
PARK CITY, UTAH • MARCH 11-13, 2021
NASH-TAG
CONFERENCE
2021

TLR Signaling Inhibition with Mosedipimod / EC-18

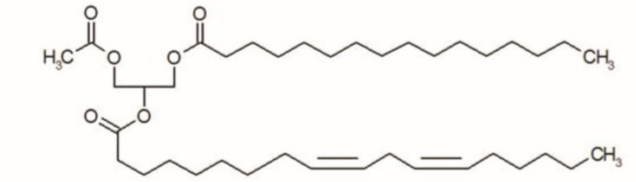
Michael Charlton MD, FRCP
University of Chicago

Contents

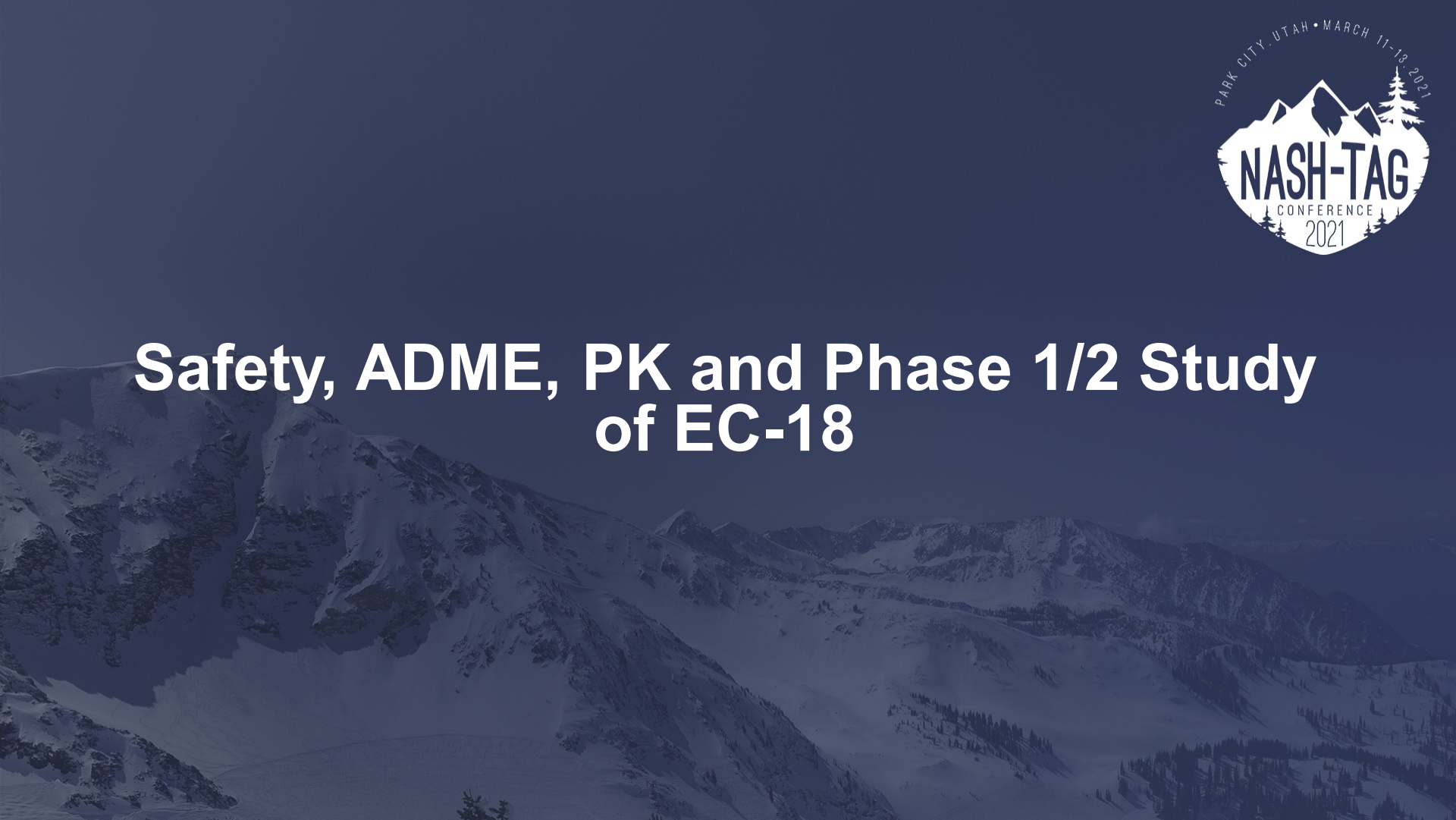
Introduction of EC-18

An oral, lipid-based, first-in-class small molecule

PLAG (1-Palmitoyl-2-Linoleoyl-3-Acetyl-rac-Glycerol)

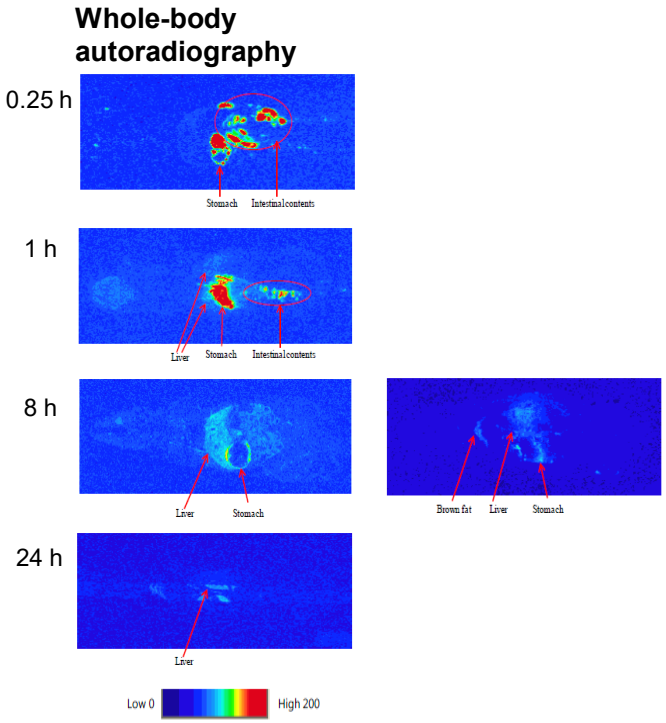


- Fully synthetic PLAG, originated from Sika deer antlers
- Orally-available, lipid-based small molecule administered daily as soft gel capsules
- 1 capsule: 500 mg of PLAG (99.5% high purity level) + 1 mg antioxidant (α-tocopherol)
- No drug-related safety issues observed in Phase I and on-going Phase II clinical studies (CRIOM, CIN)



Safety, ADME, PK and Phase 1/2 Study of EC-18

Distribution of EC-18



Whole-body autoradiograms in albino rats at 0.25, 1, 8, and 24 h after oral administration of [^{14}C]EC-18 at single dose of 200 mg/kg (N=2)

Plasma stability

Test compound	Final test conc. (μM)	% of remaining drug after 1 h incubation (N=3)	
		Human plasma	Rat plasma
EC-18	1	>99	>99
Albendazol (high plasma stability marker)	1	>99	>99
