RNA therapeutics for cardiovascular diseases

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Professor of Medicine
University of California San Diego

39th Panhellenic Congress of Cardiology

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Disclosures

Named as co-inventor and receives royalties from patents owned by the University of California San Diego on oxidation-specific antibodies

Co-Founder of Oxitope, Inc

Dual appointment at UCSD and Ionis Pharmaceuticals
Unmet need – Beyond LDL-C
FOURIER Trial Demonstrated 2% Absolute reduction in CVD events and no Effect of CVD Mortality

A Primary Efficacy End Point

Hazard ratio, 0.85 (95% CI, 0.79–0.92)
P<0.001

No. at Risk
Placebo 13,780 13,278 12,825 11,871 7610 3690 686
Evolocumab 13,784 13,351 12,939 12,070 7771 3746 689
Lipoprotein Targets in Preventing and Treating Cardiovascular Disease

Genetically Validated Lipoprotein Targets

- LDL-CapoB
- TG ApoC-III
- Lp(a) Apo(a)
- TG-LDL ANGPTL3
FDA Approvals for RNA Therapeutics
6 drug approvals to date

1998 - Vitravene (fomivirsen, Ionis) – CMV retinitis

2004 - Mucagen (pegaptanib, OSI Pharma)—VEGF

2013 - Kynamro® (mipomersen, Ionis)- HoFH

2016- Spinraza (nusinersen, Ionis) - SMA

2018- Tegsedi (inotersen-Ionis/Akcea) – TTR amyloidosis

2018- Patisiran (Onpattro- Alnylum)- TTR amyloidosis

2018- Waylivra (volanesorsen, Ionis/Akcea)
## U.S. Companies Developing RNA-targeted Therapies*

<table>
<thead>
<tr>
<th>Antisense Technology Companies</th>
<th>RNAi Technology Companies</th>
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<tbody>
<tr>
<td>Ionis Pharmaceuticals</td>
<td>Arcturus Therapeutics</td>
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<tr>
<td>Bio-Path Holdings</td>
<td>Avidity NanoMedicines</td>
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<tr>
<td>Enzo Biochem</td>
<td>Bioneer</td>
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<td>Idera Pharmaceuticals</td>
<td>Halo-Bio, RNAi Therapeutics</td>
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<tr>
<td>Rexahn</td>
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<tr>
<td>Sarepta Therapeutics</td>
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<td>Wave Therapeutics</td>
<td>miragen (subsidiary of Asuragen)</td>
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<th>Additional RNA-targeting Technology Companies</th>
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<tr>
<td>Moderna</td>
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<tr>
<td>Voyager Therapeutics</td>
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<tr>
<td>Ziopharm</td>
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</tbody>
</table>

- 24 non-U.S. companies developing RNA-targeted therapies*

* As of April 2017
RNA Therapeutics to “Silence” Genes
Fundamentally Novel Therapy Compared to Small Molecules and Antibodies

Gene

mRNA

Translation

Disease-Causing Protein

Traditional Small Molecule Drugs
Inhibitors or Agonists of proteins

↓ DISEASE

Biologics
Inhibitors or Mimics of proteins

Traditional Small Molecule Drugs
Inhibitors or Agonists of proteins

↓ DISEASE

Antisense Oligonucleotide
Inhibition of RNA function
(no production of disease causing protein)
Distinct Chemical Classes of RNA-Based Technologies & Therapeutics

- **Antisense**
  - Single-Strand
  - Multiple Mechanisms

- **siRNA**
  - Double-strand
  - RISC Mechanism

- **Aptamer**
  - Structured

- **DNA**
  - Phosphorothioate (PS)
    - 2’-MOE, 2’-OMe, cEt, LNA

- **RNA**
  - Phosphodiester
    - 2’-OMe, 2’-F
    - Aliphatic substituents

- **DNA or RNA**
  - Mixed modifications
    - Pegylation
    - (REG1 anticoagulation system)

- **Target Protein**
- **Antisense Strand**
- **Sense Strand**
- **Aptamer**
RNaseH1 and siRNA Antisense Mechanisms

