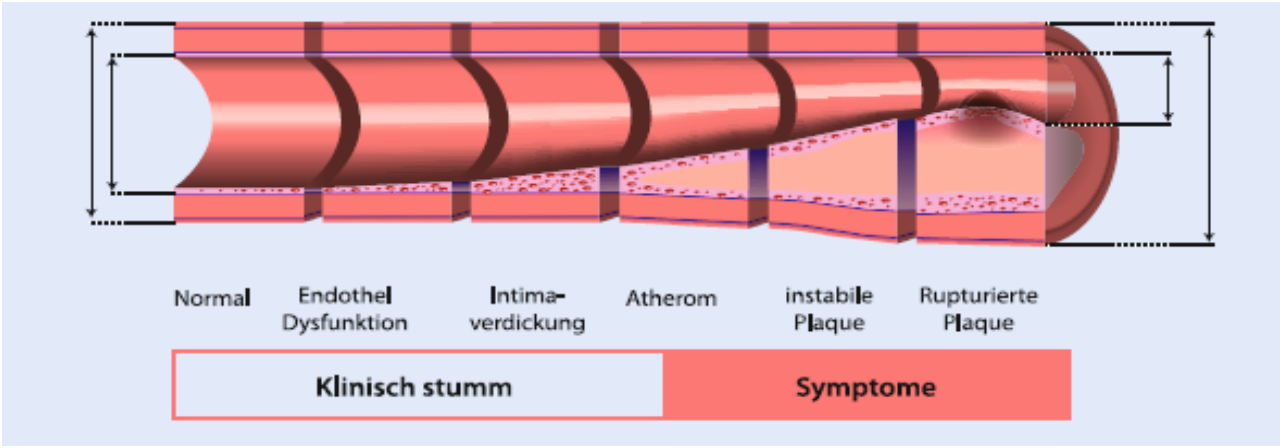
Background Atherosclerosis

- Risk factors:
  - high cholesterol/dyslipidaemia,
  - Smoking
  - Hypertonus
- Chronic inflammation, CRP?

$$R = \frac{8 * l}{\pi * r^4} * \eta$$

Impedence *R* in blood vessels

J.Steffel, T. Lüscher: Herz-Kreislauf, Springer 2014



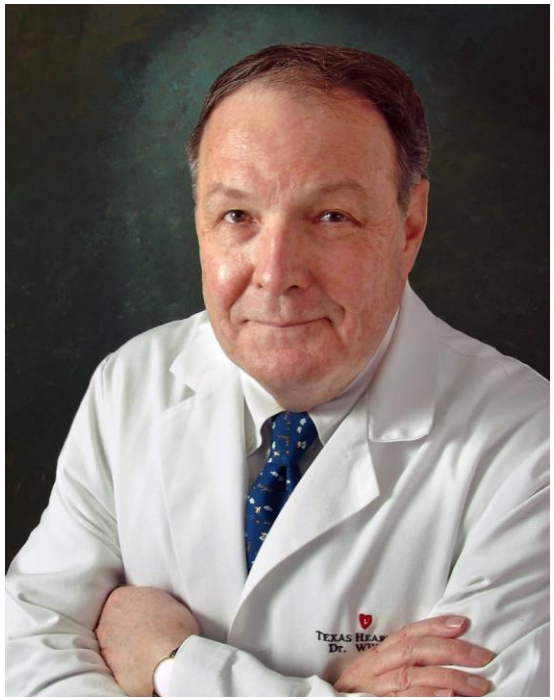
Background
Statins

- Inhibit synthesis of Cholesterol by binding to HMG-CoA-Reductase
- Lova/Simva/Prava/Atorva/Fluva/ Rosuvastatin
- Pleiotropic effects: antioxidative, antithrombotic, vasculoprotective, plaque stabilization

Antiinflammatory?