Vinpocetin (low plasma stability marker)	1	85.3 ± 3.8	52.4 ± 7.2

Plasma protein binding

Test compound	Final test conc. (μM)	% of plasma protein binding (N=3)	
		Humans	Rats
EC-18	1	>99	98.8±0.5
Warfarin (high PPB marker)	1	99.2±0.2	99.1±0.3
Atenolol (low PPB marker)	1	1.7±1.2	9.2±2.1

Summary of Phase 1 Studies

Item	EC18-001	EC18-102	EC-18-001
Study	SAD, MAD and PK (QD)	SAD, Food effect and PK (QD)	SAD and PK (QD)
Country	Republic of Korea	Republic of Korea	United States of America
Registration	KCT0001470/ NCT02532712	KCT0001845/ NCT02700360	NCT02496143
Study Period	25 Feb 2015 ~ 28 Dec 2015	24 Jul 2015 ~ 11 Oct 2015	15 July 2015 ~ 10 Dec 2015
Subjects	32 + 32 = total 64 healthy volunteers	6 per sequence = total 36 healthy volunteers	8/9 per cohort = total 33 healthy volunteers
Dosing	EC-18 500, 1000, 2000, 4000 mg + Placebo	EC-18 500, 1000, 2000 mg + Placebo	EC-18 500, 1000, 2000, 4000 mg + Placebo
Safety issue	**No SAE and SADR occurred**	**No SAE and SADR occurred**	**No SAE and SADR occurred**
Conclusion	The administration of EC-18 up to 4,000 mg was safe and tolerable	An oral absorption of EC-18 was enhanced by administration with meal	The administration of EC-18 up to 4,000 mg was safe and tolerable

