ATHEROSCLEROSIS: OMICS TO FUTURE CLINICAL CHALLENGES

Highest technologies available we still have unacceptable recurrent acute coronary events after treatment with secondary prevention.....10-20% in first 12 months

Eur Heart J. 2015;36:1163–1170

Professor Robert Chilton
University of Texas Health Science Center
San Antonio, Texas
Director of Cath Lab
Director clinical proteomics center

43 y/o male
Recent stent placement
ASA, Plavix, Statins
CONFLICTS: YES

- Research
- Clinical Trials
- International Advisory Boards
INTERHEART: IMPACT OF MULTIPLE RISK FACTORS ON CV RISK

9 modifiable factors account for 90% of first MI

NOTE: IT STARTS MAINLY AFTER 15-20YRS

Johns Hopkins: medical students cholesterol and risk of CV disease

Prospective study
N = 1017 young men
Mean age 22

**NEJM 1993;328:313**
CUMULATIVE LDL EXPOSURE (EXPRESSED AS GRAMS OF CHOLESTEROL PER YEAR) OVER A LIFETIME IN PATIENTS

- 80 Y/O COULD HAVE HAD 10 G/DL-YEARS EXPOSURE TO LDL
- <20 YEARS OF AGE FH PATIENT WOULD HAVE ALREADY HAD 10 G/DL-YEARS EXPOSURE TO LDL
Hematologic importance in plaque rupture

Translational biology of atherosclerosis

Cholesterol / fibroatheroma

Angiotensin II

Nature medicine 2013;19:1094
Austin et al Blood 2013;121:431
ACUTE CORONARY SYNDROME CHARACTERISTIC'S BY OCT

Plaque rupture

Thrombus

Thrombi

Plaque erosion

Biomarkers?

Cardiology Research and Practice: doi:10.4061/2011/312978
30 y/o/ Hispanic type 2 DM male A1c 8.5

8 months before

NIRS-IVUS

Imaging biomarker

Acute coronary syndrome
OCT CAN ESTIMATE MACROPHAGE (INFLAMMATION) ACCUMULATION WITHIN FIBROUS CAPS

Humans with unstable angina (Red outlines: fibrous cap of the OCT image)

J Am Coll Cardiol 2004;44:972–9
Biomarkers to Improve the Prediction of Death from Cardiovascular Causes

- **Uppsala Longitudinal Study of Adult Men** (ULSAM): cohort of elderly men (mean age 71)

- **Hypothesis**: Can biomarkers improve risk stratification for CV events vs conventional?

- N = 1135
  - 136 deaths were cardiovascular

- Four biomarkers (troponin I, NT-pro-BNP, C-reactive protein, and cystatin C) improved risk stratification for death from cardiovascular causes

C statistic improved significantly with four biomarkers

0.766 Biomarkers

0.664 Without biomarkers

Established risk factors

N Engl J Med 2008;358:2107-16
THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY: DIABETES LOOK

- **ARIC STUDY - PROSPECTIVE OBSERVATIONAL STUDY OF THE NATURAL HISTORY OF ATHEROSCLEROTIC DISEASES AND CARDIOVASCULAR RISK FACTORS**
- **MIDDLE-AGED (45 – 64 YEARS) (N=11,656)**
- **PARTICIPANTS WITHOUT PREVALENT CVD, WITH FURTHER CATEGORIZATION ACCORDING TO THE PRESENCE (N = 1,510) OR ABSENCE (N = 6,892) OF DIABETES**

Kaplan-Meier curves for probability of fatal and nonfatal cardiovascular events. HRs are adjusted for demographic characteristics and cardiovascular risk factors. DM, diabetes.

NTproBNP cutoff value of 125 pg/mL

**Diabetes Care 2016;39:677 – 685**
Statins

BET inhibitors

PCSK9

Microbiome

Future

Introduction-translational cardio-metabolics

New CV agents that lower BS

↓ LDL

Myositis

European Heart Journal
doi:10.1093/eurheartj/ehr224

Outline
Maestro of the CV system

Live imaging of endothelial cells at 600x magnification

Endothelial cell health

Co-shared risk factors

Cardiovascular health

Courtesy of Jerome Breslin
Statins: High CV risk patient high potency statin

LDL RECEPTOR PATHWAY

- Down regulate HMGCoA reductase
- Reduce LDL receptor synthesis
- Esterified by ACAT (storage)

LDL RECEPTOR PATHWAY:
- Binding
- Endocytosis
- Receptor Recycling
- Lysosomal Degradation
- Functions of Cholesterol