James T. Willerson

- BA UT of Austin 1961, Varsity swimming scholarship
- MD Baylor University of Medicine 1965
- Postgraduate Training at Harvard Medical School
- Fellow at Massachusetts General Hospital
- Clinical associate in Bethesda, Maryland



medonline.at, 2014

James T. Willerson

- Published over 980 articles
- Editor of *Circulation* from 1993 to 2004
- Editorial board for *New England Journal of Medicine*, *American Journal of Medicine* and others
- Member of board American Heart Associtation (AHA)
- Current President at the Texas Heart Institute

http://www.texasheart.org/AboutUs/History/willerson.cfm

Paul M. Ridker

- Eugene Braunwald Professor of Medicine at Harvard medical school
- Graduate of Brown University (1981)
- Harvard Medical School (1986)
- Principle investigator in many multi-national randomized trials
- More than 500 publications

http://www.einstein.yu.edu/medicine/cardiology/cvresearchcenter.aspx?id=16656

Pasceri V. et al. 2000

Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation. 2000;102(18):2165-8.

- HUVECs incubated + recombinant bacterial CRP →
  - 10-fold increase of ICAM-1
  - „significant expression“ of VCAM-1

Pasceri V. et al. 2000

- HUVECs incubated with highly purified human CRP →
  - „also showed biological actvity“
  - „Data not shown“?
- HCAECs incubated with CRP (unclear human or recombinant) →
  - „significant proinflammatory effects“
  - Inducing high levels of ICAM-1, VCAM-1 and E-Selectin