▪ **SAD**: Single Ascending Dose; **MAD**: Multiple Ascending Dose; **SAE**: Serious Adverse Event; **SADR**: Serious Adverse Drug Reaction

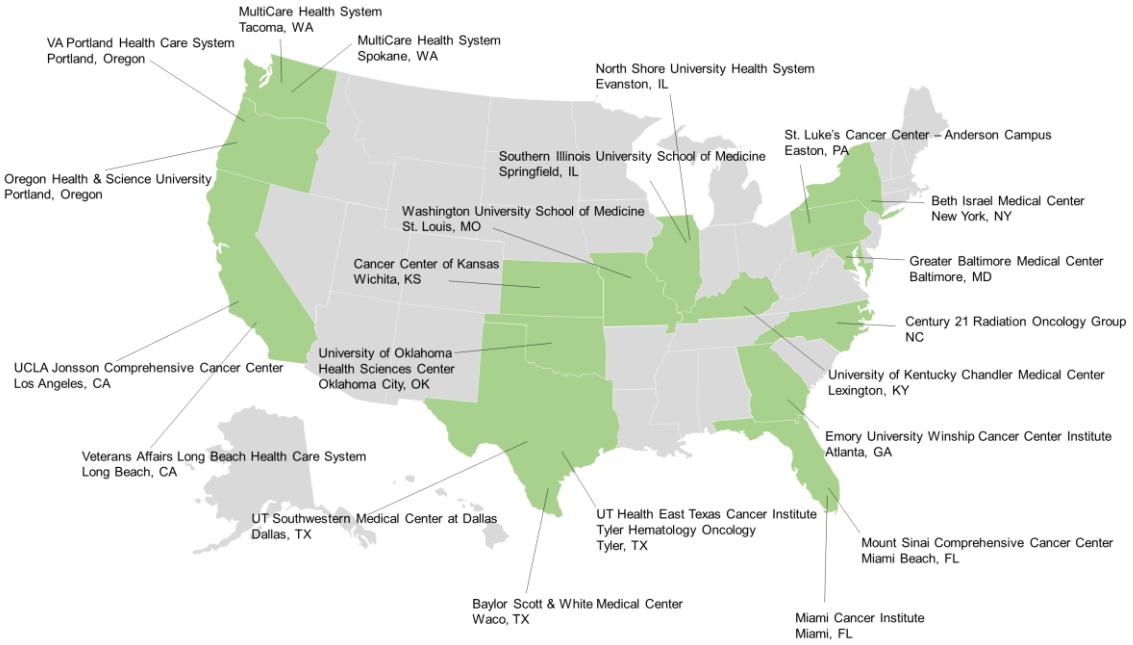
Phase 2 Study Program (CRIOM)

