Novel RNAi-Lipid Nanoparticle Therapeutics for Hypertriglyceridemia

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Keystone Symposia Conference: Liver Metabolism and Nonalcoholic Fatty Liver Disease (NAFLD)
Siling of TG-Lipid Metabolism Genes by RNAi-triggers

“RNAi-triggers for multiple genes e.g., ApoC3, ANGPTL3, expressed in the liver with role in TG/Lipid Metabolism, as novel therapy for HTG (rare/common) and NAFLD-NASH”

• Combine complementary mechanisms: a) liver uptake/clearance, b) plasma lipolysis/clearance, c) liver synthesis/secretion
• Enhance efficacy, broaden patient coverage

• RNAi-trigger
  • RNAi silences target gene by mRNA degradation through RISC
  • Tekmira Lipid Nano Particle (LNP) delivery platform enables RNA-based therapeutics e.g., RNAi, mRNA, microRNA
    • Shields RNAi from serum nucleases; Induces cellular uptake; Promotes endosomal uptake and delivery to RISC
  • RNAi-trigger, chemically modified RNAi encapsulated in LNP
### ApoC3

**Role in TG-Lipid Metabolism, Liver Steatosis**

- **Glycoprotein; synthesized mostly in the liver; secreted; 79 aa**
- **Key regulator of plasma TG levels by inhibiting...**
  - Receptor mediated uptake/clearance of plasma TG-rich particle by the liver
  - HL (↓ hepatic VLDL clearance)
  - LPL activation by ApoC2 (↓ plasma lipolysis of TG-rich particles)
- **Genetics - mouse, human**
  - Human loss-of-function: Hypo-TG/reduced CV events; gain of function: NAFLD/insulin resistance
- **Pharmacology - mouse, human**

### ANGPTL3

**Role in TG-Lipid Metabolism, Liver Steatosis**

- **Member of ANGPTL family; synthesized primarily in the liver; secreted; 460 aa**
- **Key regulator of plasma TG and lipid levels by inhibiting...**
  - Lipoprotein Lipase (LPL) mediated lipolysis and clearance of plasma TG-rich particles
    - Inhibits LPL by enhancing its cleavage by pro-convertase (PCSK5)
    - Endothelial Lipase (EL) /preventing clearance of plasma HDL-c
- **Genetics - mouse, human**
  - Human loss-of-function: Familial Combined Hypolipidemia
- **Pharmacology – mouse, human**
Hypertriglyceridemia (HTG)
Silencing of ApoC3 & ANGPTL3

1. TKM-ApoC3, the RNAi-trigger against human ApoC3
   • Characterization in human ApoC3-Tg mice
     • Gene silencing in the liver (mRNA); Plasma ApoC3 (PD marker) and TG (efficacy) lowering; Other metabolic parameters
   • Safety assessment

2. Combination of RNAi-triggers against ApoC3 & ANGPTL3
   • Silencing of two complementary/overlapping mechanisms
   • TKM-mApoC3 + TKM-mANGPTL3 (the RNAi-triggers against mouse ApoC3 & mouse ANGPTL3) in HFD mice
     • Plasma TG lowering; Gene silencing in the liver (mRNA)
TKM-ApoC3
Potent Silencing of Human ApoC3 Gene

**Human ApoC3 mRNA**

**HepG2 cells**

- **Human ApoC3 (%) of untreated/GAPD normalized**
- **log concentration (ng/ml)**
- **TKM-ApoC3**

**hApoC3-Tg mouse liver**

- **Human ApoC3 (%) Luc2-LNP**
- **Drug administration:** Single iv; 1, 0.3, 0.1, 0.03, 0.01 mg/kg; n=3 (females); Liver harvested after 24-hrs from 5 hr fasted mice

- **KD$_{50}$:** 0.18 ng/mL*
  - **KD$_{75}$:** 0.55 ng/mL*
  - *Approximate values

- **KD$_{50}$:** 0.026 mg/kg*
  - **KD$_{75}$:** 0.104 mg/kg*
  - *Approximate values

Significantly different compared to Luc2-LNP. ***p<0.001, **p=0.001-0.01, (1way-ANOVA). Mean ± SD.
**TKM-ApoC3**