- **siRNA Mechanism**

- **RNase H1 Mechanism**
The Antisense Drug-Receptor Interaction

~15-20 base pairs required for specificity and binding
Natural DNA and RNA do not make good drugs due to insufficient stability and distribution in animals
This can be addressed with appropriate chemical modification
Examples of Chemical Modifications Used in RNA Therapeutic Agents

- **Deoxy** (2'-deoxyribofuransyl)
- **Ribon** (ribofuransyl)
- **2'-Fluoro**
- **OMe** (2'-O-methyl)
- **MOE** (2'-O-methoxyethyl)
- **LNA** (locked nucleic acid)
- **S-cEt** (2',4'-constrained-2'-O-ethyl BNA)
- **Morpholino**
- **PNA** (Peptide nucleic acid)

**2'-O-methoxyethyl (MOE)**
RNAse H1 ASO Design and Ionis Clinical Experience

Chimeric RNAse H1 ASO Design

- ↑ affinity
- ↑ stability
- ↑ tolerability

RNase H1 Substrate

MOE DNA MOE

RNase H1 Terminating Mechanism

Clinical Experience with 2nd Generation ASOs

- >6000 subjects treated by IV and/or SC administration
- >60 clinical studies
- Multiple therapeutic indications
- >100 patients dosed for >1 year
- Some patients dosed for > 4 years
- Doses up to 1200 mg/wk tolerated

- Specific sequence not repeated throughout genome, reducing potential for off-target binding
Composition of Lp(a) and Relationship to Plasminogen

Tsimikas J Am Coll Cardiol 2017;69:692-711
Continental Differences in the Prevalence of Elevated Lp(a)

Lp(a) level gradient (high to low): Africa, South Asia, Europe, North America, South America, East Asia

- Estimated global population with elevated Lp(a) >50 mg/dL = 1.43 billion
  - If threshold of >30 mg/dL is used, estimate is >2 billion

- Apo(a) is synthesized in liver

- Estimates of Lp(a) >50 mg/dL or >125 nmol/L (% of Millions)
  - Africa: 20%, 1.262 billion
  - Europe: 9.9%, 738 million
  - South Asia: 24.4%, 1.876 billion
  - Rest of Asia: 35.8%, 2.807 billion
  - Oceania: 6.6%, 40.68 million
  - Latin America/Caribbean: 8.8%, 849 million
  - Northern America: 4.8%, 383 million
Baseline Lp(a) Quartile vs. MACE ODYSSEY OUTCOMES

*Adj. for age, sex, race, geographic region, time since index event, BMI, smoking history, diabetes, baseline LDL-C, and treatment assignment. Quartile interaction P-value in adjusted model: p=0.44 with treatment, p=0.67 with baseline LDL-C
What are the mechanisms through which Lpa) mediates CVD and CAVS?
OxPL-apoB and CVD Outcomes

Byun et al JACC 2015;65:1286-95
Lp(a)-OxPL and Aortic Stenosis

Oxidized Phospholipids, Lipoprotein(a), and Progression of Calcific Aortic Valve Stenosis

Romain Capoulade, PhD,* Kwan L. Chan, MD,1 Calvin Yeung, MD, PhD,1 Patrick Mathieu, MD,1 Yohan Bossé, PhD,1 Jean G. Dumesnil, MD,1 James W. Tam, MD,1 Koon K. Yeo, MBChB, PhD,1 Abhajit Mahmut, MD, MSC,1 Xiaohong Yang, BSc,1 Joseph L. Witztum, MD,1 Benjamin J. Arseneault, PhD,1 Jean-Pierre Després, PhD,1 Philippe Pibarot, DVM, PhD,1 Sotiris Tsimikas, MD,1

Lipoprotein(a)-Associated Molecules Are Prominent Components in Plasma and Valve Leaflets in Calcific Aortic Valve Stenosis

