NLA Special CME/CE Breakfast Symposium:
Advancing Therapy in the Severe FH Patient
Saturday, August 23, 2014
JW Marriott Hotel | Indianapolis, IN
Identification of the Patient with Familial Hypercholesterolemia: Genotypic and Phenotypic Considerations

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August 23, 2014
Disclosures

Board Member/Advisory Panel: Foundation of the National Lipid Association; ACC/AHA Cholesterol Guideline Panel (NHLBI ATP4 panel)

Employee: Washington University School of Medicine

Research Support: Research contracts to institution—Merck, Genzyme/ISIS/Sanofi-Aventis, Glaxo-Smith-Kline, Amgen, Amarin, Regeneron/Sanofi-Aventis, Roche/Genentech, Pfizer

Consulting: Tekmira, Astra-Zeneca, uniQure

Editorial: Merck Manual
Outline

- Definition of FH
- Genetics
- Prevalence
- Diagnostic criteria for heterozygous and homozygous FH
Familial hypercholesterolemia (FH) is an inherited genetic disorder causing high cholesterol concentrations and increased risk of premature cardiovascular disease. Lifetime exposure to high LDL levels, essentially from birth. Untreated, FH leads to substantial CVD risk in men and women, with early onset of cardiovascular disease. Early diagnosis and treatment significantly decrease the excess CVD risk.
Definition of Familial Hypercholesterolemia

- Autosomal co-dominant high LDL
  - Most families have only heterozygotes
- Gene dosage effect
  - Homozygotes (or compound heterozygotes) have much higher LDL-C and much earlier CAD onset (childhood and adolescence) than heterozygotes
- Autosomal recessive hypercholesterolemia – very rare, homozygotes for LDL receptor adaptor protein
Genetics of FH

- Genetic causes lead to impaired LDL receptor function and decreased LDL removal
  - ½ LDL catabolic rate → 2x increase in LDL
- \textit{LDLR} mutations (most common—85-90% of cases)—over 1600 known, most pathogenic
- \textit{APOB} mutations (impair LDL receptor binding)
  - familial defective apo B (FDB)--Arg3500Gln
- \textit{PCSK9} (proprotein convertase subtilisin-like / kexin type 9) gain of function mutations—rare
  - (Loss of function leads to lifelong low levels and decreased CVD risk)

Mutations

- **LDLR**
  - Missense, nonsense, insertions, deletions spread throughout *LDLR* affecting number and function

- **ApoB**
  - Most common is the apoB3500, which affects binding of LDL to the LDL receptor

- **PCSK9**
  - Gain of function leads to increased PCSK9 causing increased degradation of LDL receptors

- **LDLRAP1**
  - Facilitates the interaction between the LDL receptor and the cell machinery regulating the endocytic process; LDLR-LDL complex internalization impaired
Gene loci of familial hypercholesterolemia.

- Heterozygotes = one abnormal allele

- Homozygotes = same mutation in both alleles of the same gene

- Compound heterozygotes = different mutations in each allele of the same gene

- Double heterozygotes = mutations in two different genes affecting LDL receptor function
Proteins affecting low-density lipoprotein receptor function

Cuchel M et al. Eur Heart J
2014;eurheartj.ehu274
Proteins affecting low-density lipoprotein receptor function

Cuchel M et al. Eur Heart J 2014;eurheartj.ehu274
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Cuchel M et al. Eur Heart J 2014;eurheartj.ehu274
Familial Hypercholesterolemia

- Autosomal co-dominant high LDL
- Heterozygotes: untreated LDL-C 155 to 500 mg/dL
- Homozygotes often have untreated LDL-C >500 mg/dL
  - CAD onset in childhood and adolescence
  - Insufficient response to usual lipid lowering medication, even in combination
- Pre 1990: average age at death 18.4 years, age at first cardiac event 12.8 years
- Post 1990: average age at death 32.9 years, age first event 28.3 years

Nordestgaard B G et al. Eur Heart J 2013;eurheartj.eht273
Prevalence of FH

- Common “single gene” disease
- Heterozygous FH
  - 1 in 200 to 500 people
  - 1 in 100 in French Canadian, S. African, others
- Homozygous FH
  - 1 in 250,000 to 1 million people (more common in some groups)
- Over 12 million FH patients worldwide
- In the United States, estimated 620,000 people with FH

Populations with high prevalence of FH due to founder effect

- French Canadian (1 in 100-270)
- Christian Lebanese (1 in 100)
- Several South African populations:
  - Dutch Afrikaner (1 in 100)
  - Ashkenazi Jewish (1 in 100)
  - South Asian Indian
Estimated per cent of individuals diagnosed with FH as a fraction of those predicted, based on a frequency of 1/500

Nordestgaard B G et al. Eur Heart J 2013;eurheartj.eht273
FH prevalence based on recent genetic studies

● Historically HoFH 1 in 1 million and HeFH 1 in 500

● Prevalence using genetic testing in a central laboratory in the Netherlands
  ● HoFH estimated prevalence 1 in 300,000