Pasceri V. et al. 2000

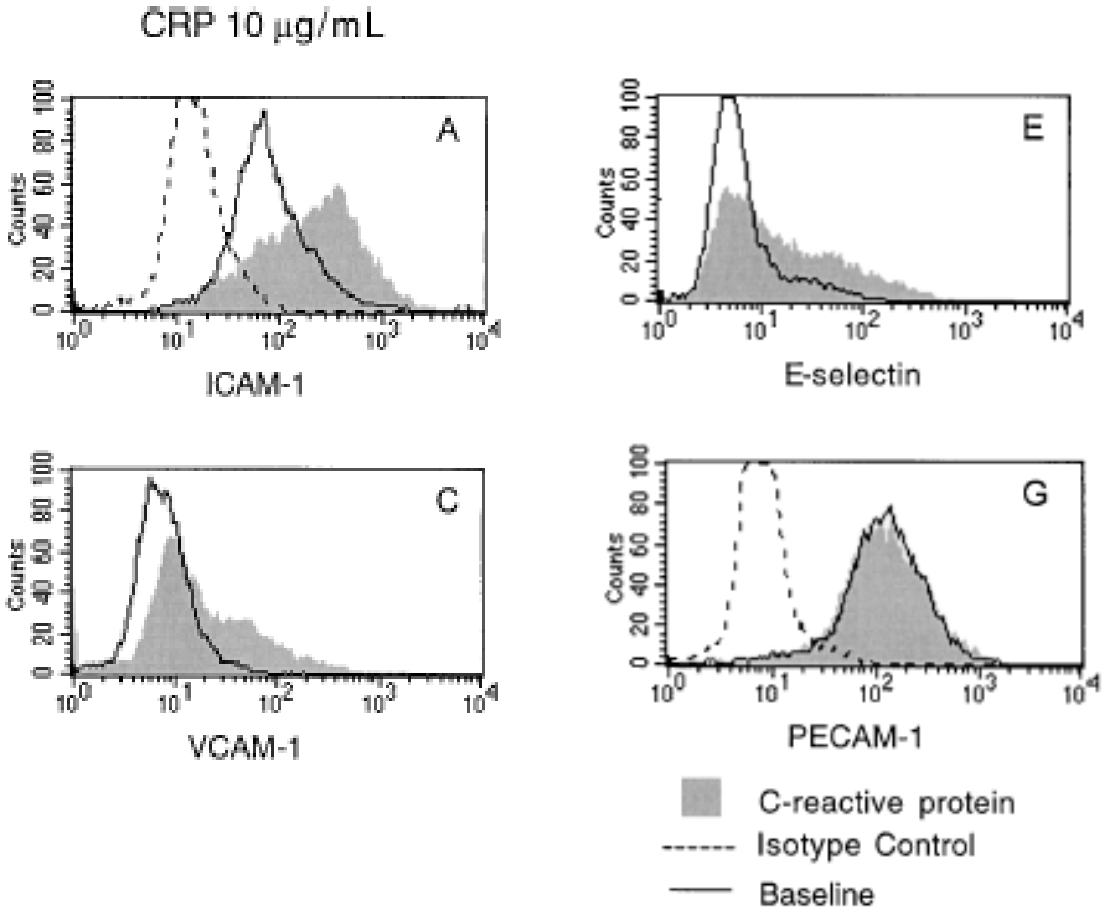


Fig. 1:Induction of adhaesion molecule expression by CRP

Pasceri V. et al. 2000

„CRP (…) induces significant expression of adhesion molecules (…) and is not merely a marker of inflammation, but has modulatory functions that may contribute to the development of inflammation/atherosclerosis“

Pasceri V et al. 2001

Pasceri V, Cheng JS, Willerson JT, Yeh ET. Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs. Circulation. 2001;103(21):2531-4.

- Incubation of HUVECS with CRP (recombinant or human?) →
  - Induced significant secretion of MCP-1
  - No increased secretion on RANTES

Pasceri V et al. 2001

Pasceri V, Cheng JS, Willerson JT, Yeh ET. Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs. Circulation. 2001;103(21):2531-4.

- Incubation of HUVECS with CRP (recombinant or human?)+ Simvastatin

→

  – Reduced to 43% of maximal MCP-1 response

Pasceri V et al. 2001

„…Our findings support the hypothesis of a direct role of CRP in the pathogenesis of inflammation/atherosclerosis and ioen the way to new pharmacological strategies for ist treatment.“

Taylor KE et al. 2005

Taylor KE, Giddings JC, van den Berg CW. C-reactive protein-induced in vitro endothelial cell activation is an artefact caused by azide and lipopolysaccharide. Arteriosclerosis, thrombosis, and vascular biology. 2005;25(6):1225-30

- In vitro CRP lack robust controls
- Authors generated own recombinant CRP
- Incubation of HUVECs with
  - Commcercial CRP (cCRP) from *E.coli*
  - Own recombinant CRP (rCRP)
    - Lipopolysaccharide (LPS) and azide-free

Taylor KE et al. 2005

- HUVECs cultured with rCRP showed morphology to controls
- HUVECs cultured with azide appeared similar to cCRP →

azide and not CRP inhibts cell proliferation!

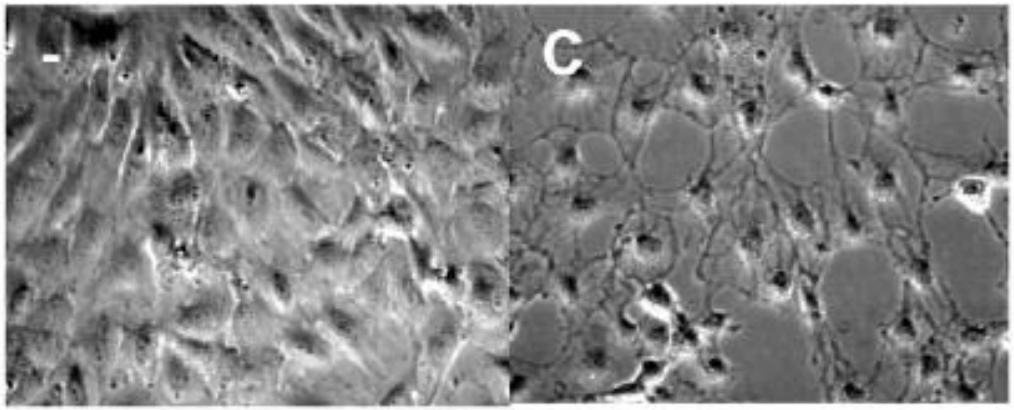


Fig. 1: Azide in cCRP is responsible for reduced cell proliferation, change in morphology and increased cell apostosis

Taylor KE et al. 2005

- ICAM-1 expression observed only in HUVECs with cCRP
- No ICAM-1 expression in rCRP
- Dialsysis of cCRP removed ICAM-1-inducing activity
  - Contamination with LPS induced ICAM-1-expression

Taylor KE et al. 2005

- Not a single direct effect of CRP on endothelial cells!

