Novel Therapies and Future Developments to Treat Familial Hypercholesterolemia

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President of Oman society of Lipid & Atherosclerosis (OSLA)
Disclosures

• Honoraria for Speakers Bureau (Pharma)
  AstraZeneca, Sanofi, Pfizer

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• Research Funding: Pfizer
Familial Hypercholesterolemia as a Prototype for Precision Medicine

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Many Patients With High CV Risk Are Not Achieving Optimal LDL-C Levels

- Statins +/- ezetimibe are the standard of care for LDL-C management
- However, many patients are not reaching LDL-C goals with standard-of-care therapy due to suboptimal reductions in LDL-C\(^{(1-3)}\)

![Pie charts showing LDL-C goal attainment](image)

**High risk patients\(^{(1)}\)**
- LDL-C Goal <100 mg/dL: 23% not at goal
- LDL-C Goal <70 mg/dL: 76% not at goal

**HeFH patients\(^{(2,3)}\)**
- LDL-C Goal <100 mg/dL: ~80% not at goal

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**Novel Gene therapies with future potential prospects for FH**

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Strategy</th>
<th>Potential Target</th>
<th>Animal experiment result</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minicircle DNA vectors</td>
<td>Small (∼4 kb), circular, nonviral plasmid derivatives</td>
<td>Longer ectopic expression, higher transfection rate, and greater resistance to shearing forces</td>
<td>LDLR</td>
<td>LDL-c levels decreased from 140-200 mg/dl to 90-150 mg/dl at 20 weeks without any detectable toxicity in C57BL/6 LDLR−/− mice</td>
<td>[32]</td>
</tr>
<tr>
<td>MicroRNAs</td>
<td>Endogenous non-coding single-stranded RNA containing 18-22 nucleotides</td>
<td>Small volume; target multiple genes</td>
<td>Genes involved in cholesterol metabolism</td>
<td>Hydrodynamic injection of pLDLR-LDLR-miR82 into Ldlr−/− mice reduced plasma levels of atherogenic lipids by ∼32% and atherosclerosis by ∼40% after 12 weeks</td>
<td>[34]</td>
</tr>
<tr>
<td>IncRNAs</td>
<td>RNA molecules that are greater than 200 nt in length</td>
<td>Easy association with homologous DNA sequences, homologous RNA sequences, and proteins</td>
<td>Genes involved in cholesterol metabolism</td>
<td>Ldlr−/− mice treated with AAV8. hTBG.LeXis exhibited reduced expression of genes involved in cholesterol biosynthesis, TC and TG levels and atherosclerotic burden</td>
<td>[36]</td>
</tr>
<tr>
<td>CRISPR/Cas9</td>
<td>A gene-editing system</td>
<td>Simple production, lower cost, high efficiency, can knock-in or knock-out multigene</td>
<td>Genes involved in cholesterol metabolism</td>
<td>Multiple studies have shown that PCSK9 targeting reduces lipid levels and atherosclerosis in Ldlr−/− mice</td>
<td>[38]</td>
</tr>
</tbody>
</table>
### Ongoing clinical trials for gene therapy for FH

<table>
<thead>
<tr>
<th>Trial</th>
<th>Vector</th>
<th>Therapeutic Agent</th>
<th>Drug Name</th>
<th>Delivery</th>
<th>Study Design</th>
<th>N</th>
<th>Primary Endpoint</th>
<th>Trial Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>AAV</td>
<td>hLDLR</td>
<td>AAV8.TBG, hLDLR</td>
<td>I.V. injection</td>
<td>Phase 1/2, Open Label</td>
<td>12</td>
<td>52 weeks safety; physical examinations; clinical laboratory parameters</td>
<td>NCT02651675</td>
</tr>
<tr>
<td>ORION-2</td>
<td>NA</td>
<td>siRNA-PCSK9</td>
<td>ALN-PCSsc</td>
<td>S.C. injection</td>
<td>Phase 2 Open Label</td>
<td>10</td>
<td>Changes in LDL-c levels at 90 or 180 days</td>
<td>NCT02963311</td>
</tr>
<tr>
<td>RADICHOL 1</td>
<td>NA</td>
<td>ASO-ApoB-100</td>
<td>Mipomersen</td>
<td>S.C. injection</td>
<td>Phase 3, RCT/Open Label</td>
<td>51</td>
<td>Changes in LDL-c levels up to week 28</td>
<td>NCT00607373, NCT00706849, NCT00794664, NCT00694109</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>ASO-ANGPTL3-IRx</td>
<td>IONIS ANGPTL3-IRx</td>
<td>S.C. injection</td>
<td>Phase 1/2, RCT</td>
<td>61</td>
<td>Safety and tolerability, pharmacokinetics, and pharmacodynamics up to day 127</td>
<td>NCT02709850</td>
</tr>
</tbody>
</table>