● In Denmark, genetic testing of Dutch Lipid Clinic defined cases, HeFH 1 in 200

Sjouke B et al. Eur Heart J 2014;eurheartj.ehu058
Recognized FH Diagnostic Criteria

- Best characterized clinical diagnostic tools:
  - US MEDPED Program
  - Simon-Broome Registry Group (UK)
  - Dutch Lipid Clinic Network

- DNA evidence:
  A mutation is not always found—can be anywhere from 20 to 50% of the time
  Useful in patients where there is a known mutation in the family
  Cost is decreasing but still an issue in US with insurance coverage
Diagnosis of FH

- Suspect FH at these LDL cholesterol levels:
  - LDL-C ≥ 250 mg/dL in a patient aged 30 or more
  - LDL-C ≥ 220 mg/dL for patients aged 20 to 29
  - LDL-C ≥ 190 mg/dL in patients under age 20
- Obtain further family history
- Rule out secondary causes: hypothyroidism, nephrotic syndrome

## Useful LDL-C cutpoints for FH diagnosis

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>LDL-C (mg/dL) cutpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>General population 95(^{th}) percentile</td>
<td>130</td>
</tr>
<tr>
<td>2</td>
<td>80% have FH in first-degree relatives</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>80% have FH in general population</td>
<td>190</td>
</tr>
<tr>
<td>4</td>
<td>99% have FH in general population</td>
<td>220</td>
</tr>
<tr>
<td>5</td>
<td>99.9% have FH in general population</td>
<td>240</td>
</tr>
</tbody>
</table>

Pedigree of a family with familial hypercholesterolemia.

Nordestgaard B G et al. Eur Heart J 2013;eurheartj.eht273
Overlap of clinical and mutation diagnosis of heterozygous familial hypercholesterolaemia.

Nordestgaard B G et al. Eur Heart J 2013;34:3478-3490
Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society


Cuchel M et al. Eur Heart J 2014;eurheartj.ehu274
Criteria for the diagnosis of homozygous familial hypercholesterolemia

Genetic confirmation of two mutant alleles at the \textit{LDLR}, \textit{APOB}, \textit{PCSK9}, or \textit{LDLRAP1} gene locus

\textbf{OR}

An untreated LDL-C $>13$ mmol/L (500 mg/dL) or treated LDL-C $\geq 8$ mmol/L (300 mg/dL)* together with either:

- Cutaneous or tendon xanthoma before age 10 years

\textbf{OR}

- Untreated elevated LDL-C levels consistent with heterozygous FH in both parents (except in ARH)

* Lower LDL-C levels, especially in children or in treated patients, do not exclude HoFH

\textit{Cuchel M et al. Eur Heart J 2014;eurheartj.ehu274}
Estimated number of individuals worldwide with homozygous familial hypercholesterolaemia by the World Health Organization region.

<table>
<thead>
<tr>
<th>Region</th>
<th>Estimated Frequency</th>
<th>Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire world</td>
<td>1/1,000,000</td>
<td>6,860</td>
</tr>
<tr>
<td>African region</td>
<td>1/160,000</td>
<td>42,880</td>
</tr>
<tr>
<td>Region of the Americas</td>
<td>1/160,000</td>
<td>830</td>
</tr>
<tr>
<td>South-East Asia region</td>
<td>1/160,000</td>
<td>5,190</td>
</tr>
<tr>
<td>European region</td>
<td>1/160,000</td>
<td>930</td>
</tr>
<tr>
<td>Eastern Mediterranean region</td>
<td>1/160,000</td>
<td>5,810</td>
</tr>
<tr>
<td>Western Pacific region</td>
<td>1/160,000</td>
<td>1,810</td>
</tr>
</tbody>
</table>

Cuchel M et al. Eur Heart J 2014;eurheartj.ehu274
Phenotypic variability in homozygous familial hypercholesterolemia

Cuchel M et al. Eur Heart J 2014;eurheartj.ehu274
Low-density lipoprotein-cholesterol levels in homozygous autosomal dominant hypercholesterolemia patients before and after lipid lowering therapy. Plus indicates patients with two null alleles.

Sjouke B et al. Eur Heart J 2014;eurheartj.ehu058

LLT = lipid lowering therapy
Not everything that looks like heterozygous FH is a monogenic disorder

Patients with phenotypic FH without a genetic diagnosis
Multiple single nucleotide mutations

“In a substantial proportion of patients with familial hypercholesterolaemia without a known mutation, their raised LDL-C concentrations might have a polygenic cause, which could compromise the efficiency of cascade testing. In patients with a detected mutation, a substantial polygenic contribution might add to the variable penetrance of the disease.”