„… all other studies in which cCRP preparations have been used are most likely artifacts to azide of LPS contamination.“

Pepys et al. 2005

Pepys MB, Hawkins PN, Kahan MC, Tennent GA, Gallimore JR, Graham D, et al. Proinflammatory effects of bacterial recombinant human C-reactive protein are caused by contamination with bacterial products, not by C-reactive protein itself. Circulation research. 2005;97(11):e97-103.

- „….Intravenous injection of highly purified, structurally intact and fully functional active human CRP (…) did not induce an acute phase response“
- „… injection of the same quantity of recombinant CRP induced acute phase response.“

The CRP-testing patent

held by PM Ridker

(12) **United States Patent**
Ridker et al.

(10) **Patent No.: US 7,964,614 B2**
(45) **Date of Patent: Jun. 21, 2011**

(54) **SYSTEMIC INFLAMMATORY MARKERS AS DIAGNOSTIC TOOLS IN THE PREVENTION OF ATHEROSCLEROTIC DISEASES AND AS TOOLS TO AID IN THE SELECTION OF AGENTS TO BE USED FOR THE PREVENTION AND TREATMENT OF ATHEROSCLEROTIC DISEASE**

(75) Inventors: **Paul Ridker**, Chestnut Hill, MA (US); **Charles H. Hennekens**, Boca Raton, FL (US)

(73) Assignee: **The Brigham and Women's Hospital, Inc.**, Boston, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1201 days.

(21) Appl. No.: **11/158,889**

(22) Filed: **Jun. 22, 2005**

(65) **Prior Publication Data**

US 2006/0104941 A1 May 18, 2006

WO 98/43630 * 10/1998
WO 98/47509 * 10/1998
WO WO 98 43630 A 10/1998

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Paul M. Ridker et al., "Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men," *The Lancet*, vol. 351, No. 9096, pp. 88-92, Jan. 10, 1988.*

M. A. Mendall et al., "C Reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study," *BMJ*, vol. 312, pp. 1065-1069, Feb. 1996.*

Russell P. Tracy et al., "Relationship of C-Reactive Protein to Risk of Cardiovascular Disease in the Elderly," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 17, No. 6, Jun. 1997.*

Paul M. Ridker et al., "Plasma Concentration of C-Reactive Protein and Risk of Developing Peripheral Vascular Disease," *Circulation*, vol. 97, pp. 425-428, 1998.*

Paul M. Ridker et al., "C-Reactive Protein Adds to the Predictive Value of Total and HDL Cholesterol in Determining Risk of First Myocardial Infarction," *Circulation*, vol. 97, pp. 2007-2011, 1998.*

Wolfgang Koenig, M.D., "C-Reactive Protein, a Sensitive Marker of Inflammation, Predicts Future Risk of Coronary Heart Disease in

CRP-testing patent

- Applied for in June 2005 by PM Ridker and Charles Hennekens
- Granted in June 2011
- Introduction of hsCRP-test by Siemens in 2004
- Siemens hsCRP-test approved by the FDA in 2009 after the JUPITER-trial
- hsCRP tests sold by Siemens and AstraZeneca

The CRP-testing patent

http://www.healthcare.siemens.com/point-of-care/poc-cardiac-topics/cardiac-assays/cardiophase-hscrp

SIEMENS

The JUPITER study Ridker et al. 2008

Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, Willerson JT et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. The New England journal of medicine. 2008;359(21):2195-207.

