

Multi-target engagement effect of a novel long-acting Glucagon/GIP/GLP-1 triple agonist (HM15211) in animal model of NASH

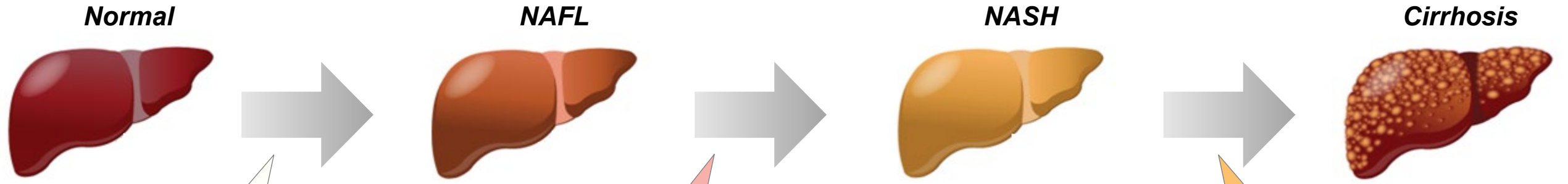
Jae Hyuk Choi, Jong Suk Lee, Jung Kuk Kim, Hyunjoo Kwon, Eun Jin Park,
Jong soo Lee, Dae Jin Kim, Younghoon Kim, In Young Choi

Hanmi Pharm. Co., Ltd., Seoul, Republic of Korea



Employee of Hanmi Pharm. Co., Ltd.

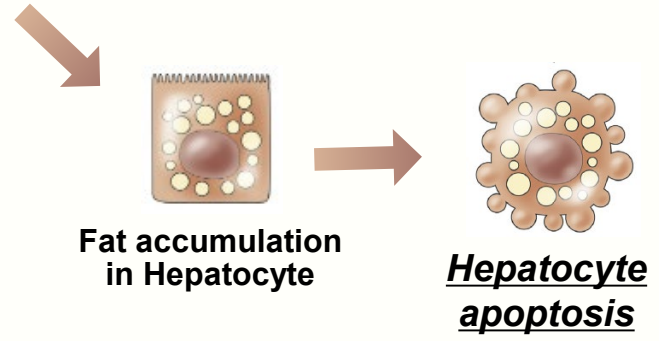
NASH progression



Steatosis

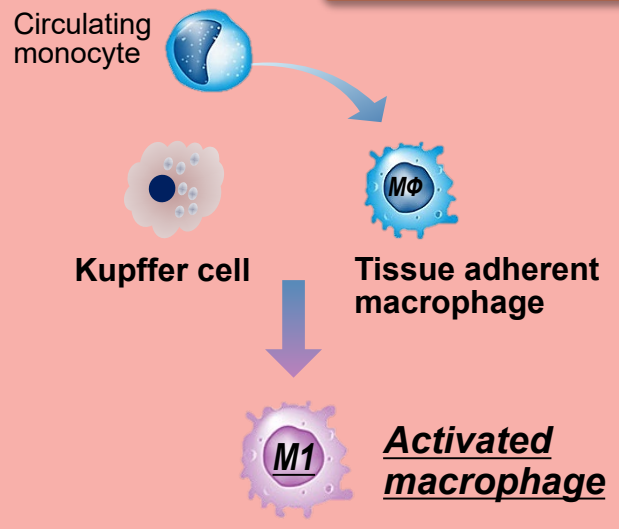
Metabolic stress

- Obesity
- Dyslipidemia
- Insulin resistance, T2DM



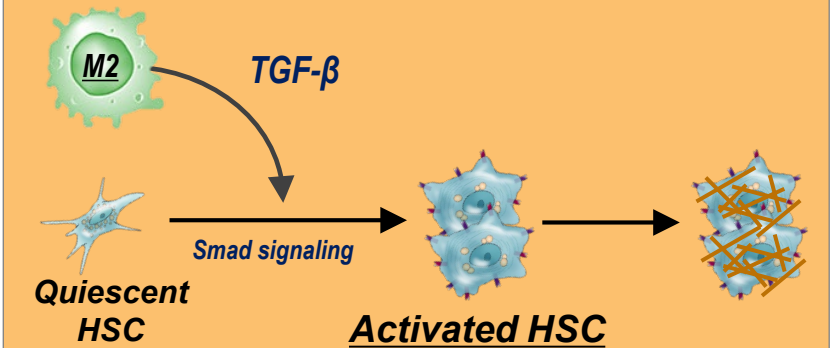
“Hepatic lipid accumulation”
→ Lipotoxicity and liver cell apoptosis

Inflammation



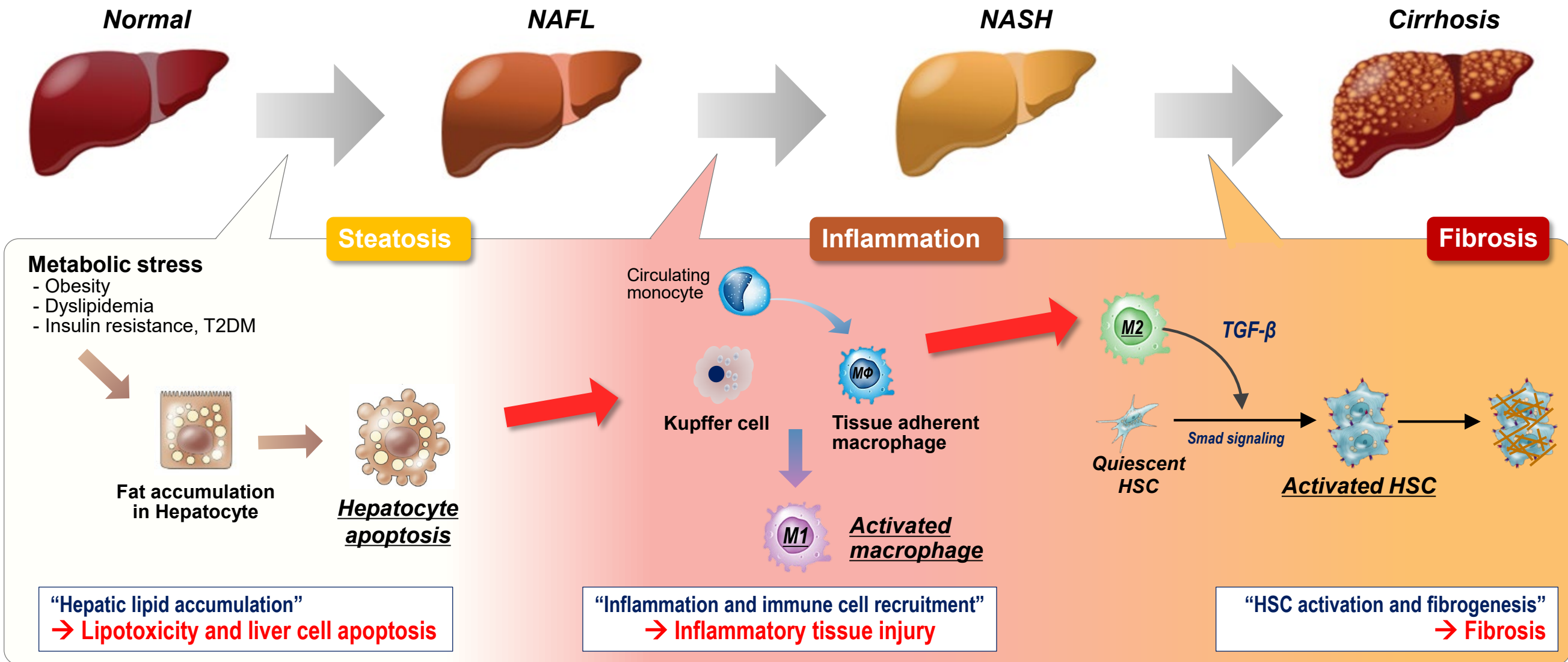
“Inflammation and immune cell recruitment”
→ Inflammatory tissue injury

Fibrosis



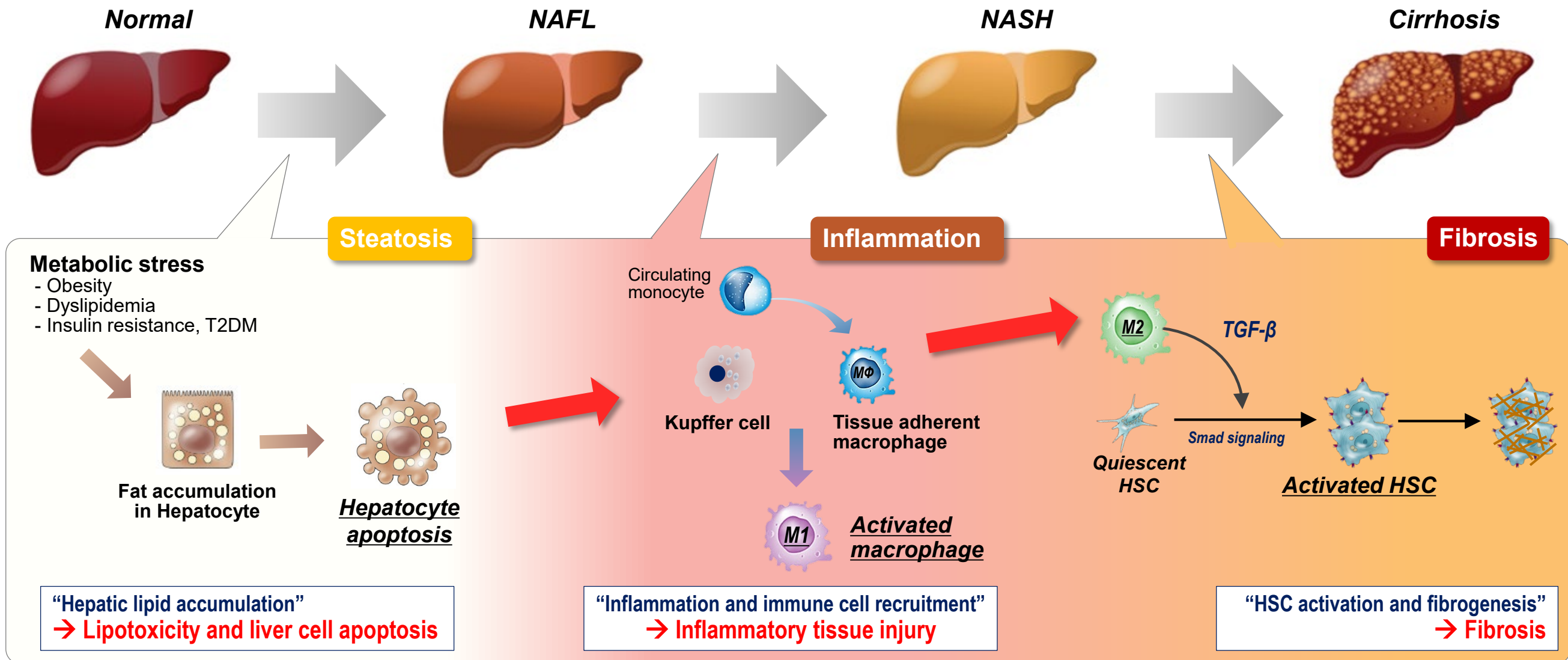
“HSC activation and fibrogenesis”
→ Fibrosis