Stage 1

- 25 sites
- 24 subjects
- 4 groups @ 500mg, 1000mg, 2000mg, placebo

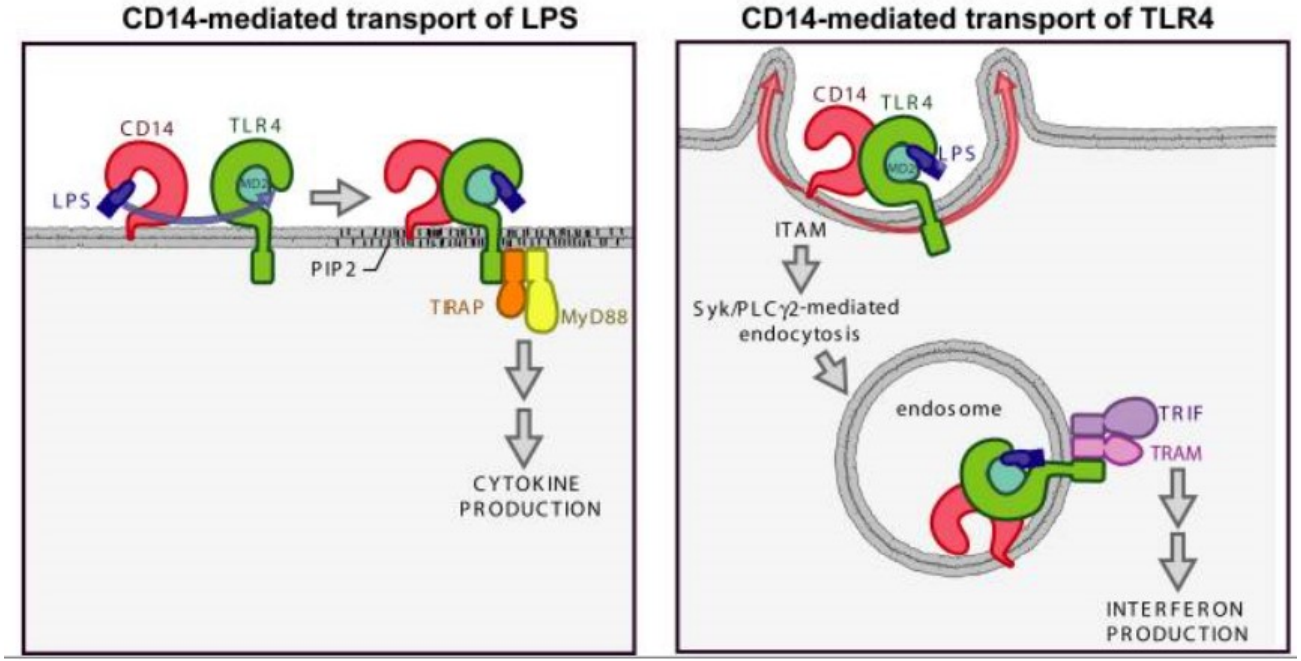
Stage 2

- 25 sites
- 80 subjects
- 2 groups @ 2000mg, placebo



Mechanism of Action

CD14 initiated PRR Endocytic Trafficking

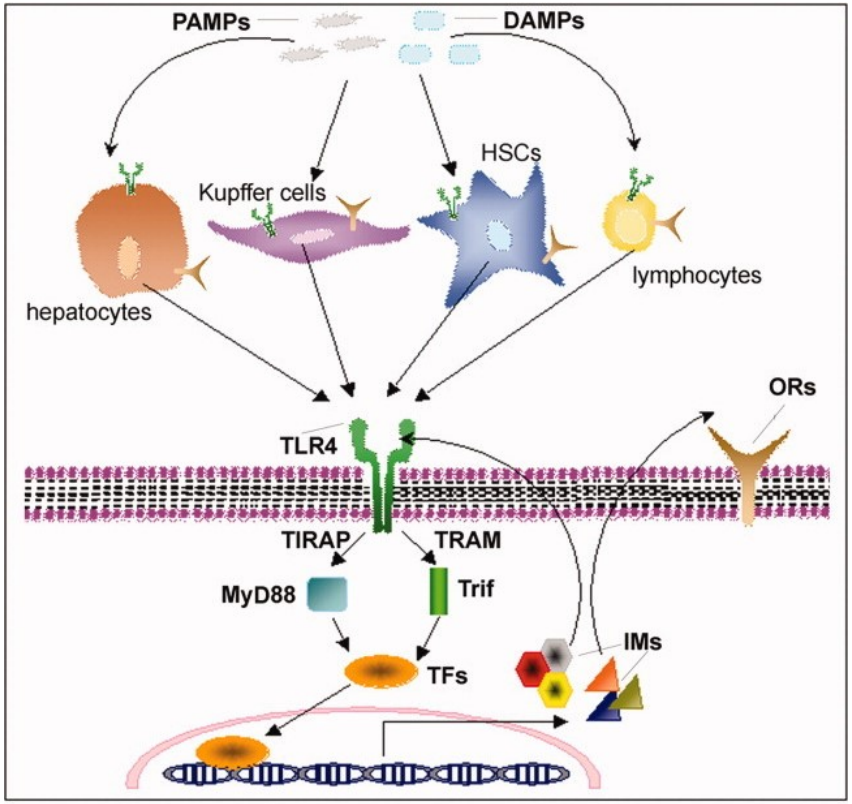


CD14 initiates a cascade of trafficking events. This cascade begins with CD14 transporting LPS to TLR4, and culminates with CD14 delivering TLR4 to the endosomal