- PCSK9 prevents LDLR recycling
LDL AND ATHEROGENESIS

LDL Readily Enter the Artery Wall Where They May be Modified

**LDL ox**

Vessel Lumen

Endothelial cells

Endothelium

Modified LDL are Pro inflammatory


- Oxidation of Lipids and ApoB
- Hydrolysis of Phosphatidylcholine to Lysophosphatidylcholine
- Aggregation
- Other Chemical Modifications
- Modified LDL

Intima
MACROPHAGES AND FOAM CELLS EXPRESS GROWTH FACTORS AND PROTEINASES

C V RISK CONTINUES EVEN WITH STATIN THERAPY

<table>
<thead>
<tr>
<th>Trial</th>
<th>Statin treatment</th>
<th>Risk reduction vs placebo</th>
<th>Remaining risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>WOSCOPS** (6595)</td>
<td>Pravastatin 40 mg</td>
<td>31%</td>
<td>69%</td>
</tr>
<tr>
<td>AFCAPS/TexCAPS** (6605)</td>
<td>Lovastatin 20 or 40 mg</td>
<td>40%</td>
<td>60%</td>
</tr>
<tr>
<td>ASCOT-LLA** (10,305)</td>
<td>Atorvastatin 10 mg</td>
<td>38%</td>
<td>62%</td>
</tr>
<tr>
<td>4S** (4444)</td>
<td>Simvastatin 20 mg</td>
<td>26%</td>
<td>74%</td>
</tr>
<tr>
<td>CARE*** (4159)</td>
<td>Pravastatin 40 mg</td>
<td>24%</td>
<td>76%</td>
</tr>
<tr>
<td>LIPID*** (9014)</td>
<td>Pravastatin 40 mg</td>
<td>24%</td>
<td>76%</td>
</tr>
<tr>
<td>HPS*** (20,536)</td>
<td>Simvastatin 40 mg</td>
<td>27%</td>
<td>73%</td>
</tr>
<tr>
<td>PROSPER*** (5804)</td>
<td>Pravastatin 40 mg</td>
<td>24%</td>
<td>76%</td>
</tr>
</tbody>
</table>

*Nonfatal myocardial infarction and coronary death; **Primary prevention trial; ***Secondary prevention trial
QUESTION:

• ARE RELATIVE RISK REDUCTIONS IN CV EVENTS WITH STATIN THERAPY ---RELATED TO BASELINE LEVELS OF LDLC?


Genetic studies suggest that even lower LDLC levels are likely to confer cardiovascular benefits regardless of starting cholesterol levels

Am Coll Cardiol 2012;60:2631-9

JUPITER primary prevention trial high-intensity statin regimen, the magnitude of percentage change in LDLC was directly related to subsequent event rates.

Eur Heart J 2016;37:1373-9
THE LARGER PERCENTAGE CHANGE IN LDL THE GREATER THE CV BENEFIT
IVUS FINDS LOWER IS BETTER FOR CHANGING PLAQUE ATEROMA VOLUME

Am Heart J 2016;176:83-92
PCSK9 (proprotein convertase subtilisin-kexin type 9)(GENE)
When PCSK9 binds to an LDLR, the receptor is destroyed along with the LDL particle.

Atherosclerosis Risk in Communities Study, a missense mutation (pcsk9) in the prodomain (R46L), was associated with a 15% reduction in LDL-C and a 46% reduction in CHD.

Trends Biochem. Sci. 32: 71

2-nonsense mutations (Y1423 and C6793), present in 2% of African-Americans, caused a 28% reduction in LDL-C and an 88% reduction in CHD.

FURTHER CARDIOVASCULAR OUTCOMES RESEARCH WITH PCSK9 INHIBITION IN SUBJECTS WITH ELEVATED RISK (FOURIER)

- **DB, RANDOMIZED:** IMPACT OF ADDITIONAL LDL-CHOLESTEROL REDUCTION ON MAJOR CARDIOVASCULAR EVENTS WHEN EVOLOCUMAB (AMG 145) IS USED IN COMBINATION WITH STATIN THERAPY IN PATIENTS WITH CLINICALLY EVIDENT CARDIOVASCULAR DISEASE

- **(N = 27,500), 5 YEARS**

- **PRIMARY ENDPOINT:** CARDIOVASCULAR DEATH, MYOCARDIAL INFARCTION, HOSPITALIZATION FOR UNSTABLE ANGINA, STROKE, OR CORONARY REVASCULARIZATION WHICHEVER OCCURS FIRST

Enrollment: 27564
Study Start Date: February 2013
Estimated Study Completion Date: November 2016
Estimated Primary Completion Date: November 2016

AHA?

