A Novel GPR119 Agonist, DA-1241 Reduces Hepatic Inflammation and Fibrosis in Pre-established Diet-induced Obese NASH mice.

Hansu Park, Seung Ho Lee, Mi-Kyung Kim*
Dong-A ST Co., Ltd., Republic of Korea

*Correspondence: MK Kim (kmk@donga.co.kr)
Declaration

All authors are employees of Dong-A ST Co., Ltd.
Background

- Non-alcoholic steatohepatitis (NASH) is characterized by steatosis, inflammation, and fibrosis in the liver
- Nearly 30% of adults have fatty liver and around 20% of them are progressed to NASH
- There is no FDA-approved drug for NASH treatment yet

- **DA-1241, a novel GPR119 agonist,** is currently underway of early clinical development for the treatment of type 2 diabetes
- DA-1241 is the most advanced GPR119 agonist with unique characteristics

- **GPR119 agonist** and **DPP4 inhibitor** are an ideal combination to maximize endogenous GLP-1 benefits
- DA-1241 prevented NASH progression in ob/ob-NASH mice with improved GLP-1 secretion compared to MBX-2982 *(see 217-LB poster presentation at ADA 2020)*
Experimental Design

To explore the combination effects of DA-1241 and a DPP4 inhibitor in pre-established DIO-NASH mice.

8 weeks with normal/Amylin diet
- Normal Control
- DIO-NASH Control
- DA-1241 alone, 30 mg/kg/day
- DA-1241 alone, 100 mg/kg/day
- DA-1241 + *DPP4 inhibitor

12 weeks with Amylin diet
(45% kcal fat, 22% fructose, 2% cholesterol)
- DIO-NASH Control
- DA-1241 alone, 100 mg/kg/day
- DA-1241 + *DPP4 inhibitor
- *DPP4 inhibitor alone

- Liver histology (HE/Sirius Red staining)
- IHC (type I collagen, galectin-3, αSMA)
- Plasma ALT/AST
- Plasma TIMP1, cytokines
- Plasma active GLP-1
- Liver RNAseq (8 week study)

*Blue-colored: only for 8-week study
*Red-colored: only for 12-week study
*Black-colored: both studies
DA-1241 Lowered Hepatic Steatosis and Liver Damage

- DA-1241 alone reduced hepatic lipid accumulation and liver enzyme levels
- DA-1241 in combination with DPP4 inhibitor augmented those responses

**Hepatic lipid area (%)**

<table>
<thead>
<tr>
<th>Group</th>
<th>DIO-NASH Co.</th>
<th>DA</th>
<th>DA+DPP4i</th>
<th>DPP4i</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic lipid area (%)</td>
<td>30±5</td>
<td>25±3*</td>
<td>20±2***</td>
<td>10±1**</td>
</tr>
</tbody>
</table>

**Liver weight (g)**

<table>
<thead>
<tr>
<th>Group</th>
<th>DIO-NASH Co.</th>
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<th>DA+DPP4i</th>
<th>DPP4i</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight (g)</td>
<td>1.5±0.2</td>
<td>1.2±0.1*</td>
<td>1.0±0.1**</td>
<td>0.8±0.1***</td>
</tr>
</tbody>
</table>

**AST (U/L)**

<table>
<thead>
<tr>
<th>Group</th>
<th>DIO-NASH Co.</th>
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<th>DA+DPP4i</th>
<th>DPP4i</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>400±50</td>
<td>300±40*</td>
<td>200±30***</td>
<td>100±20###</td>
</tr>
</tbody>
</table>

**ALT (U/L)**

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</table>

*, vs DIO-NASH Co.
#, vs DA+DPP4i combination
DA-1241 Reduced Immune Cell Infiltration in the Liver

- DA-1241 alone reduced infiltrated immune cells in the liver assessed by HE staining and IHC by anti-galectin 3Ab
- DA-1241 in combination with DPP4 inhibitor enhanced these anti-inflammatory responses

**Inflammation Score after 8-week Administration**

<table>
<thead>
<tr>
<th></th>
<th>Normal Co.</th>
<th>DIO-NASH Co.</th>
<th>DA30</th>
<th>DA100</th>
<th>DA100+DPP4i</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>3</td>
<td>2.5</td>
<td>2</td>
<td>2.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Post</td>
<td>1</td>
<td>2</td>
<td>1.5</td>
<td>1.8</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**Galectin-3+ Area (%) after 8-week Administration**