Talmud PJ, et al. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolemia: a case-control study. Lancet 2013
Conclusions

- FH is more prevalent than we thought
- The genetics are also more complicated
- LDL cholesterol levels can vary considerably in both heterozygous and homozygous FH with overlapping ranges
- More widespread genetic testing may be helpful but does not always give an answer—treat anyway based on LDL levels
- Early diagnosis and treatment are important
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Advancing Therapy in the Severe FH Patient

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Clinical Evaluation of the Severe FH Patient and Treatment Considerations

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  • Aegerion
  • Amarin

• Consultant:
  • Genzyme
  • Kaneka America
Familial Hypercholesterolemia

Definition:

A group of inherited genetic defects affecting the:
- low-density lipoprotein receptor
- apolipoprotein (Apo B)
- proprotein convertase subtilisin/kexin type 9 (PCSK9) genes

Resulting in high cholesterol concentrations in the blood and increased risk of premature coronary heart disease (CAD)

McKenney, J. & Hawkins D. Handbook on the management of Lipid Disorders. 2nd edit, 2001
Statistically Speaking

• Risk of premature CAD is elevated about 20-fold in untreated FH patients
• MIs as early as 20 to 30 years of age, often in 40s
• Only 20% of patients are diagnosed
• Of those diagnosed, ≤50% have treated cholesterol at target

Screening for FH

• Universal screening for patients with elevated serum cholesterol is recommended.

• FH is suspected when untreated fasting LDL or non-HDL cholesterols are:
  – Adults ≥ 20 years with LDL-C ≥ 190 mg/dL or non-HDL-C is ≥220 mg/dL
  – Children, adolescents and young adults (< 20 years) with LDL ≥ 160 mg/dL or non-HDL-C ≥190 mg/dL
  – For all patients with these levels, a family history of high cholesterol and heart disease in first-degree relatives should be evaluated

Lipoprotein(a) Levels in Familial Hypercholesterolemia: An Important Predictor of Cardiovascular Disease Independent of the Type of LDL Receptor Mutation

Lp(a) is considered a cardiovascular risk factor. Nevertheless, the role of Lp(a) as a predictor of CVD in patients with FH has been a controversial issue.

The aim of this study was to determine the relationship between lipoprotein(a) [Lp(a)] and cardiovascular disease (CVD) in a large cohort of patients with heterozygous familial hypercholesterolemia (FH).

Methods

A cross-sectional analysis of 1,960 patients with FH and 957 non-FH relatives recruited for SAFEHEART (Spanish Familial Hypercholesterolemia Cohort Study)

A long-term observational cohort study of a molecularly well-defined FH study group, was performed. Lp(a) concentrations were measured in plasma using an immunoturbidimetric method.

Rodrigo Alonso et al, 
Lipoprotein(a) Levels in Familial Hypercholesterolemia: An Important Predictor of Cardiovascular Disease Independent of the Type of LDL Receptor Mutation

• Results

• Patients with FH, especially those with CVD, had higher Lp(a) plasma levels compared with their unaffected relatives (p < 0.001).

• A significant difference in Lp(a) levels was observed when the most frequent null and defective mutations in LDLR mutations were analyzed (p < 0.0016).

• On multivariate analysis, Lp(a) was an independent predictor of cardiovascular disease. Patients carrying null mutations and Lp(a) levels >50 mg/dl showed the highest cardiovascular risk compared with patients carrying the same mutations and Lp(a) levels <50 mg/dl.

• Conclusions

• Lp(a) is an independent predictor of CVD in men and women with FH. The risk of CVD is higher in those patients with an Lp(a) level >50 mg/dl and carrying a receptor-negative mutation in the LDLR gene compared with other less severe mutations.

Physical Findings

Although not always present, and not necessary for a diagnosis of FH, certain physical exam findings should prompt the clinician to strongly suspect FH and order lipid measurements if unknown:

- Tendon xanthoma at any age (most common in Achilles tendon and finger extensor tendons, but can also occur in patellar and triceps tendons)
- Arcus corneae in patients < 45 years of age
- Tuberous xanthoma or xanthelasma in a patient < 20-25
Homozygous Familial Hypercholesterolemia:

5-year old

17-year old

21-year old
Diagnosis

• Age of onset of family members with CAD is critical to document in the family history

• Any physical findings can be very specific in the diagnosis, although absence does not rule out for FH

• Clinical diagnosis is most likely when 2 or more first degree relatives are found to have elevated LDL cholesterol in ranges as previously noted, when pediatric cases are identified in the family, or when the patient or a close relative has a tendon xanthoma

• Patients with FH on occasion are noted to have elevated TGs, this should not exclude the diagnosis of FH

Genetic Screening

- Genetic screening for the FH is generally NOT needed for clinical management or diagnosis but may be useful when the diagnosis is uncertain.

- A negative genetic test does NOT exclude FH.
  - 20% of clinically definite FH patients will NOT be found to have a mutation despite an exhaustive search using current methods.