Am Heart J 2016;173:94-101
Clinical trials.gov Sept 2016
ODYSSEY OUTCOMES: EVALUATION OF CARDIOVASCULAR OUTCOMES AFTER AN ACUTE CORONARY SYNDROME DURING TREATMENT WITH ALIROCUMAB

• PRIMARY: CHD death, any non-fatal MI, fatal and non-fatal ischemic stroke, unstable angina requiring hospitalization

• F/U 64 MONTHS

Estimated Enrollment: 18600
Study Start Date: October 2012
Estimated Study Completion Date: February 2018
Estimated Primary Completion Date: December 2017 (Final data collection date for primary outcome measure)
GLOBAL ASSESSMENT OF PLAQUE REGRESSION WITH A PCSK9 ANTIBODY AS MEASURED BY INTRAVASCULAR ULTRASOUND (GLAGOV)

- Impact of PCSK9 inhibition on coronary atheroma progression
- Phase 3, multicenter, double-blind, randomized, placebo-controlled trial evaluating the impact of
- Coronary atheroma volume: baseline to week 78 post randomization, as determined by intravascular ultrasound (IVUS)
- Randomized to the Evolocumab (AMG 145) arm will receive Evolocumab (AMG 145) subcutaneously every 4 weeks
- Regression (any reduction from baseline) in PAV (atheroma volume) [78 weeks]

Enrollment: 970
Study Start Date: May 2013
Study Completion Date: July 2016
Primary Completion Date: July 2016

Inclusion Criteria:
Subjects already taking statin therapy, niacin or ezetimibe at screening must have been on a stable dose for at least 4 weeks prior to screening LDL-C. Fasting LDL-C ≥ 80 mg/dL (with or without additional risk factors)

Am Heart J 2016;176:83-92
Clinical trials.gov Sept 2016
### COMPARISON OF OUTCOMES PCSK9 CLINICAL TRIALS

<table>
<thead>
<tr>
<th></th>
<th>SPIRE 1 (n = 17,000)</th>
<th>SPIRE 2 (n = 11,000)</th>
<th>FOURIER (n = 27,500)</th>
<th>ODYSSEY Outcomes (n = 18,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibody</td>
<td>Bococizumab (humanized) 150 mg Q2W</td>
<td>Bococizumab (humanized) 150 mg Q2W</td>
<td>Evolocumab (human) 140 mg Q2W 420 mg Q4W</td>
<td>Alirocumab (human) 75-150 mg Q2W</td>
</tr>
<tr>
<td>Entry LDL (mg/dL)</td>
<td>≥70</td>
<td>≥70</td>
<td>High-intensity statin preferred, minimum dose atorvastatin 20 mg or equivalent</td>
<td>≥70</td>
</tr>
<tr>
<td>Statin requirement</td>
<td>Atorvastatin 40 or 80 mg Rosuvastatin 20 or 40 mg Simvastatin 40 mg (or 80 mg if &gt;1 year) or documented intolerance to high intensity statin (SPIRE-1 and SPIRE 2) or documented complete statin intolerance (SPIRE-2)*</td>
<td>Atorvastatin 40 or 80 mg Rosuvastatin 20 or 40 mg or the maximum tolerated dose of one of these agents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-risk secondary prevention</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>High-risk primary prevention</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*Allowed not to be taking statin if intolerant to any 2 statins (one at lowest dose) or a history of statin-induced rhabdomyolysis.
HIGHLIGHTS OF STATINS AND PCSK9

- **Statins proven to work and lower appears to be better**

- **PCSK9 with statins may reduce CV events even more….but will need to wait until FOURIER reports out this year or early 2017**

- **Possibly 30% of patient might have additional benefit from PCSK9 but will need to wait until CV endpoint trials are completed**
WHAT IS THE CORRECT LDL NUMBER OR JUST HIGH INTENSITY STATIN?

