Intervention to improve LDL receptor function

*Emerging Therapies*

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Most Lipid Lowering Drugs Affect LDL-C Levels By Up-Regulating the LDLR

- statins
- ezetimibe
- bile acid sequestrants
- PCSK9 -inhibitors

B100

MTP

apoB

TG

Cuchel NLA 2017
Do we really need more LDL-C lowering drugs?

- LDL-C still not a goal with existing LLTs in a significant portion of subjects
- Drug intolerance (statins)
- Cost of new treatments
- Other reasons
Outline

• PCSK9 – beyond monoclonal antibodies
• ETC-1002 – inhibiting upstream HMGCoAR
• Replacing LDLR - Gene therapy
• ANGPTL3 inhibitors and other drug affecting LDL production
PCSK9 enhances LDLR degradation
Use of PCSK9i is complementary to that of statins

**PCSK9i**
- evolocumab
- alirocumab
- [other monoclonal ABs]

**statins**

\[ \downarrow LDL-C \]

\[ \uparrow LDLR \]

\[ \uparrow PCSK9 \]

\[ \downarrow \text{HMG-CoA} \]

\[ \downarrow \text{Cholesterol} \]
PCSK9 inhibition is very effective in lowering LDL-C
PCSK9 – beyond monoclonal Antibodies

- Monoclonal PCSK9i are expensive
- Require high doses
- Are injectable
- ?May lose responsiveness over time?
Targeting PCSK9 with novel strategies

- Vaccines
- siRNA
- Small molecules
Targeting PCSK9 with novel strategies

Vaccines

Target protein  Epitope identification  Conjugation with foreign peptide  Administration  Antibodies production

Clinicaltrials.gov NCT02508896
Vaccine against PCSK9 lowers LDL-C in primates
Targeting PCSK9 with novel strategies

siRNA

Gene silencing

SREBP-2 pathway

↑ HMGCoA Red
↑ LDL-R
↓ PCSK9

Clinicaltrials.gov NCT02597127, NCT02314442

Cuchel NLA 2017
The RNAi inhibitor of PCSK9 - inclisiran - lowers PCSK9 and LDL-C levels in humans
Targeting PCSK9 with novel strategies
Small molecules

SREBP-2 pathway

↑ HMGCoA Red
↑ LDL-R
↑ PCSK9

Adnectins
Binding Proteins to PCSK9
Targeting PCSK9 with novel strategies
Small molecules

Blocking TGN transport and/or autocleavage

SREBP-2 pathway

↑ HMGCoA Red
↑ LDL-R
↑ PCSK9

Cuchel NLA 2017
Outline

• PCSK9 – beyond monoclonal antibodies
• **ETC-1002** – inhibiting upstream HMGCoAR
• Replacing LDLR - Gene therapy
• ANGPTL3 inhibition
Inhibiting upstream HMGCoA Reductase
ETC-1002 (Bempedoic Acid)

LDL-C

\[\text{Citrate} \rightarrow \text{ACL} \rightarrow \text{Acetyl-CoA} \rightarrow \text{HMG-CoA} \rightarrow \text{HMG-R} \rightarrow \downarrow \text{Cholesterol} \]

\[\downarrow \text{LDL-C} \]

\[\text{FA} \rightarrow \text{TG} \]

\text{statins}
Inhibiting upstream HMGCoA Reductase

ETC-1002 (Bempedoic Acid)

- **Acetyl-CoA Synthase**
- **ETC-1002 CoA**
- **ACL**
- **Acetyl-CoA**
- **Citrate**
- **HMG-CoA**
- **HMG-R**
- **TG**
- **FA**
- **↓Cholesterol**
- **↓LDL-C**
- **statins**

- 18 studies listed in Clinicaltrials.gov, 5 actively recruiting
ETC-1002 lower LDL-C in patients with or without statin intolerance

Thompson J Clin Lipidol 2016. 10: 556-567,
Outline

- PCSK9 – beyond monoclonal antibodies
- ETC-1002 – inhibiting upstream HMGCoAR
- Replacing LDLR - Gene therapy
- ANGPTL3 inhibition
Homozygous Familial Hypercholesterolemia

12 Y.O. female
LDL-C=780 mg/dL, xanthomas since age 3, coronary heart disease, cardiac bypass
FH is Caused by Mutations in Genes Affecting LDL Receptor Functionality