Rapidly Lowers Plasma ApoC3 & TG in hApoC3-Tg Mice

**Human ApoC3**

\[
\mu g/mL; 24-hrs
\]

**TG**

\[
mg/dL; 24-hrs
\]

<table>
<thead>
<tr>
<th>Drug dose (mg/kg)</th>
<th>Human ApoC3</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500 *****</td>
<td>-69%*</td>
</tr>
<tr>
<td>0.3</td>
<td>300 *****</td>
<td>-83%**</td>
</tr>
<tr>
<td>0.1</td>
<td>200 ***</td>
<td>-76%**</td>
</tr>
<tr>
<td>0.03</td>
<td>100 ***</td>
<td>-78%**</td>
</tr>
</tbody>
</table>

**Drug administration**: Single iv; 1, 0.3, 0.1, 0.03 mg/kg; n=3 (females)

Plasma/Liver collected after 24-hrs from 5 hr fasted mice

Significantly different compared to Luc2-LNP. ***p<0.001, **p=0.001-0.01, *p=0.01-0.05.
(1way-ANOVA). Mean ± SD
TKM-ApoC3
Sustained Lowering of Plasma ApoC3 & TG in hApoC3-Tg Mice

**Human ApoC3**
(μg/mL; weekly)

**TG**
(mg/dL; weekly)

Drug administration: Weekly iv (Day 0, 7, 14, 21); n=4-5 (females)
Plasma collected from 5 hr fasted mice: Day -1, Day 6, Day 13, Day 20, Day 27 (terminal)

Significantly different compared to Luc2-LNP. ***p<0.001, **p=0.001-0.01
(1way ANOVA). Mean ± SE
TKM-ApoC3
Improves Overall Metabolic profile in hAPoC3-Tg Mice

**Drug administration:** Weekly iv (Day 0, 7, 14, 21); n=4-5 (females)
Blood (glucose)/Plasma (lipid) collected from 5 hr fasted mice: Day 20

Significantly different compared to Luc2-LNP. ***p<0.001, **p=0.001-0.01.
(1way-ANOVA). Mean ± SE
TKM-ApoC3
Durable Gene Silencing/TG Lowering in hApoC3-Tg Mice

**Human ApoC3**
(liver mRNA; weekly)

- Luc2-LNP 1 mg/kg
- TKM-ApoC3 1 mg/kg

**Human ApoC3**
(plasma; weekly)

- Luc2-LNP 1 mg/kg
- TKM-ApoC3 1 mg/kg

**TG**
(plasma; weekly)

- Luc2-LNP 1 mg/kg
- TKM-ApoC3 1 mg/kg

*Drug administration: Single iv; n=3 (males); Plasma collected from 5 hr fasted mice: Day 1, Day 7, Day 14*

Significantly different compared to Luc2-LNP. ***p<0.001, **p0.001-0.01, *p=0.01-0.05. (t-test). Mean ± SD
TKM-ApoC3
Preliminary Safety Assessment

• **Liver steatosis**
  • No risk based on liver lipid content compared to control treatment in hApoC3-Tg mice after repeated administration

• **Immunestimulation**
  • Low risk based on liver IFIT induction activity in mice and cytokine (MCP-1, IL-1Rα, IL-6) induction profile in human whole blood

• **Off-target activity**
  • Minimum risk against human genome (bioinformatics analysis)
    • AS strand 100% homologous to Cyno monkey (toxicity species)
TKM-mApoC3 & TKM-mANGPTL3
Durable Gene Silencing in HFD Mice

>50% silencing of Mouse ApoC3 or ANGPTL3 gene lasted for over 3 weeks

C57BL/6 mice on high-fat diet; Drug administration: Single iv dose (0.3 mg/kg), n = 3/group (females)
Liver harvested from 5-hr fasted mice (Day 1, 7, 14, 21)

NOTE: Day 21 values are expressed as % of Day 1 Untreated Control
TKM-mApoC3 + TKM-mANGPTL3

Additive Effect on Plasma TG Lowering in HFD Mice

(% of pre-treatment; Day 6)

Drug Dose: 0.5 mg/kg

Drug Dose: 0.25 mg/kg

CBA/CaJ mice on HFD; Drug administration: Single iv (Day 0); n=6 (males)

Plasma collected from 5 hr fasted mice: Day -1, Day 6

Statistical significance. ***p<0.001 Vs. Luc2-LNP. (1way ANOVA). Mean ±SE

Combo Vs. Mono: ###p<0.001 Vs. TKM-mApoC3 or TKM-mANGPTL3. (t-test).