1. **Just give high risk patients high intensity statin (Government guidelines)**
2. **Treat based on LDL number**

Your patient

1. **Just give high risk patients high intensity statin (Government guidelines)**
2. **Treat based on LDL number**

You’re the patient
BET-bromo and extraterminal (family) proteins

Bromodomains (BRDs) are protein-interaction modules that are **selectively recruited to** lysine-containing sequences

BRDs nuclear proteins: regulating transcription, chromatin modulators, and chromatin-modifying enzymes

control expression of genes that play key regulatory roles in

Cell proliferation  Apoptosis

Dysfunction of BET proteins develop tumors

**BET inhibitors- raising HDL**

Crystal structures of RVX-208 with the first and second bromodomains of human BET proteins

**CHROMATIN-BASED THERAPEUTICS FOR ATHEROSCLEROSIS**

**RVX-208: domain-selective inhibitor of BETs**

Nature 468(7327):1067–1073
Pathways known to mediate cholesterol efflux from macrophages

Cholesterol flux biomarkers

ABCA1, ABCG1, scavenger receptor B1
CHOLESTEROL EFFLUX CAPACITY, A NEW BIOMARKER WAS INVERSELY RELATED TO INCIDENCE OF CARDIOVASCULAR EVENTS

- **Population based cohort**
- **Baseline N=2924 adults free from cardiovascular disease**
- **Dallas Heart Study**
- **Primary end point was atherosclerotic cardiovascular disease,**
  - **First nonfatal myocardial infarction, nonfatal stroke, or coronary revascularization or death from cardiovascular causes**
- **Follow up 9.4 years median**

<table>
<thead>
<tr>
<th>End Point</th>
<th>All Participants (N=2416)</th>
<th>Cholesterol Efflux Capacity</th>
<th>Unadjusted Hazard Ratio (95% CI)</th>
<th>Adjusted Hazard Ratio (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary end point: atherosclerotic cardiovascular disease</td>
<td>132</td>
<td>Quartile 1 (N=602)</td>
<td>49</td>
<td>0.44 (0.27–0.73)</td>
</tr>
<tr>
<td>Myocardial Infarction‡</td>
<td>28</td>
<td>Quartile 2 (N=605)</td>
<td>35</td>
<td>0.33 (0.19–0.55)</td>
</tr>
<tr>
<td>Stroke‡</td>
<td>36</td>
<td>Quartile 3 (N=607)</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Coronary revascularization‡</td>
<td>26</td>
<td>Quartile 4 (N=602)</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Death from cardiovascular causes‡</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CHOLESTEROL EFFLUX CAPACITY, A NEW BIOMARKER WAS INVERSELY RELATED TO INCIDENCE OF CARDIOVASCULAR EVENTS

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ACS have ↑↑↑ TCFA plaques

Diabetes vulnerable plaques

Lipids
↑ Thin plaque cap & ↑ CRP
 Increased macrophages

Optical coherence tomography

Thin-cap fibroatheroma

J Am Coll Cardiol Img 2009;2:339–49
**BET BROMODOMAIN INHIBITOR, RVX-208, SHOWS REDUCTION OF Atherosclerosis IN HYPERLIPIDEMIC APOE DEFICIENT MICE**

- **HDL REDUCES CVD RISK THROUGH A PROCESS THAT INVOLVES FORMATION OF PRE-BETA PARTICLES**
  - Facilitates the removal of cholesterol from the lipid-laden macrophages in the arteries
- **BET BROMODOMAIN ANTAGONIST, RVX-208**
  - Raise apoA-I and increase pre B - HDL particles
- **HYPERLIPIDEMIC APOE -/- KO MICE**
- **Evaluated aortic atherosclerosis in 12 week study**
  - 2-fold increases in the levels of circulating HDL-C, and ~50% decreases in LDL
  - No significant changes in plasma apoA-I were observed

Western diet ≈ Univ café food

Atherosclerosis 236 (2014) 91e100
SUMMARY OF BASIC TRANSLATION RESEARCH RVX-208, BET INHIBITOR

- 2 fold increase HDL
- Reduces LDL by 50%
- Reduces inflammation
- Reduces adhesion molecules

Atherosclerosis 236 (2014) 91e100
Humans

2 trials: total of 499 subjects received either 100 mg b.i.d. of RVX-208 (n = 331) or placebo (n = 168)

No significant changes in metabolic parameters (blood pressure, high-sensitivity C-reactive protein [hsCRP] and glucose)

Clinical trials-early
CLINICAL HUMAN TRIALS WITH RVX-208 (BET INHIBITOR)

- **SUSTAIN**: DOCUMENTED STABLE CORONARY ARTERY DISEASE
- **ASSURE** STUDY (IVUS) PATIENTS WERE SCHEDULED TO UNDERGO CORONARY ANGIOGRAPHY FOR A CLINICAL INDICATION
- **Both double blind randomized trials for 26 to 28 weeks**