NASH progression



NASH progression

Targeting multiple aspects of this disease should be required for efficient management of NASH and fibrosis



GLP-1

GIP

- Insulin resistance improvement
- Glycemic control
- Weight loss by appetite regulation
- Anti-inflammation

*May be
indirect benefits*

- NASH resolution
- Fibrosis improvement (?)

GLP-1

GIP

- Insulin resistance improvement
- Glycemic control
- Weight loss by appetite regulation
- Anti-inflammation

*May be
indirect benefits*

- ➔ NASH resolution
- ➔ Fibrosis improvement (?)

Glucagon

- Glucose production
- Weight loss by energy expenditure

[Liver targeting]

- Favorable lipid metabolism reprogramming
- Bile acid production ↓
- Anti-inflammation
 - ➔ Lipotoxicity and liver injury ↓
- TGF- β production ↓
- Smad signaling ↓ in HSC
 - ➔ HSC activation and fibrogenesis ↓

GLP-1

GIP

- Insulin resistance improvement
- Glycemic control
- Weight loss by appetite regulation
- Anti-inflammation

*May be
indirect benefits*

- ➔ NASH resolution
- ➔ Fibrosis improvement

Glucagon

- Glucose production
- Weight loss by energy expenditure

[Liver targeting]

- Favorable lipid metabolism reprogramming
- Bile acid production ↓
- Anti-inflammation
 - ➔ Lipotoxicity and liver injury ↓
- TGF-β production ↓
- Smad signaling ↓ in HSC
 - ➔ HSC activation and fibrogenesis ↓

Direct benefits

GLP-1

GIP

- Insulin resistance improvement
- Glycemic control
- Weight loss by appetite regulation
- Anti-inflammation

May be indirect benefits

- ➔ NASH resolution
- ➔ Fibrosis improvement
- ➔ Off-set blood glucose elevation



Glucagon

- Glucose production
- Weight loss by energy expenditure

[Liver targeting]

- Favorable lipid metabolism reprogramming
- Bile acid production ↓
- Anti-inflammation
- ➔ Lipotoxicity and liver injury ↓

- TGF-β production ↓
- Smad signaling ↓ in HSC
- ➔ HSC activation and fibrogenesis ↓

Direct benefits



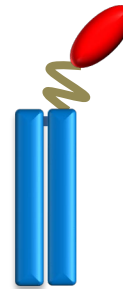
GLP-1

GIP

- Insulin resistance improvement
- Glycemic control
- Weight loss by appetite regulation
- Anti-inflammation

May be indirect benefits

- ➔ NASH resolution
- ➔ Fibrosis improvement
- ➔ Off-set blood glucose elevation



HM15211 [Ph2b, US]



Direct benefits

Glucagon

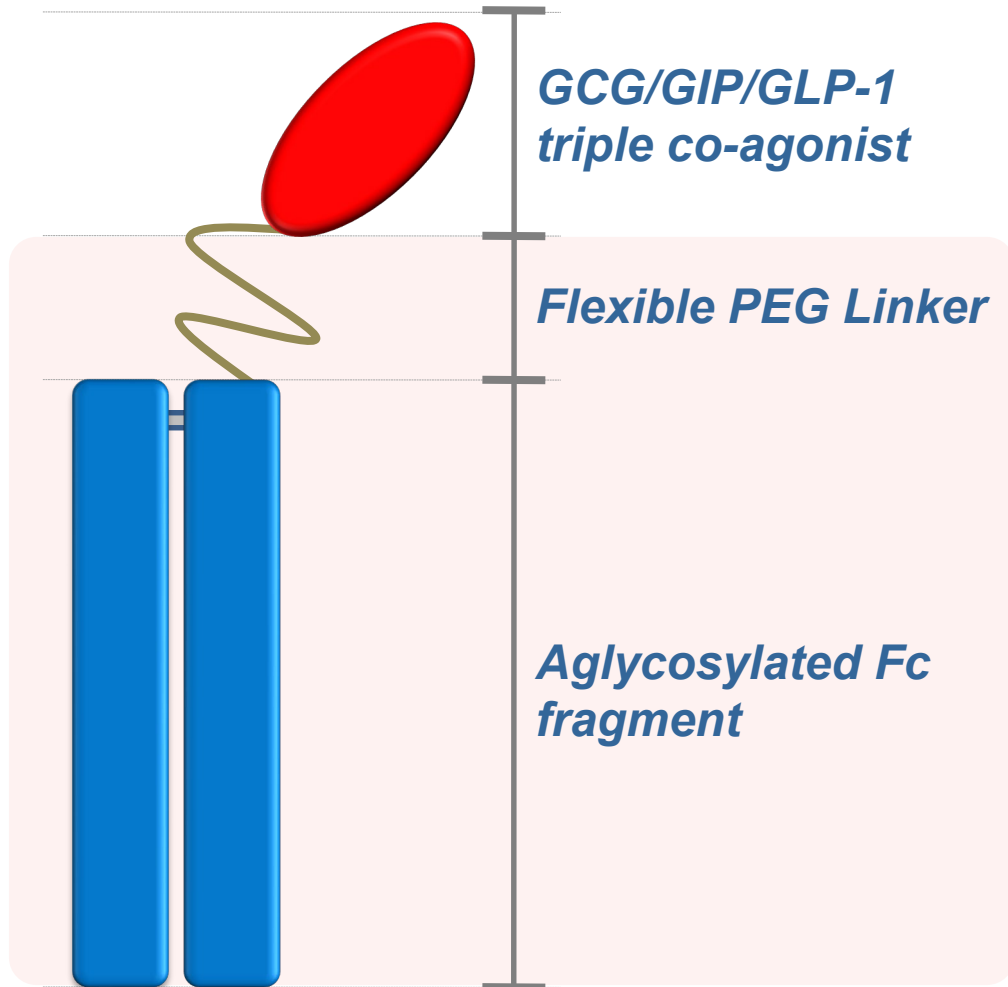
- Glucose production
- Weight loss by energy expenditure

[Liver targeting]

- Favorable lipid metabolism reprogramming
- Bile acid production ↓
- Anti-inflammation
- ➔ Lipotoxicity and liver injury ↓

- TGF-β production ↓
- Smad signaling ↓ in HSC
- ➔ HSC activation and fibrogenesis ↓

What is long-acting Glucagon/GIP/GLP-1 triple co-agonist?



Hanmi's Glucagon/GIP/GLP-1 triple co-agonist (HM15211) is conjugated with a human IgG Fc fragment *via* flexible linker

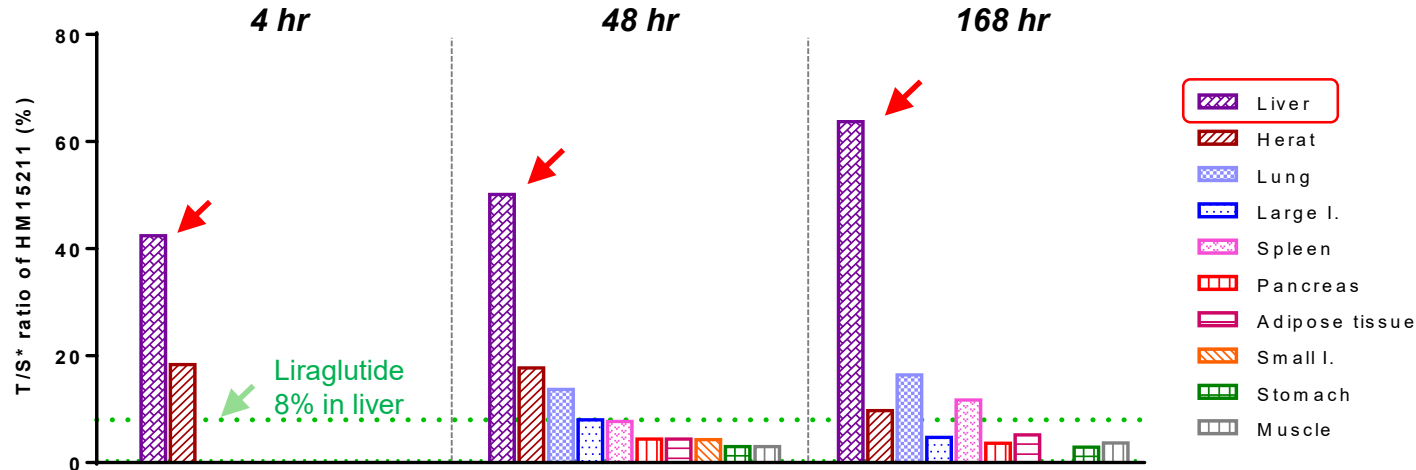
[General profile]