Justification for the **U**se of Statins in **P**rimary prevention: an **I**ntervention **T**rial **E**valuating **R**osuvastatin

The JUPITER study

- Randomized, double blind, placebo-controlled study
- 17.802 healthy men and women with low LDL<130mg/dl and CRP > 2,0mg/l
- Rosuvastatin 20mg 1/d vs. Placebo
- Follow-up of 9 years
  - End-points: myocardial infarction, stroke, combined, cardiovascular death, death from any cause

The JUPITER Study

- Early study closure after 1,9 years (393 endpoints) due to significant risk reduction for patients in the Rosuvastatin group

„… in apparently healthy men and women who did not have hyperlipidemia but had elevated levels of hsCRP, the rates of a first major cardiovascular event and death from any cause were significantly reduced among participants who received Rosuvastatin…“

The JUPITER study

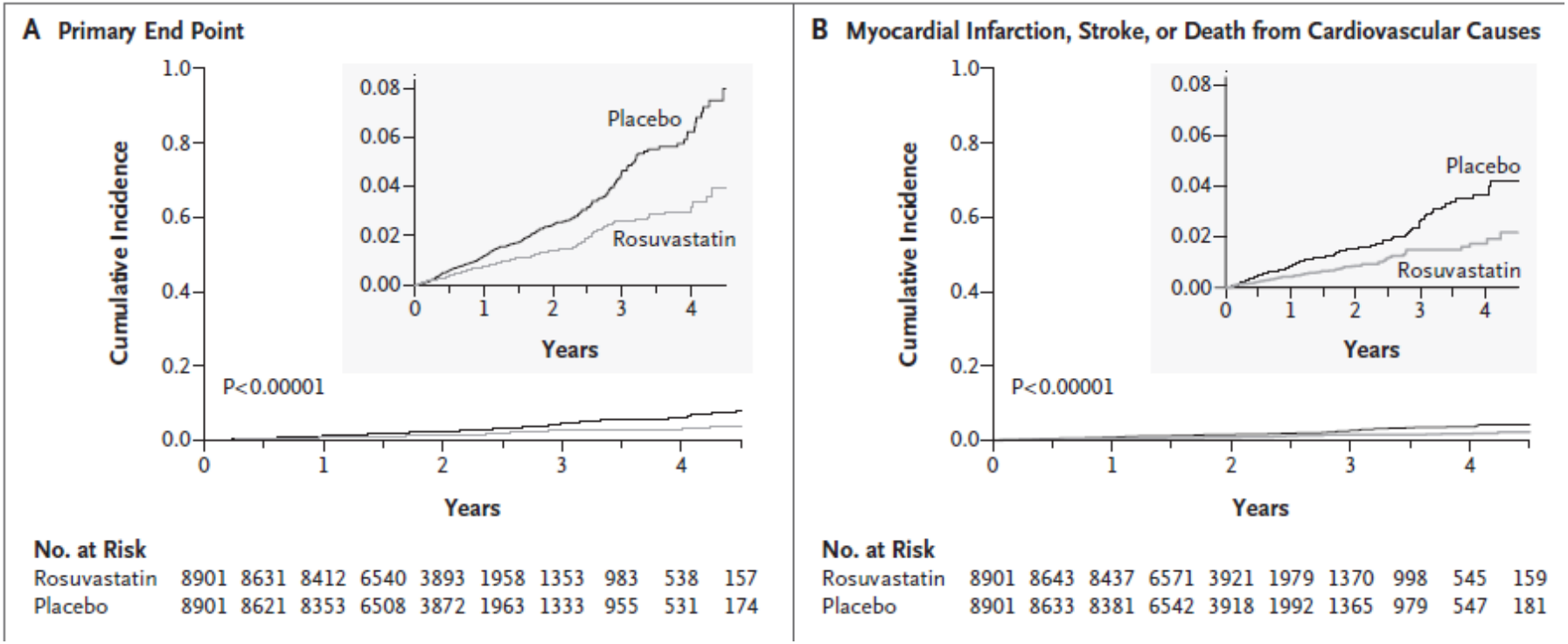
Results

Table. A Summary of the JUPITER Trial Results[a]

End Point	Rosuvastatin Group (n=8901)	Placebo Group (n=8901)
Primary end point[b]	142	251
Nonfatal myocardial infarction	22	62
Any myocardial infarction	31	68
Nonfatal stroke	30	58
Any stroke	33	64
Arterial revascularization	71	131
Hospitalization for unstable angina	16	27
Myocardial infarction, stroke, or confirmed deaths from cardiovascular causes	83	157
Death from any cause on known date	190	235