Trials evaluated: lipid, inflammatory and metabolic biomarkers as well as incidence of major adverse cardiac events (MACE) defined as death, nonfatal myocardial infarction, coronary revascularization and hospitalization for unstable angina or heart failure

Secondary outcome: HDL-c, ApoA-I and HDL-subclasses

The proApoA-I clinical data were collected in the SUSTAIN study

Primary human hepatocytes with microarrays
COMBINED TRIAL RESULTS

N=499

HDL mg/dl

P<0.0003

Drug

Control

Baseline
6 months

SUSTAIN ASSURE

Atherosclerosis 247 (2016) 48-57
**Table 1**  
Combined analysis of the ASSURE and SUSTAIN phase II trials.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>RVX-208 (n = 331)</th>
<th>Placebo (n = 166)</th>
<th>p value vs placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Change from baseline (% ▲)</td>
<td>Baseline</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>39.0</td>
<td>+3.0 (+7.69)</td>
<td>38.0</td>
</tr>
<tr>
<td>ApoA-I (mg/dL)</td>
<td>119.2</td>
<td>+12.3 (+10.3)</td>
<td>118.1</td>
</tr>
<tr>
<td>Large HDL particles (μmol/L)</td>
<td>2.4</td>
<td>+0.8 (+30.7)</td>
<td>2.1</td>
</tr>
<tr>
<td>HDL particle size (nm)</td>
<td>8.7</td>
<td>+0.1 (+1.16)</td>
<td>8.7</td>
</tr>
<tr>
<td>Total HDL particles (μmol/L)</td>
<td>27.2</td>
<td>+1.9 (+6.51)</td>
<td>26.9</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>2.4</td>
<td>-0.2 (-10.8)</td>
<td>2.3</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.8</td>
<td>+0.1 (+2.08)</td>
<td>5.7</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>2.3</td>
<td>-0.36 (-28.4)</td>
<td>2.5</td>
</tr>
</tbody>
</table>
PHASE II RESULTS: SUSTAIN & ASSURE COMBINED

Percentage

<table>
<thead>
<tr>
<th></th>
<th>Drug</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>MACE</td>
<td>5.4</td>
<td>10.1</td>
</tr>
<tr>
<td>Death</td>
<td>0.3</td>
<td>1.8</td>
</tr>
<tr>
<td>MI</td>
<td>1.2</td>
<td>0</td>
</tr>
<tr>
<td>Coronary revascularization</td>
<td>2.7</td>
<td>4.2</td>
</tr>
<tr>
<td>Hosp UA/HF</td>
<td>1.2</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Atherosclerosis 247 (2016) 48-57
Diabetes microarrays

Cryopreserved human hepatocytes were treated with 30mM RVX-208

Reduce GI absorption of glucose - SGLT 1
No change in incretins levels

Microarray analysis of RVX-208-induced gene expression changes in primary human hepatocytes

Complement Cascade
Cholesterol Biosynthesis
Fatty Acyl CoA Biosynthesis

j.metabol.2016.03.002
Atherosclerosis 247 (2016) 48-57
Microarrays from cryopreserved primary human hepatocytes

Atherosclerosis 247 (2016) 48-57
ELEVATED LIVER ENZYMES WAS OBSERVED IN RVX-208-TREATED PATIENTS (7.1 vs. 0%, P = 0.009)

PRIMARY ENDPOINT, THE CHANGE IN PERCENT ATEROMA VOLUME

- CONTROL ARM: DECREASED 0.30% (P = 0.23 COMPARED WITH BASELINE)
- RVX-208: 11.1% (P < 0.001 COMPARED WITH BASELINE)
- LDL-C DECREASED BY 17.9% VS PLACEBO (P < 0.001 COMPARED WITH BASELINE)

No greater increase in apoA-I or HDL-C or incremental regression of atherosclerosis than administration of placebo

ClinicalTrials.gov identifier-NCT01067820.
Closing comments

Treatment of atherosclerosis

Lipids

Unknown
Weight loss in humans

Adipose tissue gene expression

Magkos et al., April 2016, Cell Metabolism 23, 591–601
4007 adults undergoing cath
TAKE HOME MESSAGE

• CV EVENT REDUCTION
  • STATINS WORK!
  • PCSK9 STILL IN INVESTIGATION
  • BET INHIBITORS NOT READY
  • LIPIDS ARE ONLY A SMALL PIECE OF THE PIE

• TRANSLATIONAL MEDICINE REQUIRES MORE THAN A REDUCED “NUMBER”

• WEIGHT LOSS IS STILL THE MOST POWERFUL LIFE SAVING TREATMENT…but hardest