Gene Therapy in the FH Field

• The first use of gene therapy for FH treatment in an animal model was reported in 1991, 30% – 50% decrease in TC levels after 122 days.

• In 1995, the first clinical trial for FH treatment with recombinant retroviruses in five HoFH, LDL-c levels decreased by 6%–25% in only three patients.

• Different strategies in preclinical stage to increase the therapeutic efficacy or reduce the immunogenicity
  • Helper-dependent adenoviral vectors to deliver the VLDLR gene
  • RNA interference mediated knockdown HMG CoA reductase
Virus Vector-Mediated Gene Therapy

In Clinical Trials for FH began in 2016 and will end in 2019

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<td>NCT02651675</td>
</tr>
</tbody>
</table>
Small Interfering RNAs (siRNAs) Targeting PCSK9 Synthesis (Inclisiran)

Asialoglycoprotein receptor (ASGPR)
Inclisiran:  
siRNA conjugated to N-acetylgalactosamine  
Subcutaneous administration  
Targeted delivery to hepatocytes  
Third generation with enhanced stabilisation chemistry

Asialoglycoprotein receptor (ASGPR):  
Highly expressed in hepatocytes only.  
High rate of uptake
GalNAc-siRNA conjugates facilitate rapid hepatic uptake

**Background**

**Inclisiran:**
- siRNA conjugated to N-acetylgalactosamine
- Subcutaneous administration
- Targeted delivery to hepatocytes
- Third generation with enhanced stabilisation chemistry

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**Asialoglycoprotein receptor (ASGPR):**
- Highly expressed in hepatocytes only.
- High rate of uptake
ORION-9
Inclisiran for heterozygous familial hypercholesterolemia

<table>
<thead>
<tr>
<th>Name</th>
<th>City</th>
<th>Name</th>
<th>City</th>
<th>Name</th>
<th>City</th>
</tr>
</thead>
<tbody>
<tr>
<td>FJ Raal</td>
<td>Johannesburg</td>
<td>D Kallend</td>
<td>Zurich</td>
<td>KK Ray</td>
<td>London</td>
</tr>
<tr>
<td>W Koenig</td>
<td>Munich</td>
<td>RS Wright</td>
<td>Rochester</td>
<td>T Turner</td>
<td>Cincinnati</td>
</tr>
<tr>
<td>D Curcio</td>
<td>Parsippany</td>
<td>MJ Jaros</td>
<td>Chicago</td>
<td>PLJ Wijngaard</td>
<td>Parsippany</td>
</tr>
<tr>
<td>JJP Kastelein</td>
<td>Amsterdam</td>
<td></td>
<td></td>
<td>LA Leiter</td>
<td>Toronto</td>
</tr>
</tbody>
</table>

Presented at AHA 2019
ORION-9: Study design
Eighteen months treatment and observation