Combo 0.125+0.125 Vs. Mono 0.5: $$$p<0.001 Vs.TKM-mApoC3 or TKM-mANGPTL3 (t-test).
TKM-mApoC3 + TKM-mANGPTL3
Additive Effect on Plasma TG Lowering in HFD Mice

(% of pre-treatment; Day 20 )

Drug Dose: 0.5 mg/kg

Drug Dose: 0.25 mg/kg

CBA/CaJ mice on HFD; Drug administration: **Weekly, iv** (Day 0, 7, 14); n=6 (males)

Plasma collected from 5 hr fasted mice: Day -1, Day 20

Statistical significance. ***p<0.001 Vs. Luc2-LNP. (1way ANOVA). Mean ±SE, n=6

Combo Vs. Mono: ###p<0.001, ##p=0.001-0.01 Vs. TKM-mApoC3; +++p<0.001, ++p=0.001-0.01 Vs. TKM-mANGPTL3. (t-test).

Combo 0.125+0.125 Vs. Mono 0.5: $$$p<0.001 Vs. TKM-mApoC3; &&p=0.001-0.01 Vs. TKM-mANGPTL3 (t-test)
TKM-mApoC3 + TKM-mANGPTL3

Gene Silencing in HFD Mice – Liver mRNA

CBA/CaJ mice on HFD; Drug administration: Weekly, iv (Day 0, 7, 14, Day 21)  
n=6 (males) Liver harvested from 5 hr fasted mice: Day 27

Significantly different compared to Luc2-LNP. ***p<0.001 (1way-ANOVA). Mean ± SD
TKM-mApoC3 vs. TKM-mANGPTL3
Mechanism-of-Action

**Plasma LPL Activity (mU/mL)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LPL Activity</th>
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<tr>
<td>Luc2-LNP 1 mg/kg</td>
<td>****</td>
</tr>
<tr>
<td>TKM-mApoC3 1 mg/kg</td>
<td></td>
</tr>
<tr>
<td>TKM-mANGPTL3 1 mg/kg</td>
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</tr>
</tbody>
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**Liver TG Secretion (net increase, mg/dL)**

![Liver TG Secretion Graph]

**Liver SCD1 mRNA (difference Vs. Luc2-LNP)**

![Liver SCD1 mRNA Graph]

CBA/B6 mice on high fat diet; Drug administration: iv (Day 0, Day 7); n=5 (males); Animals fasted on Day 13 for 16h; Plasma collected prior to heparin treatment and 15min post-heparin treatment; LPL activity assessed using a fluorometric assay kit.

CBA/CaJ mice on high fat diet; Drug administration: iv (Day 0); n=5-6 (males); At Day 7, animals fasted for 4h; Plasma collected prior to and hourly till 4h following P-407 injection for plasma TG analysis.

Liver samples from ob/ob BTBR lep-/- males on regular chow diet; Drug administration: Weekly, iv (Day 1, 7, 14, 21); n=5-6. Plasma collected from 5 hr fasted mice. Liver harvested on Day 27.

Significantly different compared to Luc2-LNP. **p=0.001-0.01. (1way-ANOVA). Mean ± SE.
RNAi-Trigger Therapeutics for HTG

Summary & Conclusions

• TKM-ApoC3, the RNAi-trigger for human ApoC3
  • In human ApoC3-Tg mouse HTG model
    • Potent and durable gene silencing (liver mRNA, plasma protein)
    • Fast, potent, efficacious and sustained plasma TG lowering
    • Beneficial plasma cholesterol change; Improved glucose control
  • Safety
    • Low immune-stimulation, off-target activity and liver steatosis risk

• TKM-mApoC3 in combination with TKM-mANGPTL3
  • Additively lowers plasma TG in HFD mouse HTG model

**TKM-ApoC3, in combination with TKM-ANGPTL3, is progressing forward into IND-enabling studies**
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Thank You