Cascade Screening

- Allows for newly diagnosed patients with FH to identify other family members who should be screened
- Most cost-effective means of finding previously undiagnosed FH patients

- Cost effective in terms of cost per year of life saved provided that cholesterol-lowering treatment is begun in all those identified
Rationale for Treatment

- Individuals with FH have a very high lifetime risk of CHD
- Those with FH are at very high risk of premature onset of CHD
- Early treatment is beneficial
- FH requires lifelong treatment and regular follow-up
- Adults as well as children with LDL cholesterol ≥190 mg/dL after lifestyle management will require pharmacologic therapy
- For adult FH patients (> age 20) pharmacologic treatment to achieve an LDL cholesterol reduction of ≥ 50% should be initiated
- Long-term pharmacologic therapy of patients with FH significantly reduces or removes the excess lifetime risk of CHD, lowering the level of risk to that of the general population

Goldberg, A, MD et al. Familial Hypercholesterolemia: Screening, Diagnosis and Management of Pediatric And Adult Patients Clinical Guidance From The National Lipid Association Expert Panel on Familial Hypercholesterolemia.
Management of FH

- Risk factors should be aggressively treated as in the general population, with special attention to smoking cessation.

- Regular physical activity, a healthy diet and weight control should be emphasized (this can lead to an 18-20% lowering of Total and LDL cholesterol).

- Blood pressure should be treated to < 140/90 mm Hg.

- Low dose aspirin (75-81mg) should be considered in those at high CHD or stroke risk.

- Pharmacologic treatment
  - For adults initial treatment is moderate to high potency statins titrated to achieve LDL cholesterol reduction of ≥ 50% from baseline.
  - Low potency statins generally inadequate for FH patients.
  - If initial statin is not tolerated, consider changing to an alternative statin.
  - Combination therapy with niacin, bile acid sequestrant or cholesterol absorption inhibitors is most often required to achieve LDL goals.

# Lipid Therapy Options for Dyslipidemia

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>LDL-C (%)</th>
<th>HDL-C (%)</th>
<th>TG (%)</th>
<th>Key Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Statins</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
<td>↓ 18%–55%</td>
<td>↑ 5%–15%</td>
<td>↓ 7%–30%</td>
<td>Myositis, ↑ LFTs</td>
</tr>
<tr>
<td>Bile acid sequestrants&lt;sup&gt;1&lt;/sup&gt;</td>
<td>↓ 15%–30%</td>
<td>↑ 3%–5%</td>
<td></td>
<td>Upper/lower GI complaints (e.g., constipation)</td>
</tr>
<tr>
<td>Nicotinic acid&lt;sup&gt;1&lt;/sup&gt;</td>
<td>↓ 5%–25%</td>
<td>↑ 15%–35%</td>
<td>↓ 20%–50%</td>
<td>Flushing, hyperglycemia, hyperuricemia/gout</td>
</tr>
<tr>
<td>Fibric acid derivatives&lt;sup&gt;1&lt;/sup&gt;</td>
<td>↓ 5%–20%</td>
<td>↑ 10%–20%</td>
<td>↓ 20%–50%</td>
<td>Upper GI complaints, myopathy</td>
</tr>
<tr>
<td>Cholesterol-absorption inhibitors&lt;sup&gt;2&lt;/sup&gt;</td>
<td>↓ 18%</td>
<td>↑ 1%</td>
<td>↓ 8%</td>
<td>↑ LFTs in combination with statins; lack of outcomes data</td>
</tr>
<tr>
<td>Omega-3 fatty acids&lt;sup&gt;3*&lt;/sup&gt;</td>
<td>↑ 45%</td>
<td>↑ 9%</td>
<td>↓ 45%</td>
<td>↑ LDL-C; lack of outcomes data</td>
</tr>
</tbody>
</table>

* Based on use in patients with very high TG levels (≥500 mg/dL).

Miss R

- Father premature CAD – LDL > 400 mg off medication
- Mother currently 45 y.o. No CAD but has off treatment LDL > 400 mg
- Miss R is 10 years old – with homozygous FH – off treatment LDL in the 700 range
- No cardiac symptoms, however angiographic changes noted in aorta
- Parents had a 4 year old before this child’s birth who died with MI
Early Arterial & Heart Disease In FH

- Significant differences in compliance in the aorta of youth with FH

- International studies reveal:
  - Consistent myocardial perfusion defects in approximately 20% of youth ages 8 – 24
  - Illustrating the very early effects of high cholesterol on the blood flow to the heart

Evaluation and Diagnosis

• Findings and evaluation:
  – Bilateral achilles tendon xanthomas
  – Multiple extensor tendon xanthomas
  – Ping-Pong ball size xanthomas noted bilaterally at elbows

• Diagnosis
  – Homozygous familial hypercholesterolemia with early findings of atherosclerosis
  – Severe liver receptor deficit with poor response to statin therapy
What is LDL-Apheresis?