signaling machinery. The endocytosis of the TLR4-MD2 complex requires CD14.

Cell. 2011 Nov 11;147(4):868-80.

Bio Protoc. 2018: 08 (14); e2928.

TLR Signaling in NASH

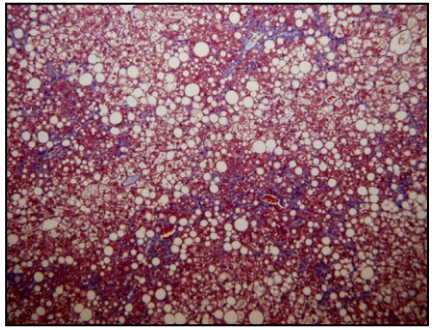


HEPATOLOGY AASLD

Editorial

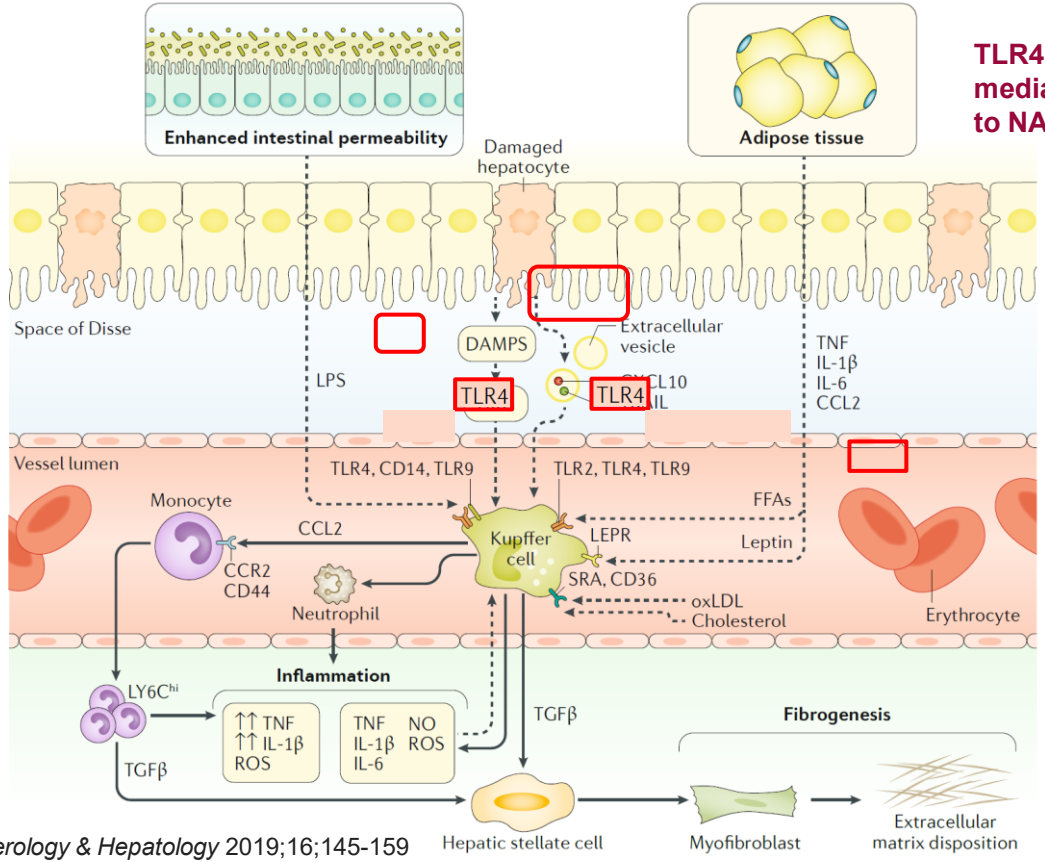
Pathogen- or damage-associated molecular patterns during nonalcoholic fatty liver disease development[†‡]

Anna Alisi Ph.D., Rita Carsetti M.D., Valerio Nobili M.D.