- Single drug moiety with triple activity
- Extended half-life allows once weekly dosing
- Rationally designed triple agonist optimized for liver targeting
- Rapid & potent liver fat reduction both in animal and human (**12 weeks MAD trial results in obese NAFLD subjects presented at 2020 EASL*)
- Multiple MoAs exist for managing inflammation and fibrosis

LAPSCOVERY : Long Acting Peptide/Protein DiSCOVERY Technology

Tissue distribution & hepatic lipid metabolism (vs. GLP-1RA)

1) Tissue distribution over time



Note. T/S* ratio: Tissue to serum ratio
Green line indicates T/S ratio range of liraglutide for liver (FDA NDA package)

1) Liver targeting

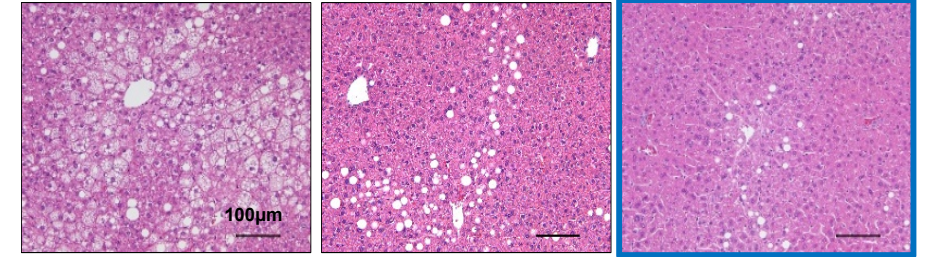
2) Increase β -oxidation

3) More liver fat reduction vs. GLP-1RA

Vehicle

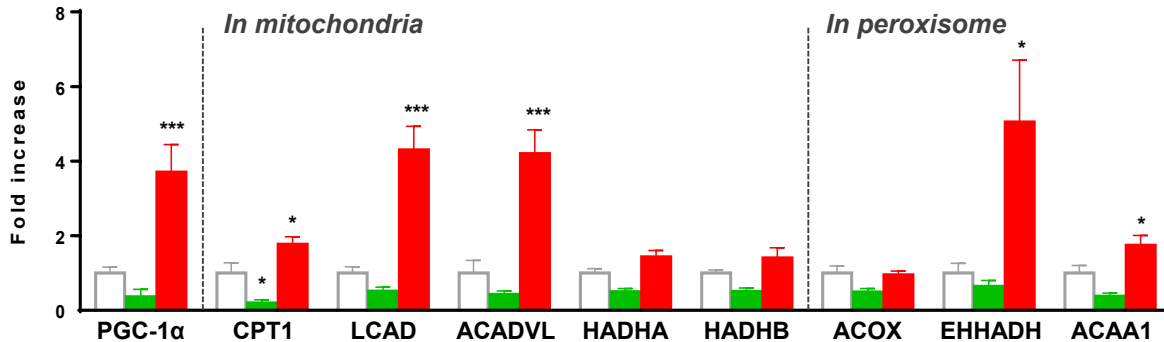
Liraglutide

HM15211

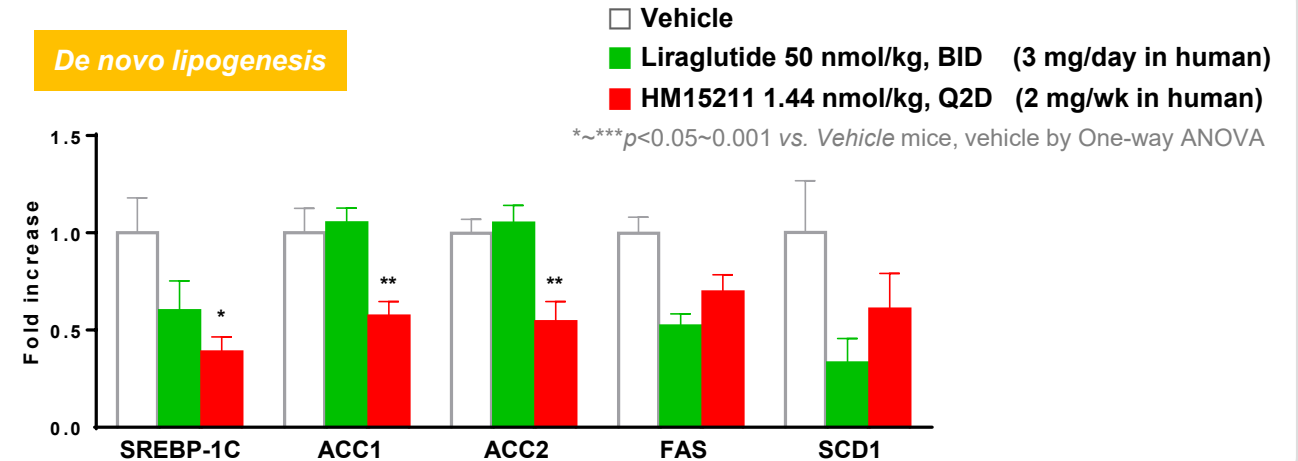


2) Hepatic lipid metabolism reprogramming

β -oxidation

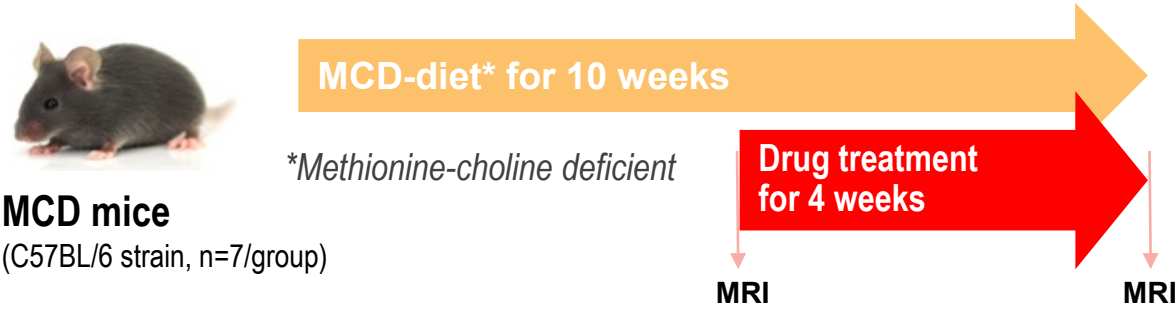


De novo lipogenesis

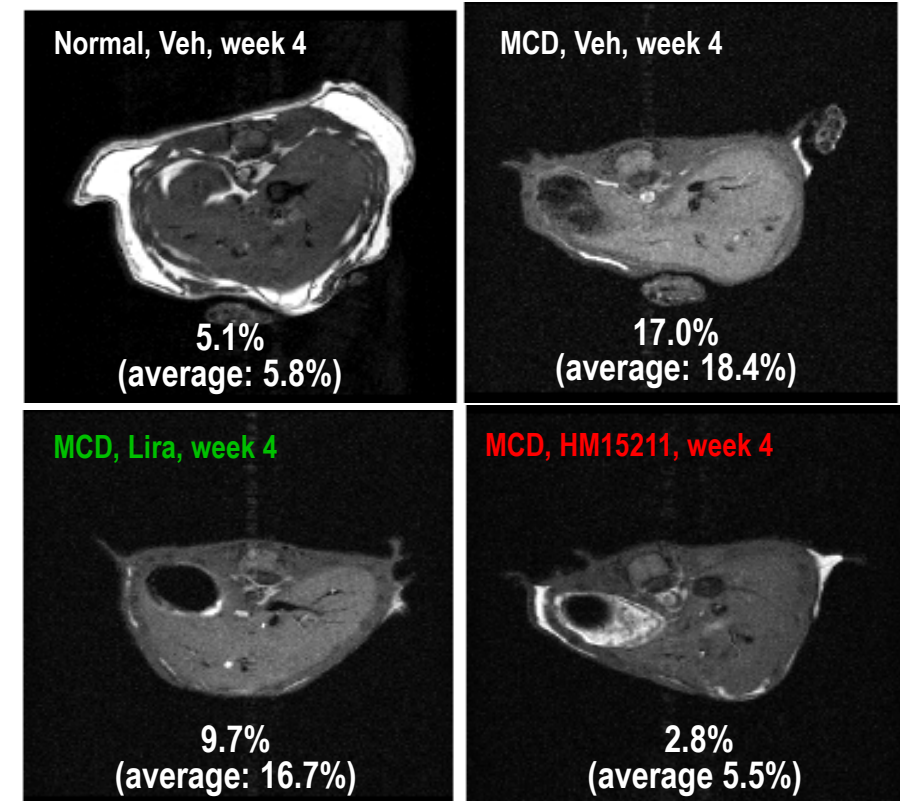


Liver fat reduction in MCD-diet mice [Lean NASH model]