- Mechanical means to remove LDL from the blood while preserving HDL and other plasma components

- Extracorporeal
  - blood taken outside of the body
  - returned to the patient without need for albumin or other blood products

Rader, D., Ross J. Special treatment for high cholesterol: What is LDL apheresis and who can benefit from its use?. Inherited High Cholesterol Foundation Newsletter, 11(5). Salt Lake City, UT 1997
# LDL Apheresis System

<table>
<thead>
<tr>
<th>Lipid/Lipoprotein</th>
<th>Acute Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>61 - 71</td>
</tr>
<tr>
<td>LDL-C</td>
<td>73 - 83</td>
</tr>
<tr>
<td>HDL-C</td>
<td>3 - 14</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>53 - 76</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>47 - 68</td>
</tr>
</tbody>
</table>
LDL Apheresis

Treatment Frequency
- Once every 2 weeks

Plasma Volume Treated
- 4 - 5 liters

Heparin Dosage
- Priming, loading and continuous dose
# LDL Apheresis

## PATIENT REACTIONS
(During Clinical Study)

(74 Patients  4,936 Treatments)

<table>
<thead>
<tr>
<th>Events</th>
<th>Episodes</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypotension</td>
<td>41</td>
<td>0.8%</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>27</td>
<td>0.5%</td>
</tr>
<tr>
<td>Flushing</td>
<td>20</td>
<td>0.4%</td>
</tr>
<tr>
<td>Angina</td>
<td>10</td>
<td>0.2%</td>
</tr>
<tr>
<td>Fainting</td>
<td>9</td>
<td>0.2%</td>
</tr>
</tbody>
</table>
Summary

- FH is a common problem that goes unidentified and treated in the U.S.

- Those with FH will require lifestyle management and medication in the form of statin therapy to reduce the atherogenic LDL cholesterol

- It may be necessary to implement polypharmacy in those with severe forms of FH to achieve targeted goals

- When traditional therapies do not yield adequate results or when traditional therapies are unable to be tolerated, LDL apheresis may be considered

- In patients with FH, early identification and long term treatment to targeted LDL goals significantly reduces or removes the excess lifetime risk of CHD, lowering the level of risk to that of the general population

Goldberg, A, MD et al. Familial Hypercholesterolemia: Screening, Diagnosis and Management of Pediatric And Adult
Patients Clinical Guidance From The National Lipid Association Expert Panel on Familial Hypercholesterolemia
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New Treatments for Homozygous Familial Hypercholesterolemia

James A. Underberg MS, MD, FACP, FACPM, FNLA
NYU Center for Prevention of Cardiovascular Disease
Director, Bellevue Hospital Lipid Clinic
HoFH Disease Overview

• HoFH is a serious genetic disease characterized by extremely elevated blood LDL-C levels, and premature atherosclerosis.¹

• HoFH usually presents in childhood, but patients may go undiagnosed until adulthood.²,⁴,⁵

• Diagnostic criteria for HoFH in the literature are variable and not universally defined. However, the clinical diagnosis typically consists of the following:³
  – Significantly elevated levels of LDL-C
  – Cutaneous and tendon xanthomas and corneal arcus
  – Parental history of significant hypercholesterolemia and/or premature CVD

**Traditional Cholesterol-Lowering Drugs in HoFH**

<table>
<thead>
<tr>
<th>Class</th>
<th>Major Effect</th>
<th>Typical LDL-C-Lowering Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statins (e.g. atorvastatin, rosuvastatin)</td>
<td>↑ LDLR activity</td>
<td>&lt;10 to 25%</td>
</tr>
<tr>
<td>Bile acid sequestrants (e.g. cholestyamine, colestipol)</td>
<td>↑ LDLR activity</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>Cholesterol absorption inhibitors (e.g. ezetimibe)</td>
<td>↑ LDLR activity</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>Nicotinic acid (i.e., niacin)</td>
<td>Unknown</td>
<td>&lt;10%</td>
</tr>
</tbody>
</table>

Juxtapid is an adjunct therapy to low-fat diet and lipid lowering therapies.

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MOA of Traditional Lipid Lowering Therapies

Statins\(^1\)
- Inhibit HMG-CoA reductase, the rate limiting step in cholesterol synthesis

Ezetimibe\(^2\)
- Localizes at the brush border of the small intestine and inhibits the absorption of cholesterol\(^2\)

Bile Acid Sequestrants\(^1\)
- Form nonabsorbable complex with bile acid, inhibit enterohepatic reuptake and increase fecal loss of bile salts

All ultimately increase expression of LDL receptors

1. Lexicomp Drug Information Handbook. 7th Edition
Altered lipid metabolism in HoFH and points of drug action

Clin. Lipidol. (2014) 9(1)
Novel Drug Targets for HoFH

APO B Technologies for HoFH

• Lomitapide
• Mipomersen
MTP Inhibitors – Mechanism of Action

MTP inhibitors¹,²

• Prevent the assembly of apo B-containing lipoproteins in hepatocytes and enterocytes. This inhibits the synthesis of VLDL and chylomicrons.

• The inhibition of the synthesis of VLDL and intestinal chylomicron secretion lowers plasma lipids.