Randomized 1:1 inclisiran 300 mg vs. placebo – with maximally tolerated statins

**Screening**
- Day -14 to -1

**Visit 1**
- Day 1

**V2**
- Day 30

**V3**
- Day 90

**V4**
- Day 150

**V5**
- Day 270

**V6**
- Day 330

**V7**
- Day 450

**V8**
- Day 510

End of Study
- Day 540 (V9)
  - 90 days post last dose
## ORION-9: Patients

**High-risk phenotypes balanced by randomization**

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>Placebo</th>
<th>Inclisiran</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ITT population</strong></td>
<td>N = 240</td>
<td>N = 242</td>
</tr>
<tr>
<td>Age median (IQR) – years</td>
<td>56 (47, 63)</td>
<td>56 (46, 64)</td>
</tr>
<tr>
<td>Female gender</td>
<td>125 (52%)</td>
<td>130 (54%)</td>
</tr>
<tr>
<td>Atherosclerotic cardiovascular disease</td>
<td>73 (30%)</td>
<td>59 (24%)</td>
</tr>
</tbody>
</table>

### Lipid management treatment

<table>
<thead>
<tr>
<th>Statins</th>
<th>217 (90%)</th>
<th>219 (91%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Of which high intensity statins given</td>
<td>171 (79%)</td>
<td>185 (84%)</td>
</tr>
<tr>
<td>Ezetimibe use</td>
<td>135 (56%)</td>
<td>120 (50%)</td>
</tr>
</tbody>
</table>

| Baseline LDL-C mg/dL (±SD)²                      | 155 (58)  | 151 (50)  |

1. All patients who were randomized, analyzed according to randomization
2. SD is standard deviation
# ORION-9: Patients

**Genotyping results for 432 patients giving consent**

<table>
<thead>
<tr>
<th>Genetic variants</th>
<th>Placebo</th>
<th>Inclisiran</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 240</td>
<td>N = 242</td>
</tr>
<tr>
<td><strong>Genetic testing performed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDLR variants</td>
<td>211</td>
<td>221</td>
</tr>
<tr>
<td>Of which</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathogenic</td>
<td>131 (55%)</td>
<td>125 (52%)</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>118 (90%)</td>
<td>113 (90%)</td>
</tr>
<tr>
<td>Uncertain significance</td>
<td>9 (7%)</td>
<td>8 (6%)</td>
</tr>
<tr>
<td>Two variants (‘double’)</td>
<td>4 (3%)</td>
<td>4 (3%)</td>
</tr>
<tr>
<td>APOB variants</td>
<td>11 (5%)</td>
<td>12 (5%)</td>
</tr>
<tr>
<td>PCSK9 gain of function variant</td>
<td>0</td>
<td>1 (0.4%)</td>
</tr>
<tr>
<td>No variant detected</td>
<td>54 (23%)</td>
<td>61 (25%)</td>
</tr>
</tbody>
</table>

1. All patients who were randomized, analyzed according to randomization
ORION-9: Efficacy
Durable and potent effect over 18 months

Percent and absolute change in LDL-C over time – observed values in ITT patients

- Time-averaged Δ 45% for 63 mg/dL
- Δ 50% for 71 mg/dL

P-value for placebo – inclisiran comparison at each time point <0.001

1. All 95% confidence intervals are less than ±2% and therefore are not visible outside data points.
ORION-9: Summary and conclusions
Inclisiran lowered LDL-C durably and safely in HeFH

Well-powered 18 month double-blind randomized placebo controlled HeFH trial

ORION-9 met all primary and secondary efficacy endpoints
• 71 mg/dL (50%) observed LDL-C lowering at day 510
• 63 mg/dL (45%) observed time-adjusted LDL-C lowering day 90-540
• On top of statins (>90%) and ezetimibe (>50%)
• Robust reduction in LDL-C with all underlying FH genotypes

Safety profile of inclisiran was similar to placebo in a high-risk population
• Adverse event incidence and laboratory values not different
• Injection site events were ~13% higher on inclisiran – mostly mild and all transient

Inclisiran shows potential to address the unmet need of high risk HeFH patients
ORION-2: Phase II HoFH
Robust, durable effects in homozygous familial hypercholesterolemia

Asp227Glu/Asp227Glu variant

Absolute LDL-C reduction
- 184 mg/dL at day 60
- 276 mg/dL at day 120
- 242 mg/dL at day 180

Standard dose
ORION-2: Phase II HoFH
Robust, durable effects in homozygous familial hypercholesterolemia