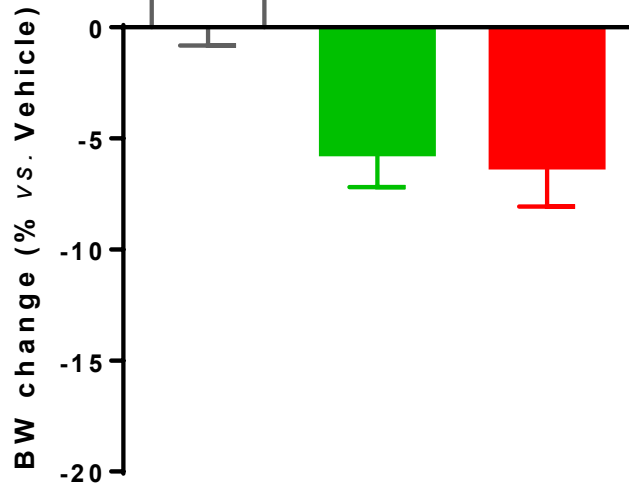
Experimental scheme



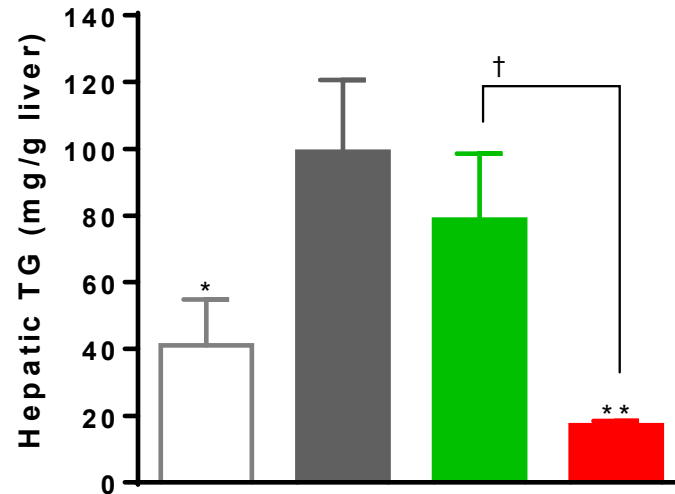
Liver fat by MRI/MRS



BW change at EOT



Liver TG




- Normal, Vehicle
- MCD, Vehicle
- MCD, Liraglutide 50 nmol/kg, BID (3 mg/day in human)
- MCD, HM15211 0.72 nmol/kg, Q2D (1 mg/wk in human)

*~**p<0.05~0.01 vs. MCD mice, vehicle by One-way ANOVA; †p<0.05 vs. liraglutide by One-way ANOVA

HM15211, long-acting Glucagon/GIP/GLP-1 triple agonist, might have therapeutic potential for NASH and fibrosis

- The efficacy in NASH and fibrosis was evaluated in various rodent disease models


Species / Strain	Induction method		Expected disease status	Abstract #
 C57BL/6 mice	1. Diet-induced	AMLN diet	Obesity; NASH	#191 (Oral)
	2. Surgery-induced	Bile duct ligation	Liver inflammation and necrosis	#668 (Poster)
	3. Chemical-induced	TAA treatment*	Moderate to severe Fibrosis	

Note.

*TAA (Thioacetamide, hepatotoxin) → Liver inflammation → Fibrosis ↑

HM15211, long-acting Glucagon/GIP/GLP-1 triple agonist, might have therapeutic potential for NASH and fibrosis

- The efficacy in NASH and fibrosis was evaluated in various rodent disease models

Species / Strain	Induction method		Expected disease status	Abstract #
 C57BL/6 mice	1. Diet-induced	AMLN diet	Obesity; NASH	#191 (Oral)
	2. Surgery-induced	Bile duct ligation	Liver inflammation and necrosis	#668 (Poster)
	3. Chemical-induced	TAA treatment*	Moderate to severe Fibrosis	

Note.

*TAA (Thioacetamide, hepatotoxin) → Liver inflammation → Fibrosis ↑

Change of NASH prognosis markers in AMLN-diet mice [Study #1]

Experimental scheme



AMLN mice[†]
(C57BL/6 strain, n=7/group)

AMLN-diet* for 49 weeks

*40% kcal from fat
22% fructose, 2% cholesterol

Drug treatment
for 12 weeks

[†]Except for baseline biopsy, same disease model w/ Gubra's NASH mice

[Group assignment]

Normal, Vehicle

AMLN, Vehicle

AMLN, OCA 30 mg/kg, QD

AMLN, HM15211 2.6 nmol/kg, Q2D (4 mg/wk in human)

Change of NASH prognosis markers in AMLN-diet mice [Study #1]

Experimental scheme



AMLN mice[†]
(C57BL/6 strain, n=7/group)

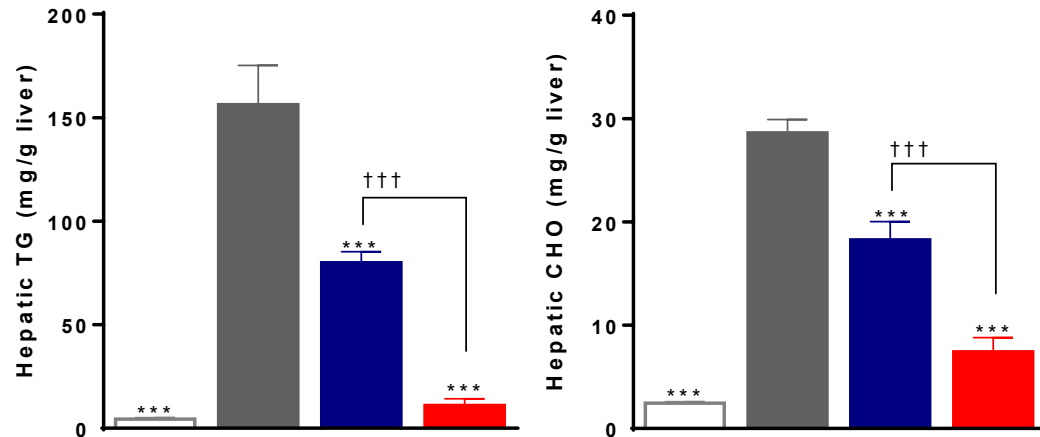
AMLN-diet* for 49 weeks

*40% kcal from fat
22% fructose, 2% cholesterol

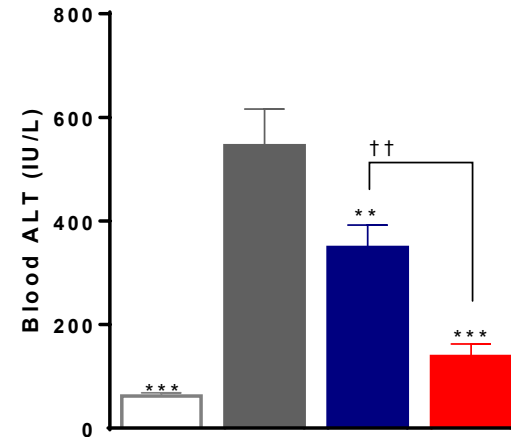
Drug treatment
for 12 weeks

[†]Except for baseline biopsy, same disease model w/ Gubra's NASH mice

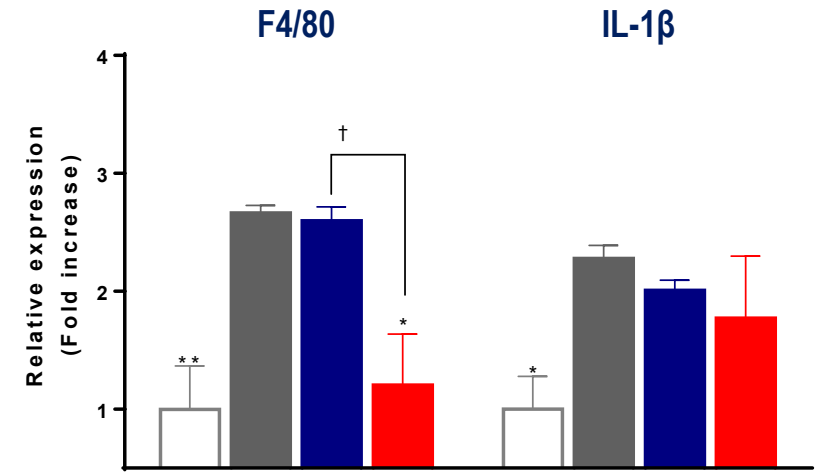
Hepatic TG and CHO



Blood ALT



Hepatic inflammation markers (by qPCR)



□ Normal, Vehicle ■ AMLN, Obeticholic acid 30 mg/kg, QD (244 mg/day in human, ~9.8XHED)
 ■ AMLN, Vehicle ■ AMLN, HM15211 2.6 nmol/kg, Q2D (4 mg/wk in human)

*~***p<0.05~0.001 vs. AMLN mice, vehicle by One-way ANOVA; †††p<0.01 vs. OCA by One-way ANOVA

Note. TG: Triglyceride, CHO: Cholesterol

Change of NAFLD activity score in AMLN-diet mice [Study #1]

Experimental scheme



AMLN mice†
(C57BL/6 strain, n=7/group)

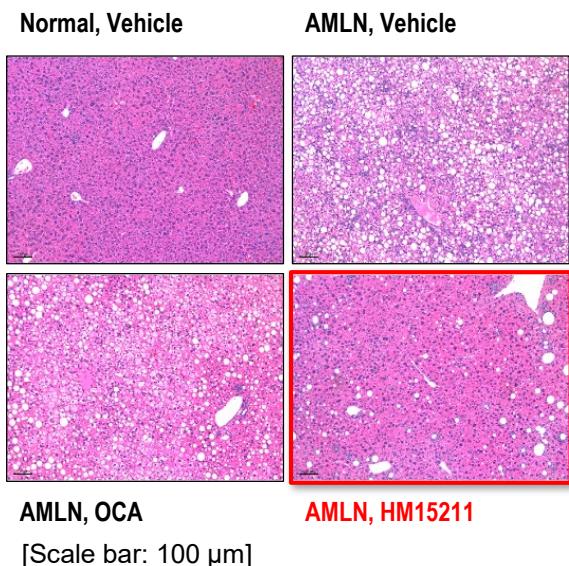
AMLN-diet* for 49 weeks

*40% kcal from fat
22% fructose, 2% cholesterol

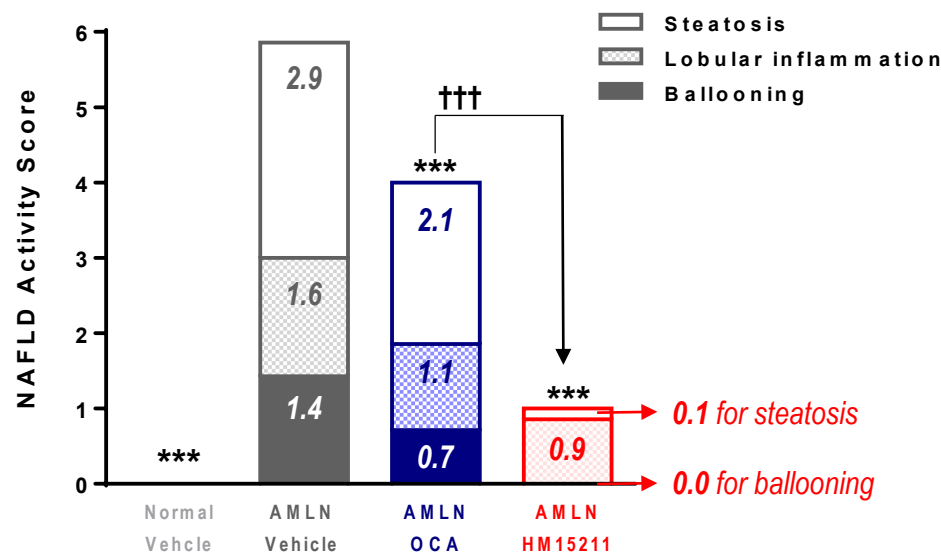
Drug treatment
for 12 weeks

†Except for baseline biopsy, same disease model w/ Gubra's NASH mice

H&E staining (representative image)