Predicted Effects of MTP Inhibition

Predicted Effects of MTP Inhibition

Predicted Effects of MTP Inhibition

Liver Cell

- ↑TG results in ↑ hepatic fat

- ↑TG contributes to GI tolerability issues

Intestinal Epithelial Cell

- ↓TG results in ↓ hepatic fat

- ↓TG contributes to GI tolerability issues

Hypothesized Clinical Effects of MTP Inhibition

Based on the physiological role of MTP, inhibition of MTP could be anticipated to have the following outcomes:

• **Efficacy**
  – Potential to produce a significant reduction in VLDL synthesis and, subsequently, LDL levels
  – Potential to produce a significant reduction in chylomicrons and, subsequently, chylomicron remnant levels, which may indirectly lower LDL-C
  – Potential to produce a significant reduction in Apo B levels

• **Safety and Tolerability**
  – Potential to produce GI side effects
  – Potential for increased hepatic fat (triglycerides)
  – Potential to reduced absorption of fat-soluble vitamins (e.g., vitamin E) and essential fatty acids (e.g., omega 3 and 6 fatty acids)
Microsomal Triglyceride Transfer Protein (MTP) Inhibitors

- Microsomal triglyceride transfer protein (MTP) is required for transport of lipids to apoB to produce lipoproteins
- An inhibitor of MTP, BMS-201038, has been tested in a clinical trial of 6 patients with homozygous FH
- Patients receiving a 1 mg dose of the MTP inhibitor had significant reductions ($P < 0.001$ for all comparisons) compared with baseline in
  - Total cholesterol: ↓ 58.4%
  - LDL: ↓ 50.9%
  - VLDL: ↓ 78.7%
  - Triglycerides: ↓ 65.4%
- Treatment of FH with the MTP inhibitor was associated with transient diarrhea, increase in aminotransferase, and fat in the liver
- Study limitations: Large variability in patients of aminotransferase levels and hepatic fat content

Efficacy and Safety of Lomitapide in HoFH: Single-Arm, Open-Label, Phase 3 Study

• LDL-C ↓ 50%, ApoB ↓ 49%, Lp(a) ↓ 15%, TG ↓ 45%
• Mean hepatic fat measure by NMRS (n = 20) increased from 1% at baseline to 8.6% at week 26, 5.8% at week 56, and 8.3% at week 78

Lomitapide (Juxtapid)
Approved December 24, 2012

• Microsomal triglyceride transfer protein inhibitor that decreases lipoprotein production

• Approved as adjunct to diet and other lipid-lowering treatments to reduce LDL-C, TC, apo B, non-HDL-C in homozygous familial hypercholesterolemia (HoFM)

• Dosing: 5 mg daily titrated to a maximum of 60 mg daily

• Adverse effects: hepatic steatosis, elevated hepatic transaminases; GI adverse reactions (93%) could decreased absorption of vitamin E, ALA, linoleic acid, EPA, DHA (supplement recommended)

• Only available through a restricted Risk Evaluation and Mitigation Strategy (REMS) program
ApoB Antisense Therapy: Mipomersen

- A 20-mer, phosphorothioate antisense oligonucleotide complementary in sequence to segment of ApoB mRNA$^{1,2}$

- 2′-O-(2-methoxyethyl)—modified ends provide high biological stability and binding affinity to mRNA$^2$

- Mipomersen has significant distribution to the liver, where ApoB-100 is synthesized$^3$

Mipomersen: 
Mechanism of Action

• Mipomersen crosses the hepatocyte and nuclear membranes to target ApoB mRNA\(^1,2\)

Mipomersen’s Mechanism of Action In The Hepatocyte

A Mipomersen is a single-stranded antisense oligonucleotide designed to correspond to the ApoB gene as a target for gene silencing.

B mRNA-antisense duplex is formed: Once Mipomersen reaches the hepatocyte, it penetrates the cell membrane and nucleus (unclear mechanism) and binds to ApoB messenger RNA (mRNA) with a high degree of fidelity.

C RNase H cleaves mRNA: After hybridization with target mRNA, the mipomersen-mRNA duplex is recognized and cleaved by endogenous RNase H, an enzyme involved in DNA replication/repair.

D Cleavage of ApoB mRNA results in decreased ApoB synthesis and lower LDL.
Phase 2:  
Monotherapy for Hypercholesterolemia  
Dose-Dependent Reduction of LDL-C  

Once-Weekly Dosing

*P = 0.000 vs Placebo

The Role of mipomersen therapy in the treatment of FH
Roda Plakogiannis, Lisa Cioce, Edward A Fisher & James A Underberg
Clin. Invest 2012 , 2(10) 1033-1037