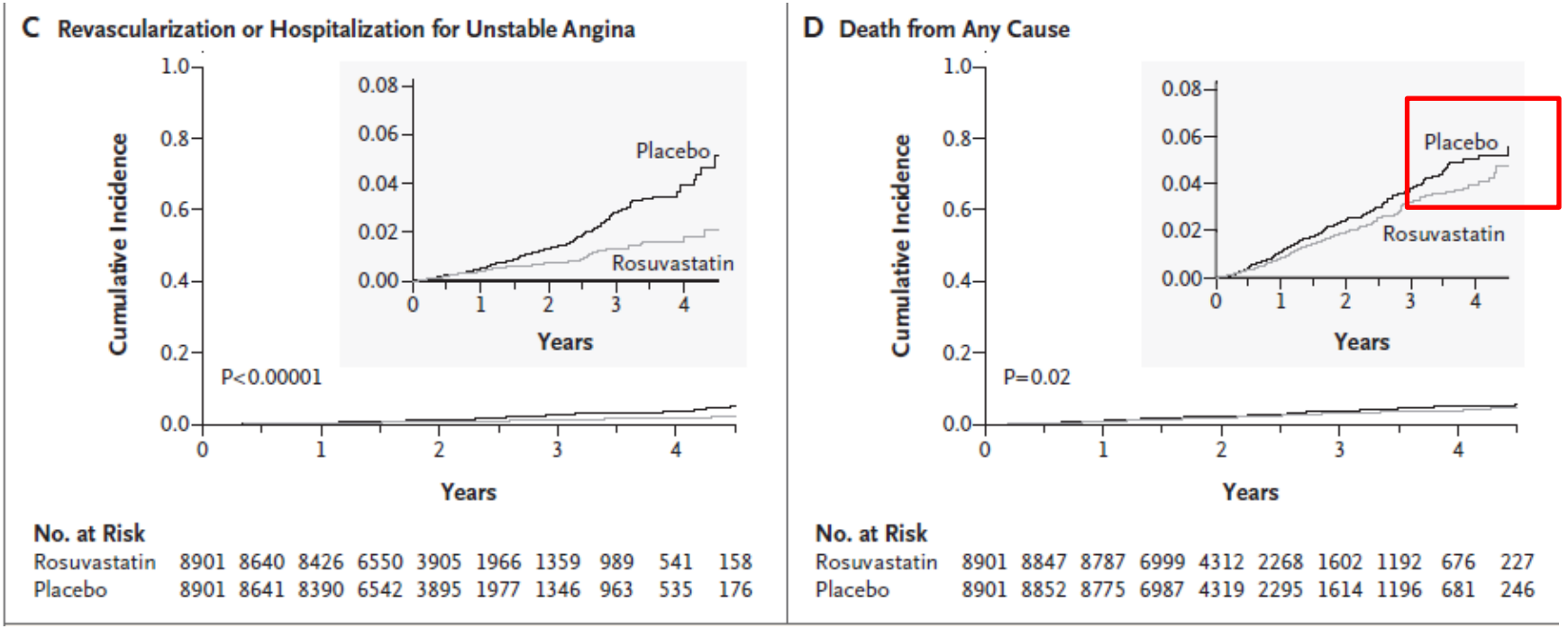
DeLorgeril et al. 2010
Adapted from Ridker et al.