NAFLD activity score (NAS)



- Normal, Vehicle
- AMLN, Obeticholic acid 30 mg/kg, QD (244 mg/day in human, ~9.8XHED)
- AMLN, Vehicle
- AMLN, HM15211 2.6 nmol/kg, Q2D (4 mg/wk in human)

***p<0.001 vs. AMLN mice, vehicle by One-way ANOVA; †††p<0.001 vs. OCA by One-way ANOVA

Change of NAFLD activity score in AMLN-diet mice [Study #1]

Experimental scheme



AMLN mice†
(C57BL/6 strain, n=7/group)

AMLN-diet* for 49 weeks

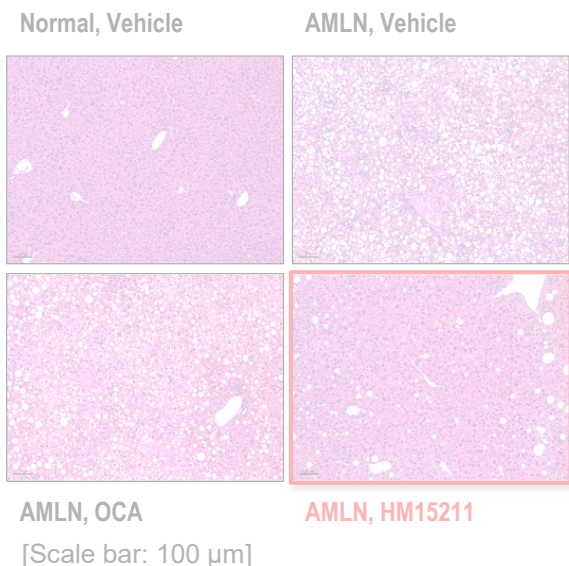
*40% kcal from fat
22% fructose, 2% cholesterol

Drug treatment
for 12 weeks

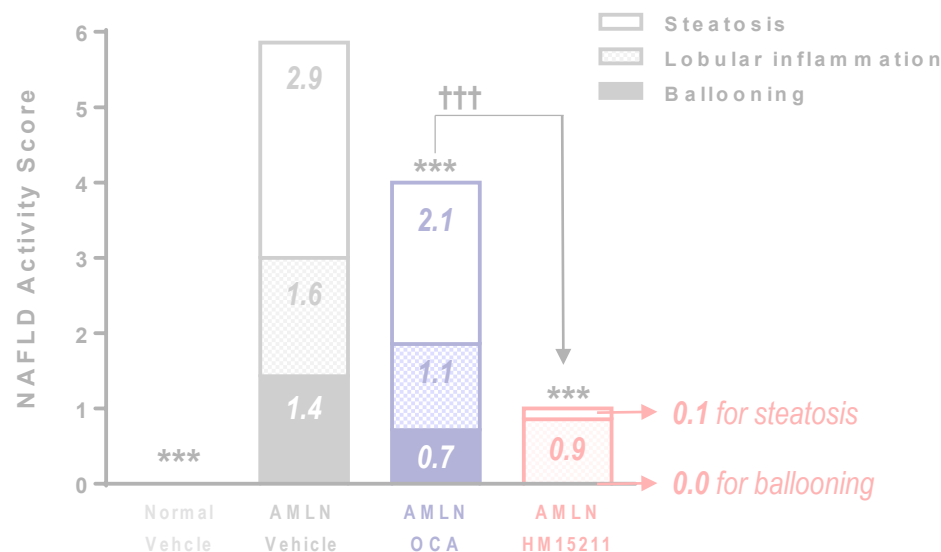
†Except for baseline biopsy, same disease model w/ Gubra's NASH mice

Definition of NASH resolution
- Inflammation score: 0 or 1
- Ballooning score: 0

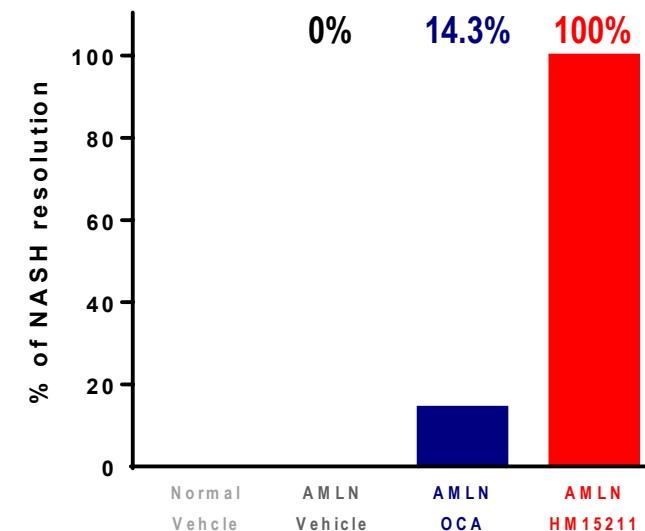
H&E staining (representative image)



NAFLD activity score (NAS)



Portion for NASH resolution



Normal, Vehicle
 AMLN, Obeticholic acid 30 mg/kg, QD (244 mg/day in human, ~9.8XHED)

AMLN, Vehicle
 AMLN, HM15211 2.6 nmol/kg, Q2D (4 mg/wk in human)

***p<0.001 vs. AMLN mice, vehicle by One-way ANOVA; †††p<0.01 vs. OCA by One-way ANOVA

Change of NAFLD activity score in AMLN-diet mice [Study #2]

Experimental scheme



AMLN mice
(C57BL/6 strain, n=7/group)

AMLN-diet* for 37 weeks

*40% kcal from fat
22% fructose, 2% cholesterol

Drug treatment
for 12 weeks

[Group assignment]

Normal, Vehicle

AMLN, Vehicle

AMLN, Acylated GLP-1 20.5 nmol/kg, Q2D (2.4 mg/wk in human)

AMLN, Acylated GLP-1/GIP 109.5 nmol/kg, Q2D (15 mg/wk in human)

AMLN, Acylated GLP-1/GCG 19.5 nmol/kg, QD (0.6 mg/day in human)

AMLN, HM15211 2.6 nmol/kg, Q2D (4 mg/wk in human)

[Current NASH studies of incretin analogs in human]

	Phase	Dose	Note
Semaglutide	Phase 3	<u>2.4 mg/week</u>	On-going, NCT04822181
Tirzepatide	Phase 2b	5, 10, or <u>15 mg/week</u>	On-going, NCT04166773
Cotadutide	Phase 2a	~ <u>0.6 mg/day</u>	Pending for top-line results, NCT04019561

Change of NAFLD activity score in AMLN-diet mice [Study #2]

Experimental scheme



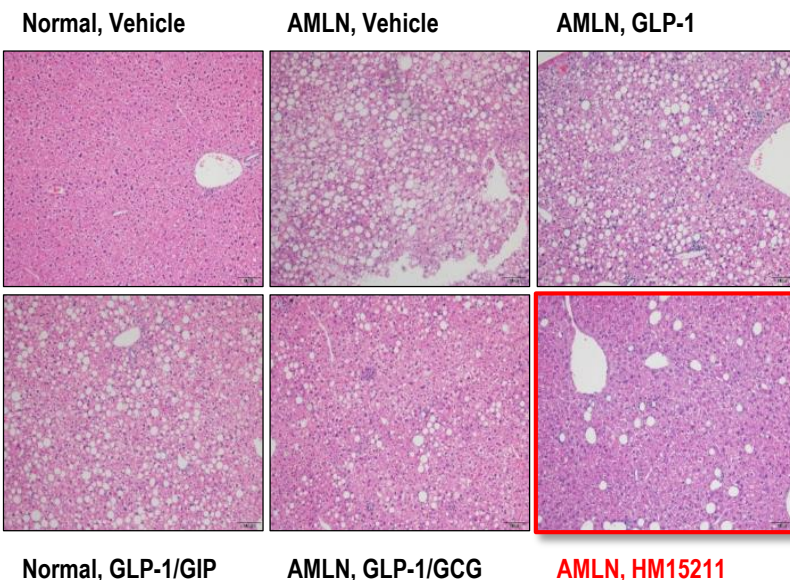
AMLN mice
(C57BL/6 strain, n=7/group)