Table 1. Clinical trials evaluating the efficacy of mipomersen therapy.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Patient (n)</th>
<th>Population</th>
<th>Mipomersen dose (mg/week)</th>
<th>Results (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LDL-C</td>
<td>ApoB</td>
</tr>
<tr>
<td>Akdim et al. (2010)</td>
<td>44</td>
<td>Heterozygous FH</td>
<td>50,100,200,300</td>
<td>-13, -11, -21**, -34**</td>
<td>-10, -8, -23, -33*</td>
</tr>
<tr>
<td>Tardif et al. (2011)</td>
<td>58</td>
<td>Heterozygous FH</td>
<td>200</td>
<td>-36*</td>
<td></td>
</tr>
<tr>
<td>Duell et al. (2012)</td>
<td>141</td>
<td>FH</td>
<td>200</td>
<td>-26 to 28</td>
<td>-28 to -31</td>
</tr>
</tbody>
</table>

Statistically significant at *p < 0.05; **p < 0.01; ***p < 0.001.
FH: Familial hypercholesterolemia; IHTG: Intrahepatic triglyceride; TG: Triglycerides.
Inj. Site Reactions
Mipomersen sodium (Kynamro)
Approved January 30, 2013

• Antisense oligonucleotide that decreases secretion of apo B containing lipoproteins from the liver
• Approved as adjunct to lipid-lowering medications and diet to reduce LDL-C, apo B, TC, non-HDL-C in patients with homozygous familial hypercholesterolemia (HoFH)
• Dosing: 200 mg once weekly subcutaneous injection
• Adverse effects: injection site reactions (84%), flu-like symptoms (30%), elevated hepatic transaminases
• Only available through a restricted Risk Evaluation and Mitigation Strategy (REMS) program
## Other studies in HoFH

<table>
<thead>
<tr>
<th>Study</th>
<th>Study phase</th>
<th>Sponsor</th>
<th>Investigational medicinal product</th>
<th>Trial identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>MODE study: a multicenter, open-label study of the effect of CER-001 on plaque volume in subjects with homozygous familial hypercholesterolemia</td>
<td>Clinical Phase II study</td>
<td>Cerenis Therapeutics</td>
<td>CER-001 is a recombinant human ApoA1-I/phospholipid complex mimicking the action of natural nascent, discoidal pre-β HDL particles</td>
<td>NCT01412034</td>
</tr>
<tr>
<td>A worldwide, multicenter, double-blind, randomized, placebo-controlled, 12-week study to assess the efficacy and tolerability of anacetrapib when added to ongoing lipid-lowering therapy in adult patients with homozygous familial hypercholesterolemia with a 52-week open-label extension (MK042)</td>
<td>Clinical Phase III study</td>
<td>Merck Sharp &amp; Dohme</td>
<td>Anacetrapib is a CETP-inhibitor The investigational medicinal product at 100 mg/placebo is given daily for 12 weeks</td>
<td>NCT01841684 EudraCT 2012-002434-37</td>
</tr>
<tr>
<td>TAUSSIG: a multicenter, open-label study to assess the long-term safety, tolerability, and efficacy of AMG145 on LDL-C in subjects with severe familial hypercholesterolemia</td>
<td>Clinical Phase II study</td>
<td>Amgen</td>
<td>AMG145 is a human monoclonal IgG2 that binds specifically to PCSK9 The investigational medicinal product is given as 420 mg subcutaneous injections 2-weekly or monthly for 5 years, or until AMG145 becomes commercially available</td>
<td>NCT01624142 EudraCT 2011-005400-15</td>
</tr>
</tbody>
</table>
Proposed Mechanism of CETPi on Lipid Exchange Between Lipoprotein Particles

CETP–mediated lipid exchange between lipoprotein particles

Proposed effects of CETP inhibition on lipid exchange

JAMA. 2011;306(19):2099-2109
PCSK9 Inhibition Background and Rationale

- Despite the widespread availability of statins, many patients fail to reach recommended LDL-C targets in clinical practice, even in combination with other lipid-lowering agents.
- In PCSK9 human population studies:
  - Gain-of-function mutations result in hypercholesterolemia.
  - Loss-of-function mutations associated with low LDL-C and low prevalence of CHD events.
- SAR236553/REGN727 is a highly specific, fully human monoclonal antibody (mAb) to PCSK9.
- A SAR236553/REGN727 Phase 1 trial* in familial and non-familial hypercholesterolemia:
  - Demonstrated dose dependently reduced LDL-C by 39% to 61% in atorvastatin-treated patients.
  - Safe and well-tolerated.

LDL Receptor Function and Life Cycle
The Role of PCSK9 in the Regulation of LDL Receptor Expression
Impact of an PCSK9 mAb on LDL Receptor Expression
Strategies to Inhibit PCSK9

• Inhibiting antibodies
  – human monoclonal Abs that bind to PCSK9 and inhibit its interaction with the LDL-R

• Therapeutic gene silencing / antisense oligonucleotides
  – all trials so far terminated early

• Small molecules that inhibit PCSK9 interactions with the LDL-R
  – all are in preclinical development

Giugliano; Lancet 2012;380:2007
Duff; Exp Opin Ther Target 2011; 10.1517/14728222.2011.547480
Evolution of Therapeutic Monoclonal Antibodies