<table>
<thead>
<tr>
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<th>Normal Co.</th>
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<th>DA100</th>
<th>DA100+DPP4i</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>0</td>
<td>2.5</td>
<td>2.2</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Post</td>
<td>0</td>
<td>1</td>
<td>1.5</td>
<td>1.8</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**Inflammation Score after 12-week Administration**

<table>
<thead>
<tr>
<th></th>
<th>Normal Co.</th>
<th>DIO-NASH Co.</th>
<th>DA30</th>
<th>DA100</th>
<th>DA100+DPP4i</th>
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<tr>
<td>Pre</td>
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<td>2.5</td>
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<td>2</td>
<td>1.5</td>
<td>1.8</td>
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**Galectin-3+ Area (%) after 12-week Administration**

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<td>2.5</td>
<td>2.2</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Post</td>
<td>0</td>
<td>1</td>
<td>1.5</td>
<td>1.8</td>
<td>1.5</td>
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&: p<0.05 vs. Normal Co.
*: vs DIO-NASH Co.
#: vs DA+DPP4i combination
DA-1241 alone lowered systemic pro-inflammatory cytokine and chemokines that recruit monocytes and neutrophils.

DA-1241 alone exhibited submaximal response even at 30 mg/kg/day after 8-week administration.

DA-1241 in combination with DPP4 inhibitor increased those response in an additive manner.

DA-1241 also Improved Systemic Inflammation

- & vs. Normal Co.
- * vs. DIO-NASH Co.
- # vs. DA+DPP4i combo.

Plasma Cytokine (% of DIO-NASH Co.)

8 weeks

12 weeks

TNFα, CCL2, CXCL1, CXCL2, CXCL10

0 50 100 150

0 50 100 150

& p<0.05 vs. Normal Co.
* p<0.05 vs. DIO-NASH Co.
# p<0.05 vs. DA+DPP4i combo.

ADA 2020 Virtual Conference, 216-LB
**DA-1241 Reduced Hepatic Fiber Content**

- DA-1241 alone lowered type I collagen content in the liver
- DA-1241 alone exhibited submaximal response at 30 mg/kg/day
- DA-1241 in combination with DPP4 inhibitor suppressed stellate cell activation more

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**Type I Collagen Content (mg)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Normal Co.</th>
<th>DIO-NASH Co.</th>
<th>DA50</th>
<th>DA100</th>
<th>DA100+DPP4i</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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**Type I Collagen content (mg)**

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</tr>
</thead>
<tbody>
<tr>
<td>12 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**αSMA** Area (%)

<table>
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<th>DA</th>
<th>DA+DPP4i</th>
<th>DPP4i</th>
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<td></td>
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</table>

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**Plasma TIMP-1 (ng/ml)**

<table>
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<th>DA</th>
<th>DA+DPP4i</th>
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<td></td>
<td></td>
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</table>

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**Plasma PINP (pg/ml)**

<table>
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*PINP: procollagen type I N-term fragment*
Additional Benefits are Attributed to Increased Active GLP-1

- DA-1241 treatment increased GLP-1 secretion assessed by plasma total GLP-1 (217-LB poster presentation)
- However, DA-1241 alone did NOT affect plasma active GLP-1 due to rapid inactivation by DPP4 enzyme
- Combination of DA-1241 and DPP4 inhibitor synergistically increased plasma active GLP-1 levels

![Graph showing plasma active GLP1 levels](image)

**Plasma Active GLP1 (pM)**

- **DIO-NASH Co.**
- **DA**
- **DA+DPP4i**
- **DPP4i**

**Intact GLP-1** (active)

**Truncated GLP-1** (inactive)

**Meal**

**GPR119**

**DA-1241**

**DPP4i**

Confidential
Summary

- DA-1241 alone reduced established steatosis, hepatic inflammation and fibrosis
- DA-1241 plus DPP4 inhibitor improved NASH phenotypes more in histological, biochemical, and RNAseq data due to enhanced plasma active GLP-1 levels

Inflammation signaling (n=17)

Stellate cell activation (n=22)