The JUPITER study
Results



Primary endpoints: occurence of a first major cardiovascular event, defined as nonfatal myocardial infarction, nonfatal stroke, hospitalization for unstable angina, an arterial revascularization procedure, or confirmed death from cardiovascular causes

The JUPITER study
Results



The JUPITER study

Results

- NNT (2y) = 95, NNT (5y) = 31

- Serious adverse events: similar in the rosuvastatin and placebo group
- „Small but significant increase in the rate of physician-reported diabetes with Rosuvastatin“

The JUPITER study

Conflicts of interest

- Study sponsored by AstraZeneca
- AstraZeneca holder of Rosuvastatin (Crestor®)
- 11/14 authors report receiving grants and/or consulting/lecture fees from AstraZeneca

The JUPITER study

Conflicts of interest

- PM Ridker coinventor of patents in the use of hsCRP, licensed to AstraZeneca
- „…The sponsor collected the trial data and monitored the study sites…“
- Increase of sales number of statins from AstraZeneca after reporting the trial results

DeLorgeril et al. 2010

de Lorgeril M, Salen P, Abramson J, Dodin S, Hamazaki T, Kostucki W, et al. Cholesterol lowering, cardiovascular diseases, and the rosuvastatin-JUPITER controversy: a critical reappraisal. Archives of internal medicine. 2010;170(12):1032-6.

- Results: „The trial was flawed.“
- …“major discrepancy between reduction of nonfatal stroke and myocardial infarction but no effect on mortality“
- Troubling questions concerning the role of commercial sponsors

DeLorgeril et al.

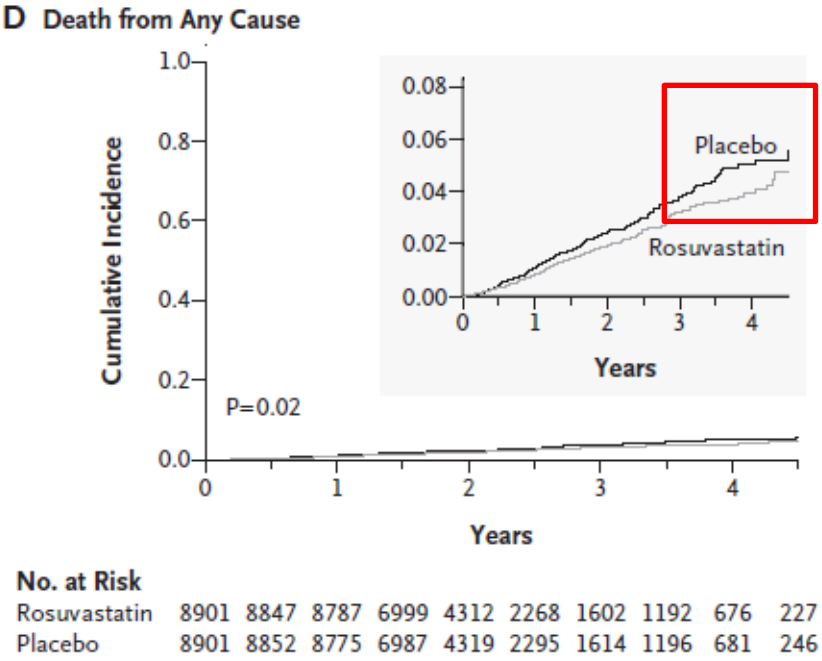
Methologial flaws

- Early study closure: closed after only 240 hard end points
- prespecified rules for study closure not published in study protocol
- all cause mortality curves were converging