Asp227Glu/Asp227Glu variant

Absolute LDL-C reduction
- 184 mg/dL at day 60
- 276 mg/dL at day 120
- 242 mg/dL at day 180

Standard dose
GalNAc-siRNA conjugates facilitate rapid hepatic uptake

**Background**

**Inclisiran:**

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- Subcutaneous administration
- Targeted delivery to hepatocytes
- Third generation with enhanced stabilization chemistry

**Asialoglycoprotein receptor (ASGPR):**

- Highly expressed in hepatocytes only.
- High rate of uptake
Angiopoietin like 3 (ANGPTL3) Inhibitors
Lipoprotein Metabolism in ANGPTL3 Deficiency

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% Difference</th>
<th>p-value</th>
<th>% Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C</td>
<td>-8.6%</td>
<td>0.007</td>
<td>-67.2%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>TG</td>
<td>-21.1%</td>
<td>0.005</td>
<td>-71.2%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-16.8%</td>
<td>p&lt;0.001</td>
<td>-39.0%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>ApoB</td>
<td>-7.2%</td>
<td>0.008</td>
<td>-48.4%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>ApoA1</td>
<td>-13.1%</td>
<td>0.001</td>
<td>-95.1%</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Other clinical characteristics of homozygotes:
- No increase in prevalence of fatty liver compared to controls
- No increased atherosclerosis or other manifestations of CVD
- Improved insulin sensitivity, lower plasma glucose, and lower incidence of T2DM

Minicocci I, et al. JLR, 2013
Antisense Oligonucleotides (ASOs) Therapy to Block Translation of ANGPTL3 Protein

RNA-Targeted Antisense Drugs Block the Translation of ANGPTL3 Protein

DNA

Transcription

mRNA

Translation

Disease-associated Protein

Traditional Drug

RNase H1

Degradation

mRNA = No Translation
Antisense Oligonucleotides (ASOs) Therapy to Block Translation of ANGPTL3 Protein (IONIS-ANGPTL3\textsubscript{RX})

Conclusion from Phase 1 Trial on IONIS-ANGPTL3\textsubscript{RX}

- IONIS-ANGPTL3-L\textsubscript{RX} reduced plasma levels of ANGPTL3 up to 83% in healthy volunteers with elevated triglyceride levels.

- Significant mean reductions were noted in TGs (-66%), apoC-III (-68%), LDL-C (-35%), total cholesterol (-36%), HDL-C (-25%) and non-HDL-C (-40%).

- Among all known therapies that lower TG levels, this is associated not only with reduced levels of LDL-C but total apoB as well.

- No safety concerns were identified related to target reduction or drug administration.

- IONIS-ANGPTL3-L\textsubscript{RX} is a promising candidate for patients with poorly controlled LDL-C, elevated TG and possibly in patients with hepatic steatosis or NASH.
Study of AKCEA-ANGPTL3-LRX (ISIS 703802) in Patients With Homozygous Familial Hypercholesterolemia (HoFH)

The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Read our disclaimer for details.

ClinicalTrials.gov Identifier: NCT03455777

Recruitment Status: Withdrawn (Study withdrawn due to lack of available patients meeting entry criteria)
First Posted: March 7, 2018
Last Update Posted: December 3, 2018

Sponsor:
Akcea Therapeutics

Collaborator:
Ionis Pharmaceuticals, Inc.

Information provided by (Responsible Party):
Akcea Therapeutics
Table 1. Low-Density Lipoprotein (LDL) Receptor Function and Responses to Evinacumab at 4 Weeks.*