- The impaired LDLR functionality leads to a decreased clearance of LDL particles from plasma

HeFH
LDL-C > 190 mg/dl

HoFH
LDL-C > 500 mg/dl

Cuchel NLA 2016
FH is Most Frequently Caused by \textit{LDLR} Mutations

- Mutations in \textit{LDLR} are found in \textasciitilde 95\% of the confirmed cases of HoFH
- Other genes are: \textit{APOB, PCSK9, LDLRAP1}
- More than 1,000 \textit{LDLR} mutations have been reported
- Based on the mutations patients can be divided in receptor negative (<2\% activity) or receptor defective
- The type of mutations affect the LDL-C levels and the response to treatment
Penn HoFH Cohort:  
LDLR Negative Subjects Have a More Severe Phenotype

<table>
<thead>
<tr>
<th>Median (range)</th>
<th>LDLR negative (n=18)</th>
<th>LDLR defective (n=16)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr) at visit 1</td>
<td>11.5 (3.3 - 29)</td>
<td>28.1 (3.3 - 44.6)</td>
<td>0.008</td>
</tr>
<tr>
<td>Age at 1st xanthomas</td>
<td>2.0 (0.25 - 4)</td>
<td>7.0 (1 - 15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (yr) at FH dx</td>
<td>3.0 (0.5 - 7)</td>
<td>8.0 (2 - 17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC (mg/dl) at dx</td>
<td>895 (602-1260)</td>
<td>686 (519-900)</td>
<td>0.002</td>
</tr>
<tr>
<td>Age (yr) at start of Rx</td>
<td>5.0 (1.2 - 10)</td>
<td>16.0 (2 - 31)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (yr) at CAD</td>
<td>12.5 (6 - 16)</td>
<td>22.0 (16 - 37)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Lipid profile at visit 1  
Mean (SD)  
<table>
<thead>
<tr>
<th>TC (mg/dl)</th>
<th>LDLR negative (n=18)</th>
<th>LDLR defective (n=16)</th>
<th>0.025</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C (mg/dl)</td>
<td>535 (214)</td>
<td>393 (159)</td>
<td>0.040</td>
</tr>
<tr>
<td>Apo B (mg/dl)</td>
<td>388 (142)</td>
<td>293 (92)</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Kolansky DM, Am J Cardiol 2008; 102:14-38-43  
Cuchel NLA 2017
New Lipid Lowering Drugs Affect LDL-C Levels by Inhibiting VLDL Secretion

mipomersen

apoB

B100

TG

MTP

lomitapide

TG
Would liver-directed gene therapy of LDLR for HoFH work?
Liver transplantation normalizes LDL-C in patients with Homozygous Familial Hypercholesterolemia

Gene Therapy
Viruses Are Natural Gene Delivery Vehicles

- Viruses have evolved to deliver genes efficiently to cells
- Viruses can be engineered to both express the human gene of interest and impair their ability to replicate. These engineered viruses are often referred to as viral vectors.

Liver directed \textit{ex vivo} gene therapy in patients with HoFH

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<table>
<thead>
<tr>
<th>LDL cholesterol</th>
<th>Pre + statin</th>
<th>Pre - statin</th>
<th>Post - statin</th>
<th>Post + statin</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH1</td>
<td>448 ± 30 (6)</td>
<td>482 ± 19 (7)</td>
<td>372 ± 35 (51)</td>
<td>380 ± 29 (17)</td>
</tr>
<tr>
<td>FH2</td>
<td>510 ± 24 (3)</td>
<td>516 ± 15 (5)</td>
<td>515 ± 43 (14)</td>
<td>477 ± 38 (56)</td>
</tr>
<tr>
<td>FH3</td>
<td>804 ± 49 (5)</td>
<td>792 ± 37 (6)</td>
<td>745 ± 82 (23)</td>
<td>803 ± 75 (5)</td>
</tr>
<tr>
<td>FH4</td>
<td>421 ± 44 (5)</td>
<td>530 ± 28 (5)</td>
<td>525 ± 55 (20)</td>
<td>448 ± 20 (7)</td>
</tr>
<tr>
<td>FH5</td>
<td>786 ± 103 (2)</td>
<td>737 ± 34 (12)</td>
<td>595 ± 41 (18)</td>
<td>664 ± 42</td>
</tr>
</tbody>
</table>