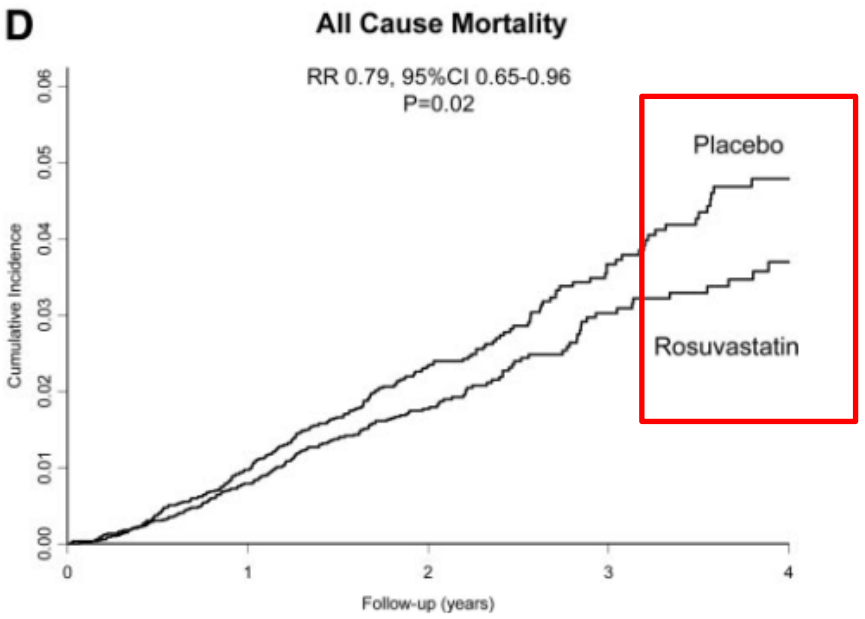
→ not published in subsequent paper

DeLorgeril et al.

Methologial flaws



Ridker PM et al., NEJM 359;21 2008



Ridker PM. The JUPITER trial: results, controversies, and implications for prevention. Circulation Cardiovascular quality and outcomes. 2009;2(3):279-85.

DeLorgeril et al.

Epidemiological problems

- Surprisingly low cardiovascular mortality:
- case-fatality rate
  - To be expected: 40%
  - JUPITER placebo group: 8,8%
  - JUPITER Rosuvastatin group: 29%
- „JUPITER-patients highly resistant to ischemia and infarction?“
- No mention on numbers of sudden cardiac death

DeLorgeril et al.

Statistical inconsistencies

Table. A Summary of the JUPITER Trial Results[a]

End Point	Rosuvastatin Group (n=8901)		Placebo Group (n=8901)	
Primary end point[b]	142		251	
Nonfatal myocardial infarction	22	=9	62	=6
Any myocardial infarction	31		68	
Nonfatal stroke	30	=3	58	=6
Any stroke	33		64	
Arterial revascularization	71	=12	131	=12
Hospitalization for unstable angina	16		27	
Myocardial infarction, stroke, or confirmed deaths from cardiovascular causes	83	31	157	37
Death from any cause on known date	190		235	

No significant difference in mortality!

Other Rosuvastatin studies

- ASTEROID: high intensity statin therapy (40mg Rosuvastatin) may regress coronary atherosclerosis
- AURORA: reduction of LDL, but no significant effect on primary outcome in patients with hemodialysis
- COMETS: significantly greater effect of Rosuvastatin than Atorvastatin

Other Rosuvastatin studies

- CORONA: no reduction of primary outcome, but less hospitalization
- GISSI-HF: no effect of Rosuvastatin in chronic heart failure

The heart protection study 2011

Jonathan E, Derrick B, Emma L, Sarah P, John D, Jane A, et al. C-reactive protein concentration and the vascular benefits of statin therapy: an analysis of 20,536 patients in the Heart Protection Study. Lancet (London, England). 2011;377(9764):469-76.

- 20,536 Patients in the UK categorized into CRP-baseline groups
- Simvastatin 40mg daily vs. Placebo
- Reduction of cardiovascular risk by a quarter in the Simvastatin group
- But: CRP baseline does not modify vascular benefits of statin therapy

ESC/EAS Guidelines
for dyslipidaemias

Reiner Z, Catapano AL, De Backer G, Graham I, Taskinen MR, Wiklund O, et al. ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). European heart journal. 2011;32(14):1769-818.

- „Presently, hs-CRP as a secondary target of therapy is <u>not recommended for everybody</u>; based on available data, it may be useful in people close to high risk category to better stratefy their total CV risk. “

ESC/EAS Guidelines
for dyslipidaemias

- „The contribution of hs-CRP to absolute CV risk estimation for individual patients is generally modest.“
- WHO guideline 2007:
  „CVD risk may be higher than indicated in the presence of (…) raised levels of CRP“

Conclusion, Comments

- Trying to find out the „truth“ can be quite confusing
- Commercial interests are a scientific and clinical issue and raise troubling questions
- First-glance good scientific practice might not be reliable after all
- Statistics „too good to be true“ are to be questioned and evaluated critically

Fragen, Kritik, Anregungen?

Herzlichen Dank!

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