- **Fully Mouse**
  - 100% mouse
  - Highly Immunogenic

- **Chimeric**
  - ~30% mouse
  - Still immunogenic

- **Humanized**
  - 5%–10% mouse
  - Can be time-consuming to create
  - Least immunogenic

- **“Fully” Human**
  - 100% human
  - Least immunogenic

---

### PCSK9 Inhibitors In Development

<table>
<thead>
<tr>
<th>Mechanism of Action</th>
<th>Agent</th>
<th>Company/Sponsor</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibodies</td>
<td>SAR236553/REGN727</td>
<td>Sanofi/Regeneron</td>
<td>3</td>
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<tr>
<td></td>
<td>AMG 145</td>
<td>Amgen</td>
<td>3</td>
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<tr>
<td></td>
<td>RN316</td>
<td>Pfizer</td>
<td>2</td>
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<tr>
<td></td>
<td>RG7652</td>
<td>Roche/Genentech</td>
<td>2</td>
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<td></td>
<td>LGT-209</td>
<td>Novartis</td>
<td>2</td>
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<tr>
<td></td>
<td>1D05-IgG2</td>
<td>Merck</td>
<td>Pre-clinical</td>
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<tr>
<td></td>
<td>1B20</td>
<td>Merck</td>
<td>Pre-clinical</td>
</tr>
<tr>
<td></td>
<td>J10, J16</td>
<td>Pfizer</td>
<td>Pre-clinical</td>
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<tr>
<td></td>
<td>J17</td>
<td>Pfizer</td>
<td>Pre-clinical</td>
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<tr>
<td>Adnectins</td>
<td>BMS-962476</td>
<td>Bristol-Myers Squibb/Adnexus</td>
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<tr>
<td>Mimetic peptides</td>
<td>EGF-AB peptide fragment</td>
<td>Schering-Plough</td>
<td>Pre-clinical</td>
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<td>LDLR (H306Y) subfragment</td>
<td>U.S. National Institutes of Health</td>
<td>Pre-clinical</td>
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<tr>
<td></td>
<td>LDLR DNA construct</td>
<td>U.S. National Institutes of Health</td>
<td>Pre-clinical</td>
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<tr>
<td>Small-molecule inhibitors</td>
<td>SX-PCK9</td>
<td>Serometrix</td>
<td>Pre-clinical</td>
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<td></td>
<td>TBD</td>
<td>Shifa Biomedical</td>
<td>Pre-clinical</td>
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<tr>
<td>Antisense oligonucleotides</td>
<td>ISIS 394814</td>
<td>Isis</td>
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<td>SPC4061</td>
<td>Santaris-Pharma</td>
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<td></td>
<td>SPC5011</td>
<td>Santaris-Pharma</td>
<td>1 (terminated)</td>
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<tr>
<td>RNA interference</td>
<td>ALN-PCS02</td>
<td>Alnylam</td>
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</tr>
</tbody>
</table>
Trial evaluating evolocumab, a pcsk9 antibody, in patients with homozygous fh (tesla): Results of the randomized, double-blind, placebo-controlled trial

At the end of 12 weeks, HoFH patients who were LDL receptor-defective (either one or two alleles) had a 29.6% reduction in LDL cholesterol (versus 11.2% in placebo, placebo-corrected reduction 40.8%).

Efficacy was greater in patients with two defective alleles (31.8% versus +15.1% with placebo, treatment difference 46.9%) compared with one defective allele: 21.0% versus 3.5%, treatment difference 24.5%).

Both differences were highly statistically significant versus placebo (p<0.001, p=0.013, respectively).

There was no response to evolocumab in the patient with receptor-negative status, consistent with findings from the proof-of-concept study.
Cumulative LDL effects of statin, ezetimibe, adjunctive mipomersen, lomitapide or evolocumab, and lipoprotein apheresis in HoFH
Aggressive Medical Management Of HoFH with LDLR Activity
Algorithm for management of HoFH

Homozygous Familial Hypercholesterolaemia
   LDL-C targets:
   <2.5 mmol/L [<100 mg/dL] (adults)
   <3.5 mmol/L [<135 mg/dL] (children)
   <1.8 mmol/L [<70 mg/dL] if clinical CVD

At diagnosis
Lifestyle and Diet + Statin
   (most efficacious at highest dose depending on tolerability)

Ezetimibe 10 mg + resins or other drugs*
   *Fibrate, nicotinic acid, probucol (use of these additional treatments may be limited by tolerability and drug availability)

New Therapeutic options

Future Therapeutic options
   PCSK9 inhibitors
   CETP inhibitors
   Gene therapy

LDL-Apheresis
   As early as possible if available (by 5 years, no later than 8 years)
   every 1 or 2 weeks

In selected patients
Liver Transplant

Lomitapide
   Approved by FDA, EMA

Mipomersen
   Approved by FDA

NLA Special CME/CE Breakfast Symposium:

Advancing Therapy in the Severe FH Patient

Saturday, August 23, 2014
JW Marriott Hotel | Indianapolis, IN