AMLN-diet* for 37 weeks

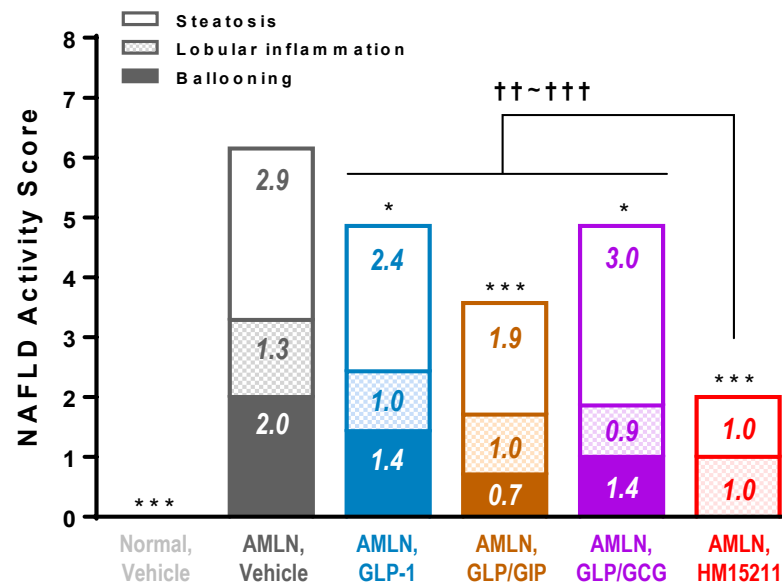
*40% kcal from fat
22% fructose, 2% cholesterol

Drug treatment
for 12 weeks

H&E staining (representative image)



NAFLD activity score (NAS)



Normal, Vehicle
 AMLN, Acylated GLP-1 20.5 nmol/kg, Q2D (2.4 mg/wk in human)
 AMLN, Acylated GLP-1/GCG 19.5 nmol/kg, Q2D (0.6 mg/wk in human)

AMLN, Vehicle
 AMLN, Acylated GLP-1/GIP 109.5 nmol/kg, Q2D (15 mg/wk in human)
 AMLN, HM15211 2.6 nmol/kg, Q2D (4 mg/wk in human)

*~***p<0.05~0.001 vs. AMLN mice, vehicle by One-way ANOVA; †~†††p<0.01~0.001 vs. acylated incretins by One-way ANOVA

Change of NAFLD activity score in AMLN-diet mice [Study #2]

Experimental scheme



AMLN mice
(C57BL/6 strain, n=7/group)

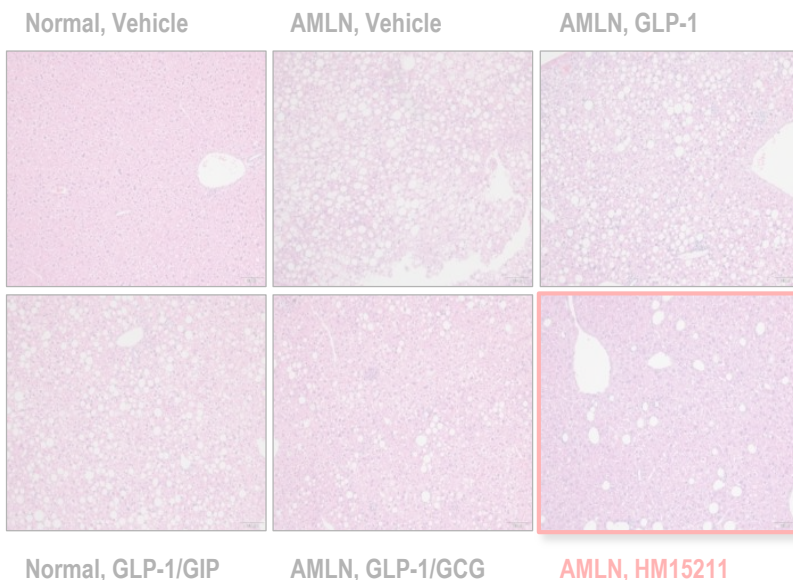
AMLN-diet* for 37 weeks

*40% kcal from fat
22% fructose, 2% cholesterol

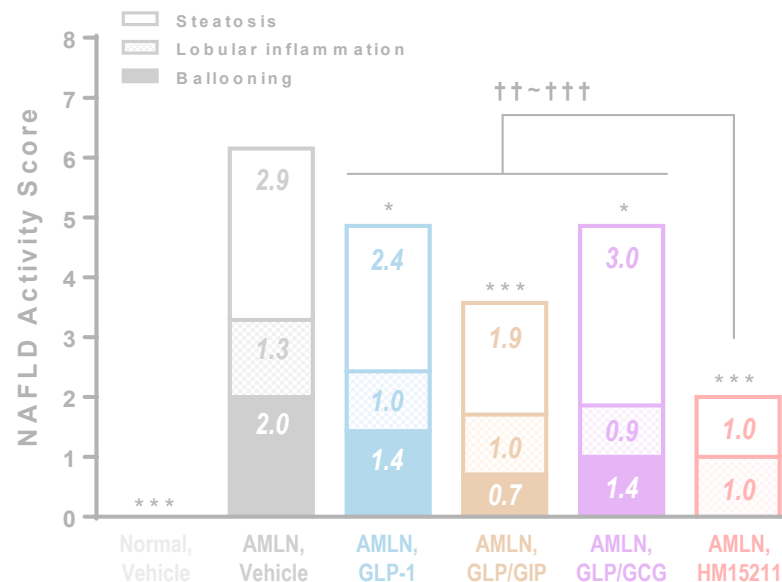
Drug treatment
for 12 weeks

Definition of NASH resolution
- Inflammation score: 0 or 1
- Ballooning score: 0

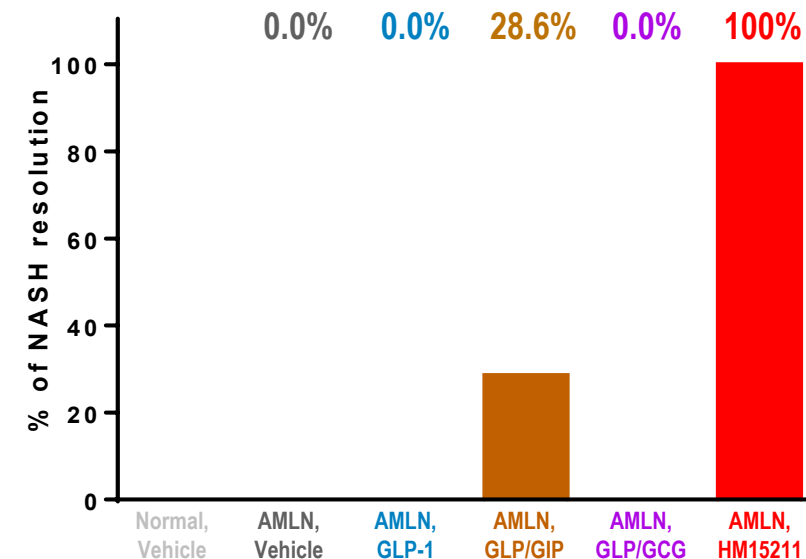
H&E staining (representative image)



NAFLD activity score (NAS)



Portion for NASH resolution



Normal, Vehicle
 AMLN, Acylated GLP-1 20.5 nmol/kg, Q2D (2.4 mg/wk in human)
 AMLN, Acylated GLP-1/GCG 19.5 nmol/kg, Q2D (0.6 mg/wk in human)

AMLN, Vehicle
 AMLN, Acylated GLP-1/GIP 109.5 nmol/kg, Q2D (15 mg/wk in human)
 AMLN, HM15211 2.6 nmol/kg, Q2D (4 mg/wk in human)

*~***p<0.05~0.001 vs. AMLN mice, vehicle by One-way ANOVA; ++~+++p<0.01~0.001 vs. acylated incretins by One-way ANOVA

Change of NAFLD activity score in AMLN-diet mice [Study #2]

Experimental scheme



AMLN mice
(C57BL/6 strain, n=7/group)