Michael Torzewski, MD,1 Amir Ravandi, MD, PhD,1 Calvin Yeung, MD, PhD,1 Andreas Edel, PhD,1 Rahul Bhindi, MD,1 Stefan Karch, MD,1 Laura Twardowski, MD,1 Jens Schmid, PhD,1 Xiaohong Yang, BSc,1 Ulrich F.W. Franke, MD,1 Joseph L. Witztum, MD,1 Sotiris Tsimikas, MD1

Autotaxin interacts with lipoprotein(a) and oxidized phospholipids in predicting the risk of calcific aortic valve stenosis in patients with coronary artery disease

M. J. Nsialia1, A. Mahmut1, M.-C. Boulanger1, B. J. Arsèneault2, R. Bouchareb1, S. Simard3, J. L. Witztum4, M.-A. Clavel1, P. Pibarot1, Y. Bossé1, S. Tsimikas1 & P. Mathieu1

From the 1Laboratory of Cardiovascular Pathobiology Quebec Heart and Lung Institute/Research Center, Department of Surgery; 2Department of Medicine, 3Statistical Consulting Service Unit at the Quebec Heart and Lung Institute/Research Center, Laval University, Quebec, Canada; 4University of California San Diego, La Jolla, CA, USA; and 5Department of Molecular Medicine, Laval University, Quebec, Canada

Oxidized Phospholipids and Risk of Calcific Aortic Valve Disease

The Copenhagen General Population Study

Pia R. Kamstrup, Ming-Yow Hung, Joseph L. Witztum, Sotiris Tsimikas, Berge G. Nordestgaard
Unifying Hypothesis of Lp(a)-OxPL-ATX axis in CAVS

Torzewski et al JACC BTS 2017
### 2016 ESC/EAS Guidelines for the Management of Dyslipidemias

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Class&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Level&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC is to be used for the estimation of total CV risk by means of the SCORE system.</td>
<td>I</td>
<td>C</td>
</tr>
<tr>
<td>LDL-C is recommended to be used as the primary lipid analysis for screening, risk estimation, diagnosis and management. HDL-C is a strong independent risk factor and is recommended to be used in the HeartScore algorithm.</td>
<td>I</td>
<td>C</td>
</tr>
<tr>
<td>TG adds information on risk and is indicated for risk estimation.</td>
<td>I</td>
<td>C</td>
</tr>
<tr>
<td>Non-HDL-C is a strong independent risk factor and should be considered as a risk marker, especially in subjects with high TG.</td>
<td>I</td>
<td>C</td>
</tr>
<tr>
<td>ApoB should be considered as an alternative risk marker whenever available, especially in subjects with high TG.</td>
<td>IIa</td>
<td>C</td>
</tr>
<tr>
<td>Lp(a) should be considered in selected cases at high-risk, in patients with a family history of premature CVD, and for reclassification in subjects with borderline risk.</td>
<td>IIa</td>
<td>C</td>
</tr>
<tr>
<td>The ratio apoB/apoA1 may be considered as an alternative analysis for risk estimation.</td>
<td>IIb</td>
<td>C</td>
</tr>
<tr>
<td>The ratio non-HDL-C/HDL-C may be considered as an alternative but HDL-C used in HeartScore gives a better risk estimation.</td>
<td>IIb</td>
<td>C</td>
</tr>
</tbody>
</table>

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**Box 7** Individuals who should be considered for lipoprotein(a) screening

- Individuals with:
  - Premature CVD
  - Familial hypercholesterolaemia
  - A family history of premature CVD and/or elevated Lp(a)
  - Recurrent CVD despite optimal lipid-lowering treatment
  - ≥5% 10-year risk of fatal CVD according to SCORE

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*Catapano et al Eur Heart J 2016;37:2999-3058*

Also endorsed by the 2016 Canadian Lipid Guidelines
Lp(a) – The Sword of Damocles?
IONIS-APO(a)-L\textsubscript{Rx} Produced Dose-dependent Significant Reductions in Lp(a)

- Well tolerated with no safety concerns

Up to 99% Reduction in Lp(a), with Mean Reduction of 92%

Mean Lp(a) reductions:
- 10 mg = ↓ 68%
- 20 mg = ↓ 80%
- 40 mg = ↓ 92%

Phase 2
ISIS 681257-CS6 STUDY
(AKCEA APO(a)-L_{Rx})