Recombinant AAV Vectors for Gene Therapy

- Adeno Associated Virus (AAV) are non-pathogenic in humans
- AAV are much less immunogenic than vectors used before
- Very unlikely to integrate into the host cell genome
- *In vivo* approach is possible
- Several AAVs are used in gene therapy for several genetic conditions
- AAV8 can produce efficient and prolonged gene transfer in the liver
## Clinical Experience With AAV Gene Therapy

<table>
<thead>
<tr>
<th>Disease</th>
<th>AAV vector</th>
<th>Route of administration</th>
<th>Target organ</th>
<th>Clinical outcome</th>
<th>Latest Reported Duration of Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoprotein lipase (LPL) deficiency(^1)</td>
<td>Glybera(^\circ), alipogene tiparvovec, AAV1-LPL(^{S447X})</td>
<td>Multiple intramuscular injections</td>
<td>Muscle</td>
<td>Reduction in pancreatitis</td>
<td>6+ years</td>
</tr>
<tr>
<td>Hemophilia B(^2)</td>
<td>AAV8-FIX</td>
<td>Single intravenous infusion</td>
<td>Liver</td>
<td>Reduction in bleeding episodes and in FIX infusions</td>
<td>4+ years</td>
</tr>
</tbody>
</table>

AAV-LDLR administration reduce LDL-C levels and atherosclerotic burden in animal models

*humanized mouse model: LDLR-Apobec-DKO/hApoB-Tg mice

Kassim SH et al Human Gene Therapy. 2013, 24: 19-26
Kassim SH et al Plos One. 2010,
FHGT002 - Phase I/II Clinical Trial in HoFH

Objectives:
1. To determine the safety of AAV-LDLR administration in patients with HoFH
2. To assess efficacy (% changes in LDL-C levels)
3. To assess immune response following vector administration

Key inclusions criteria:
1. Male or female ≥ 18 years of age.
2. **LDLR mutations** at both alleles, clinical presentation consistent with HoFH.
3. NAbs titer <1:10
4. Stable concurrent allowed lipid lowering medications:
   - statins, ezetimibe, bile acid seq., PCSK9i, apheresis

Clinicaltrials.gov NCT02651675
Outline

• PCSK9 – beyond monoclonal antibodies
• ETC-1002 – inhibiting upstream HMGCoAR
• Replacing LDLR - Gene therapy
• ANGPTL3 inhibitors (and other drug affecting LDL production)
Lowering LDL-C levels by decreasing LDL production

- Mipomersen
- ApoB
- B100
- LPL
- ApoC-III
- ANGPTL3
- Acetyl-CoA Carboxylase
- Lomitapide
- TG inhibitors
- LDL-C
- Gemcabene
ANGPTL3 inhibitor as a treatment for HoFH

- ANGPTL3 is a liver secreted protein that inhibits Lipoprotein Lipase and possibly Endothelial Lipase
- Carriers of ANGPTL3 mutations have combined hypolipidemia

ANGPTL3 inhibitor as a treatment for HoFH

• ANGPTL3 inhibition reduces LDL in ldlr -/- mice

• Can ANGPTL3 inhibition be effective in reducing LDL-C in HoFH?

Wang Y. JLR 2015. 56: 1296-1307
ANGPTL3 inhibitor as a treatment for HoFH

- Main study of REGN1500 (evinacumab) in HoFH subjects is completed. OLE study is ongoing (NCT02265952)
- Preliminary data show that it is effective in reducing LDL-C levels in HoFH
- Evinacumab received Breakthrough Therapy Designation
Lowering LDL-C levels by decreasing LDL production

apoB

MTP

TG

B100

LPL

TG

ANGPTL3

inhibitors
Summary

- Upregulation of the LDL Receptor is a valid and proven approach to lower LDL-C levels
- The LDL receptor continue to be an active drug target
- Alternative approaches to block PCSK9 activity are actively pursued
- ETC-1002, an inhibitor of ACL, is effective in reducing LDL-C levels
- LDL receptor gene replacement using AAV vectors is being currently pursued
- ANGPTL3 inhibition is a promising approach to lower LDL-C via non LDLR-mediated mechanisms.