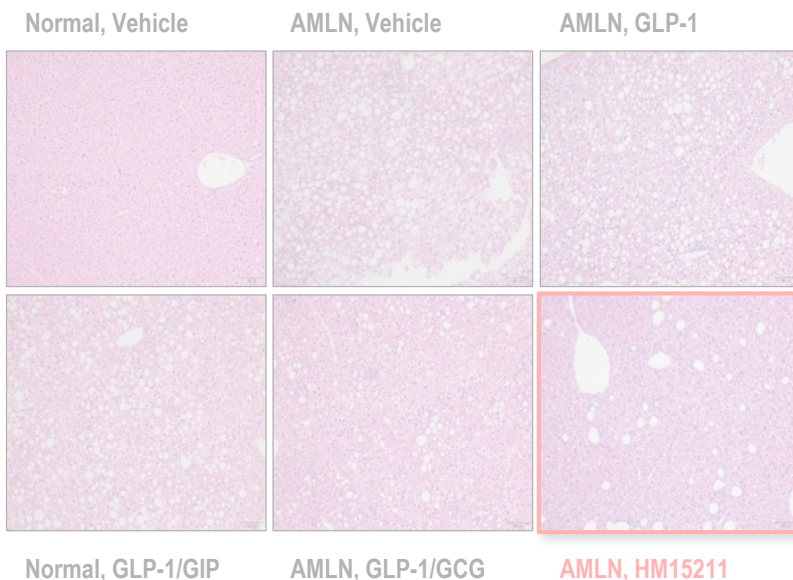
AMLN-diet* for 37 weeks

*40% kcal from fat
22% fructose, 2% cholesterol

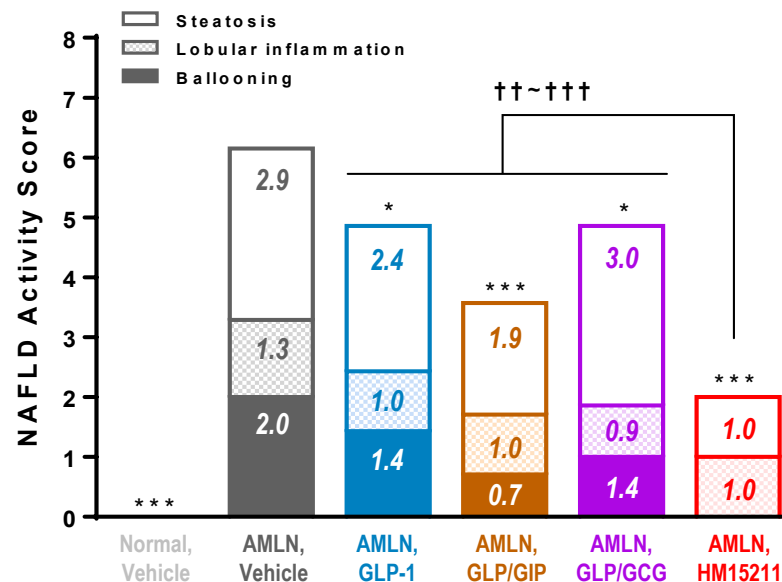
Drug treatment
for 12 weeks

Definition of NASH resolution
- Inflammation score: 0 or 1
- Ballooning score: 0

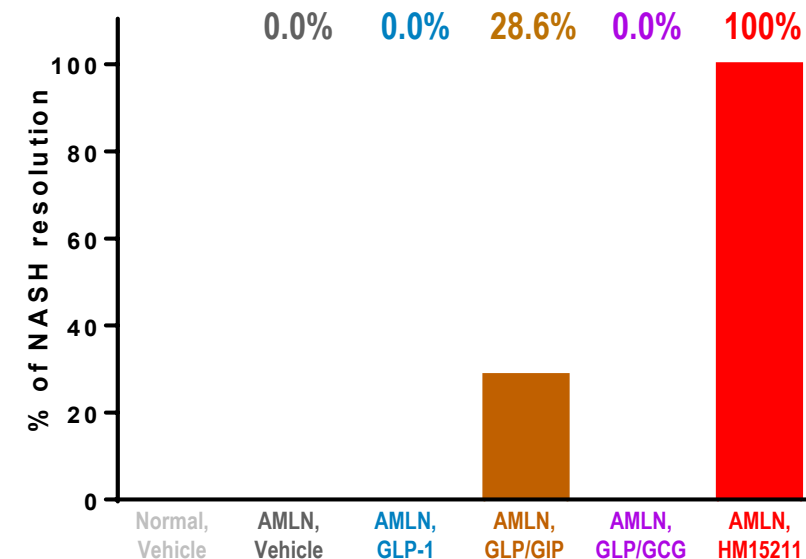
H&E staining (representative image)



NAFLD activity score (NAS)



Portion for NASH resolution

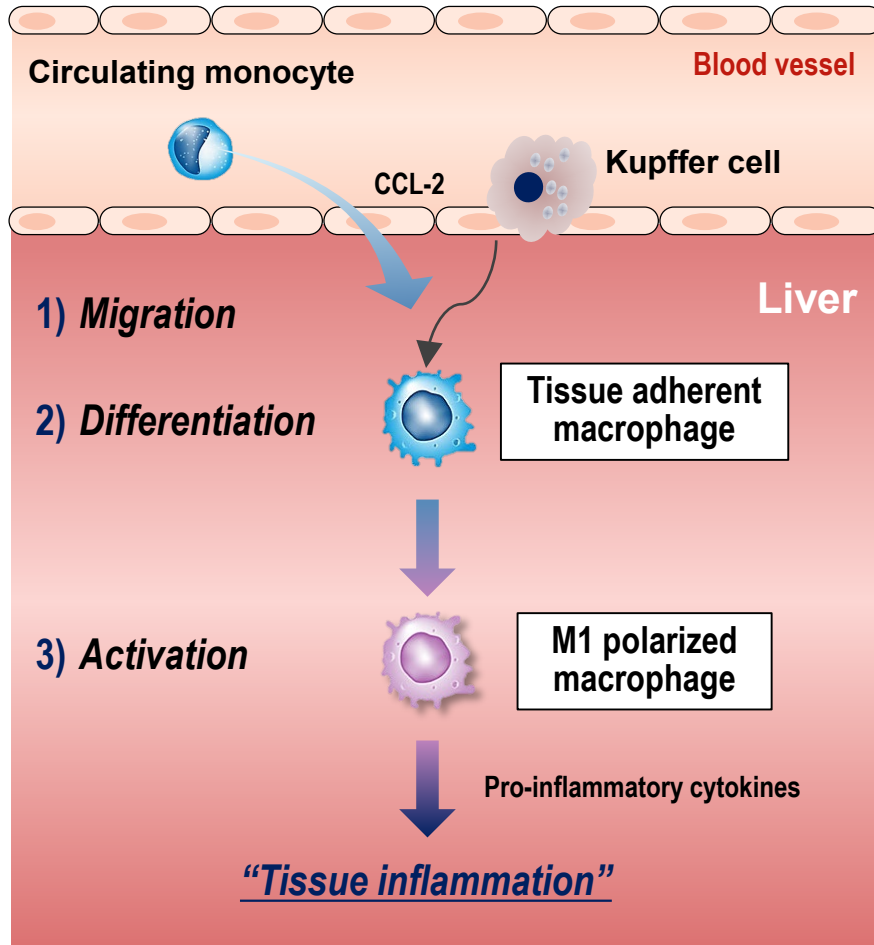


Normal, Vehicle
 AMLN, Acylated GLP-1 20.5 nmol/kg, Q2D (2.4 mg/wk in human)
 AMLN, Acylated GLP-1/GCG 19.5 nmol/kg, Q2D (0.6 mg/wk in human)

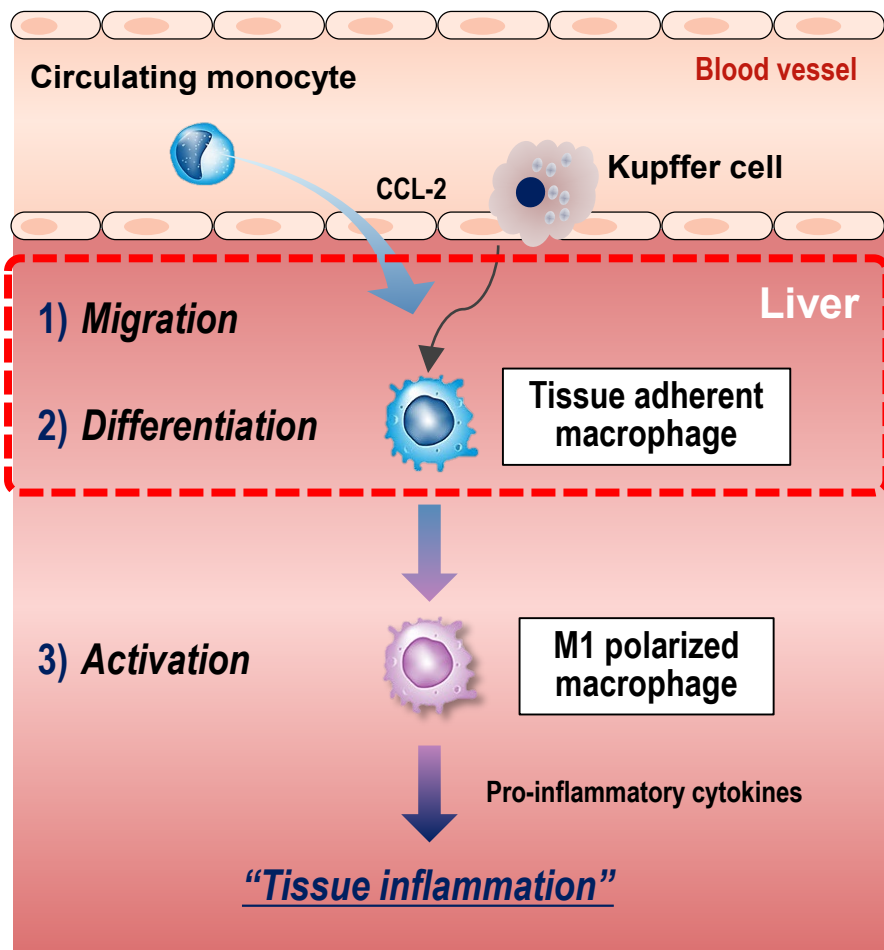
AMLN, Vehicle
 AMLN, Acylated GLP-1/GIP 109.5 nmol/kg, Q2D (15 mg/wk in human)
 AMLN, HM15211 2.6 nmol/kg, Q2D (4 mg/wk in human)

*~***p<0.05~0.001 vs. AMLN mice, vehicle by One-way ANOVA; †~†††p<0.01~0.001 vs. acylated incretins by One-way ANOVA

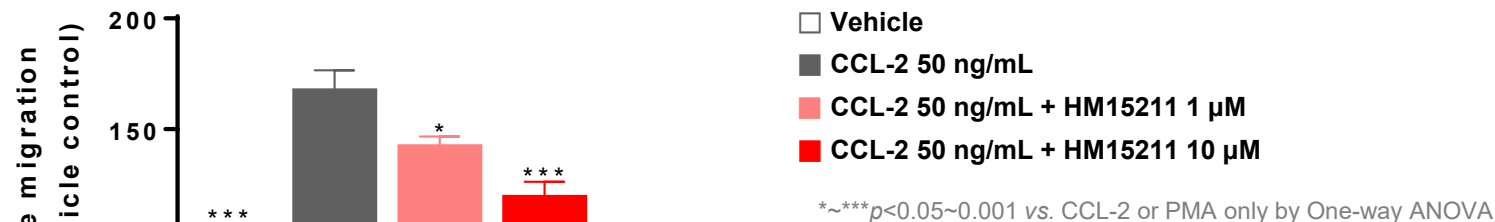
Proposed mechanism for anti-inflammatory effects of HM15211