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)

Accepted as a Late Breaking Clinical Trial at AHA
Nov 12 2018
Lp(a) Cardiovascular Outcomes Trial

Inclusion Criteria:
Lp(a) >70 mg/dL, guideline recommended
LDL-lowering therapy, prior hx CVD

Randomize to:

Lp(a) lowering therapy vs. Placebo

4 yr median f/u

Primary Endpoint: MACE+ (CV Death, MI, Revascularization)
Potential RCT of Mild-Mod Aortic Stenosis

Lp(a) AS Trial (n=600)
Lipoprotein(a) Directed Antisense Therapy to Reduce Progression of Aortic Stenosis Trial

Inclusion Criteria:
Lp(a) >60 mg/dl, AVA 1.3-1.7 mm², EF >50%

ASO to apo(a) (sc monthly) vs. Placebo

Primary Endpoint: 3 Year change in AVA or mean AV gradient by echocardiography

Secondary Endpoints:
1- Rate of AVR
2- Progression of AV calcification measured by AoV calcium and Na¹⁸F uptake
Potential RCT of Aortic Stenosis

OxPL AS Trial (n=600)
OxPL Directed Therapy to Reduce Progression of Aortic Stenosis Trial

Inclusion Criteria:
OxPL-apoB in upper tertile, AVA 1.3-1.7 mm², EF >50%

OxPL-ATX directed Rx vs. Placebo

Primary Endpoint: 3 Year change in AVA or mean AV gradient by echocardiography

Secondary endpoints:
1- Rate of AVR
2- Progression of AV calcification measured by AoV calcium and Na¹⁸F uptake
We did it Jenkins! We found the Higgs boson!

Now let’s search for a medical cure for aortic valve stenosis

The future?...
APOC3 as a Target for CHD Risk Reduction

40% Reduction in apoC-III

40% Reduction in TG

40% Reduction in CHD Risk
FCS (Familial Chylomicronemia Syndrome)
A Rare Genetic Disease Due to Mutations in LPL

European and FDA Orphan Drug Designation

- FCS is associated with extremely high levels of TG, often >2,000 mg/dL
- FCS patients are at extreme risk for acute pancreatitis events and other serious conditions

- Approximately 3,000 – 5,000 patients
- No currently available therapies
Familial Chylomicronemia Syndrome (FCS): A Severe and Rare Genetic Disorder

- **Background:**
  - Rare autosomal recessive disorder (orphan disease)
  - Caused by null loss-of-function LPL, GPIHPB1, APOC2, APOA5, LMF1 gene mutations

- **Clinical expression/risk:**
  - TG most often > 1000 mg/dl (11.1 mmol/L)
  - Signs and symptoms:
    - Abdominal pain (recurrent, often severe)
    - Plasma lactescence and viscosity
    - Lipemia retinalis
    - Hepatosplenomegaly
    - Eruptive xanthomas
    - Acute pancreatitis
    - Pancreatic insufficiency
    - Diabetes, other complications
Patients had an average 77% reduction in triglycerides at 3 months on volanesorsen, as compared to an average of 18% increase on placebo, p<0.001, for an absolute difference in average percent change of -94%.

Volanesorsen (N=33)
- Baseline TG* 2267 (1259)
- Month 3 TG* 590 (497)

Placebo (N=33)
- Baseline TG* 2152 (1153)
- Month 3 TG* 2367 (1315)

94% Treatment Effect
(-121.7, -66.6)
p-value < 0.001
Relationship of TG levels and CVD in dal-Outcomes
Potential Effect of IONIS-APOCIII-L_{Rx} (now in Phase I)

![Graph showing risk reduction for IONIS-APOCIII-L_{Rx} compared to Placebo across different quintiles of triglycerides (mg/dL). Adjusted for age, sex, smoking, diabetes, HDL-C, BMI.]

Schwartz et al., *J Am Coll Cardiol* 2015;65:2267-75
Acknowledgments

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