<table>
<thead>
<tr>
<th>Patient</th>
<th>LDL Receptor Genotype†</th>
<th>Baseline LDL Cholesterol Level‡</th>
<th>Decrease from Baseline in LDL Cholesterol Levels at Wk 4</th>
<th>Absolute Decrease from Baseline in LDL Cholesterol Level at Wk 4</th>
<th>LDL Cholesterol Level at Wk 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Homozygous (non-null/non-null)</td>
<td>516 mg/dl</td>
<td>25 %</td>
<td>128 mg/dl</td>
<td>388 mg/dl</td>
</tr>
<tr>
<td>B</td>
<td>Compound heterozygous (non-null/null)</td>
<td>297 mg/dl</td>
<td>27 %</td>
<td>81 mg/dl</td>
<td>216 mg/dl</td>
</tr>
<tr>
<td>C</td>
<td>Homozygous (non-null/non-null)</td>
<td>153 mg/dl</td>
<td>90 %</td>
<td>138 mg/dl</td>
<td>15 mg/dl</td>
</tr>
<tr>
<td>D</td>
<td>Compound heterozygous (non-null/null)</td>
<td>357 mg/dl</td>
<td>77 %</td>
<td>275 mg/dl</td>
<td>82 mg/dl</td>
</tr>
<tr>
<td>E</td>
<td>Homozygous (null/null)</td>
<td>746 mg/dl</td>
<td>26 %</td>
<td>193 mg/dl</td>
<td>553 mg/dl</td>
</tr>
<tr>
<td>F</td>
<td>Homozygous (null/null)</td>
<td>312 mg/dl</td>
<td>42 %</td>
<td>132 mg/dl</td>
<td>180 mg/dl</td>
</tr>
<tr>
<td>G</td>
<td>Compound heterozygous (null/null)</td>
<td>736 mg/dl</td>
<td>44 %</td>
<td>323 mg/dl</td>
<td>413 mg/dl</td>
</tr>
<tr>
<td>H</td>
<td>Compound heterozygous (non-null/non-null)</td>
<td>152 mg/dl</td>
<td>51 %</td>
<td>77 mg/dl</td>
<td>75 mg/dl</td>
</tr>
<tr>
<td>I</td>
<td>Compound heterozygous (non-null/non-null)</td>
<td>117 mg/dl</td>
<td>61 %</td>
<td>71 mg/dl</td>
<td>46 mg/dl</td>
</tr>
<tr>
<td>Overall mean ±SD</td>
<td>—</td>
<td>376±241 mg/dl</td>
<td>49±23 %</td>
<td>157±90 mg/dl</td>
<td>219±191 mg/dl</td>
</tr>
<tr>
<td>Overall median (IQR)</td>
<td>—</td>
<td>312 (153 to 516) mg/dl</td>
<td>44 (27 to 61) %</td>
<td>132 (81 to 193) mg/dl</td>
<td>180 (75 to 388) mg/dl</td>
</tr>
</tbody>
</table>

* IQR denotes interquartile range.
† All reported mutations cause familial hypercholesterolemia. Details are provided in Table S1 in the Supplementary Appendix.
‡ Levels were measured while patients were taking baseline lipid-lowering therapy. Details are provided in Table S1 in the Supplementary Appendix.
The ELIPSE HoFH trial evaluating the efficacy of Evinacumab (Regeneron) in the treatment of patients with homozygous familial hypercholesterolemia (HoFH)

- 65 HoFH
- Evinacumab IV (n=43) every 4 weeks vs placebo (n=22)
- Background LLTs
  - statins (98%)
  - PCSK9 inhibitors (81%)
  - ezetimibe (75%)
  - LDL apheresis (33%)
  - lomitapide (26%)
- Results: LDL-C reduced by 49% in evinacumab vs placebo
- The most common treatment-associated adverse events included influenza-like illness and rhinorrhea.
Evinacumab does not currently have Marketing Authorisation in the EU/UK for any indication.

Evinacumab has the following regulatory designations/ awards:

- An orphan drug in the USA in 2016 for the treatment of homozygous familial hypercholesterolemia.\(^{19}\)
- A Breakthrough Therapy by the FDA for the treatment of homozygous familial hypercholesterolemia in March 2017.\(^{20}\)
Conclusion

• Familial Hypercholesterolemia is a Prototype for Precision Medicine.

• Many patients with severe heterozygous FH and Homozygous FH are not reaching the therapeutic LDL-C goals.

• Gene therapy has become one of the most promising research directions for contemporary life sciences and is a potential treatment option for FH.