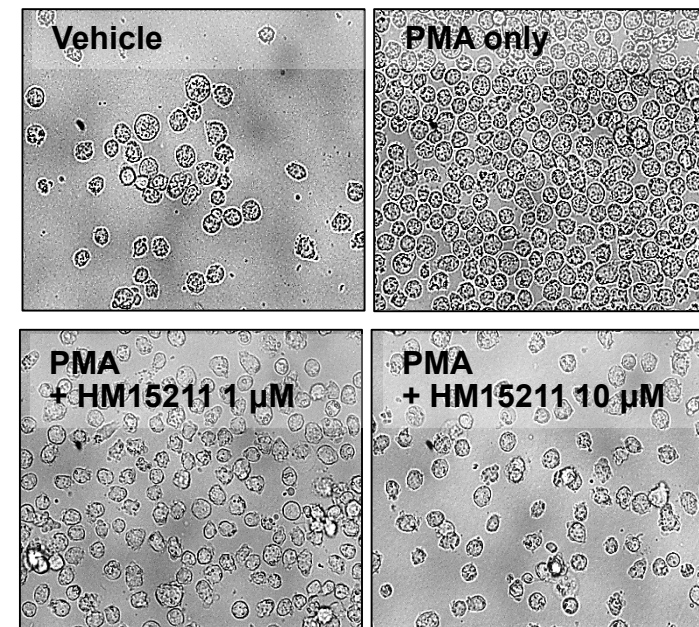
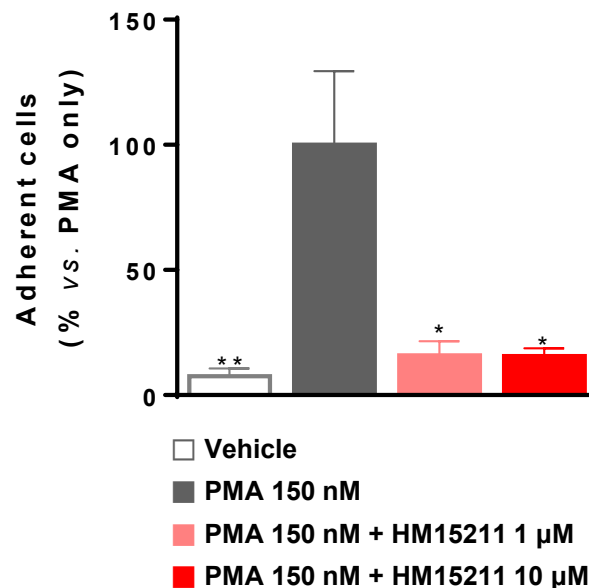
Proposed mechanism for anti-inflammatory effects of HM15211



THP-1 monocyte migration

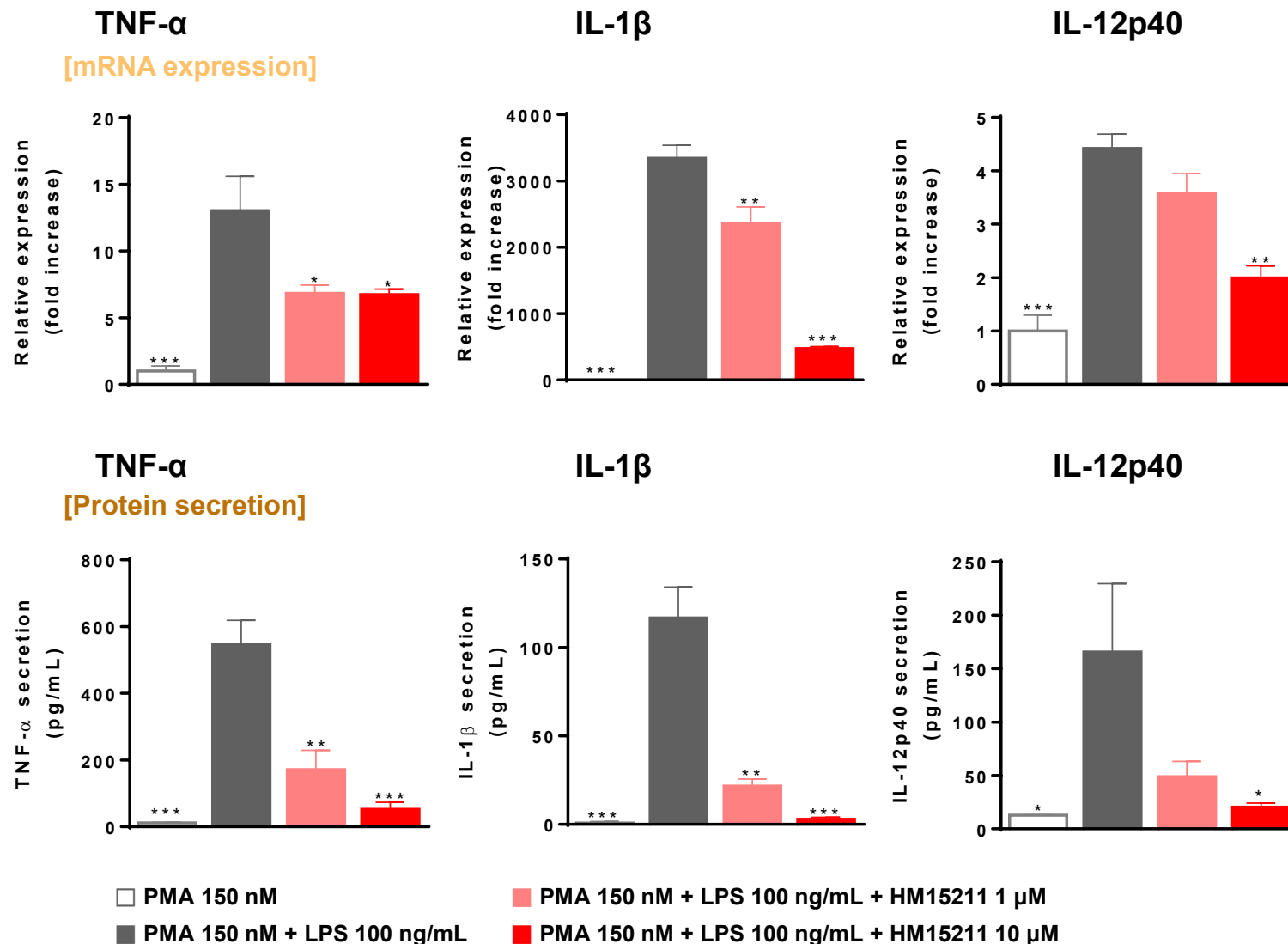
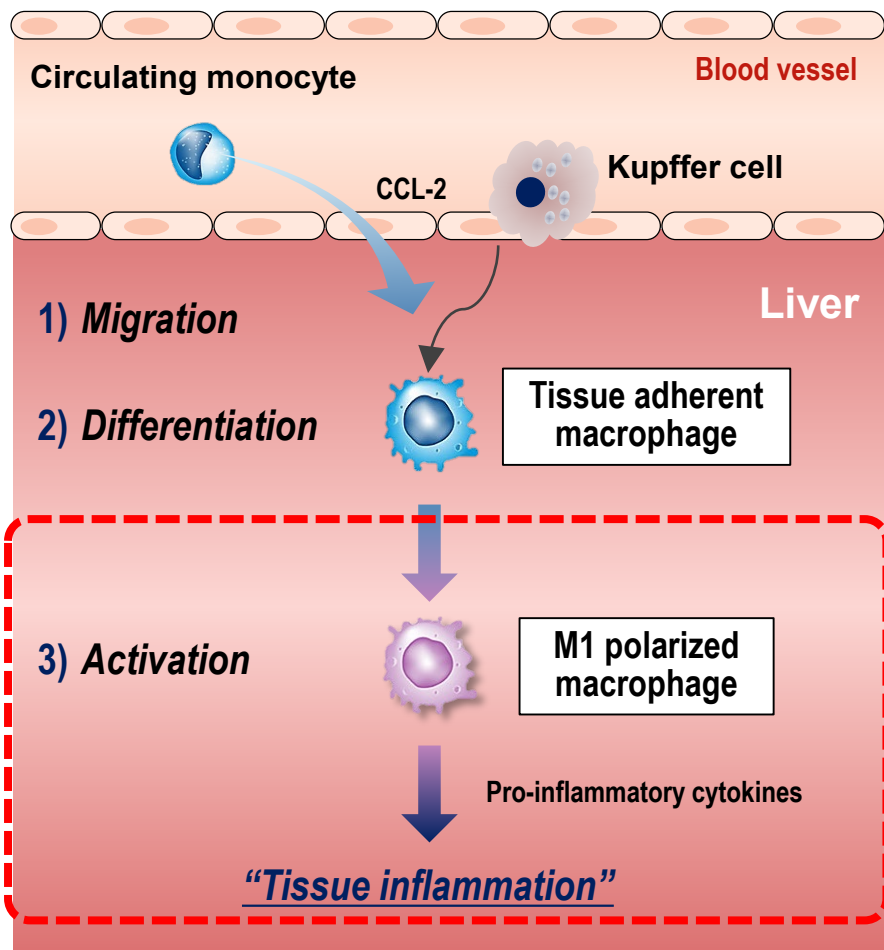


THP-1 monocyte adhesion (surrogate of macrophage differentiation)



Proposed mechanism for anti-inflammatory effects of HM15211

Pro-inflammatory cytokine expression & secretion by THP-1 macrophage



*~***p<0.05~0.001 vs. PMA + LPS only by One-way ANOVA

- **HM15211 is Glucagon/GIP/GLP-1 triple agonist with unique activity features designed to treat NASH and fibrosis by targeting multiple aspects of this disease**
- **Previous studies demonstrated that HM15211 showed greater liver fat reduction than GLP-1RA via hepatic lipid metabolism reprogramming after liver preferential distribution**
- **In AMLN-diet mice, HM15211 treatment efficiently reduced both steatosis, inflammation, and ballooning, leading to greater reduction in composite NAS than FXR agonist and other long-acting incretin analogs**
- **Mechanistic studies showed the inhibitory effects of HM15211 on THP-1 monocyte migration, macrophage differentiation and M1 polarization, which explains enhanced NASH resolution effects of HM15211 in animal model**

**With multi-modal action, HM15211 might provide improved efficacy for the treatment of NASH
Fast-track granted, and P2b clinical study is on-going in biopsy-proven NASH subjects (US)**

Please note short-oral presentation reporting more information about HM15211:

#668: Anti-fibrotic potential of a novel long-acting Glucagon/GIP/GLP-1 triple agonist (HM15211) in preclinical models of fibrosis

Contact information: jeahyuk.choi@hanmi.co.kr