PAMP/DAMP and TLR4 signaling in liver-resident cells during NAFLD development

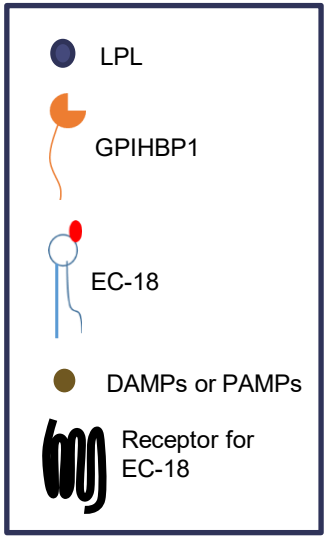
TLRs in the Pathogenesis of NASH

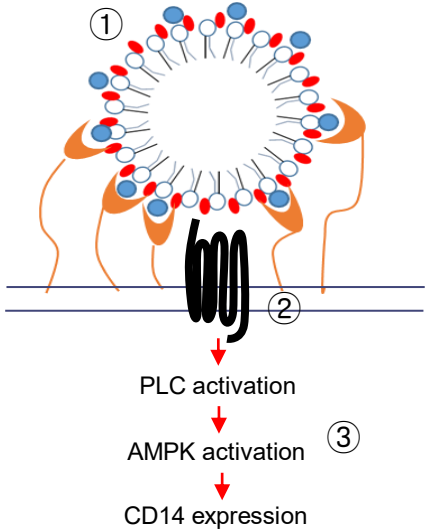


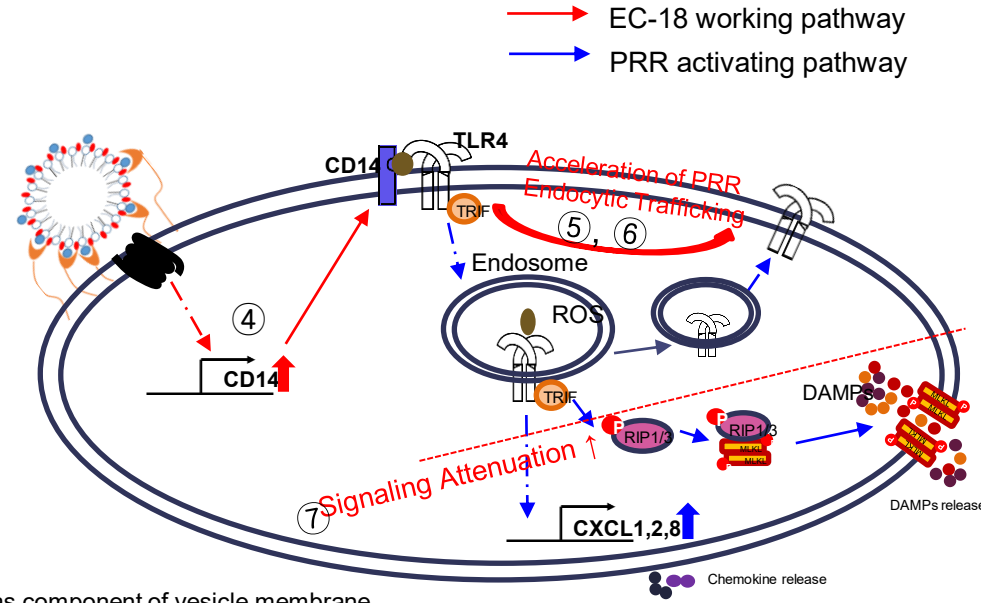
TLR4 in NASH is an important mediator of disease and essential to NASH pathogenesis

Nature Reviews Gastroenterology & Hepatology 2019;16;145-159

Therapeutic Target: Acceleration of PRR Endocytic Trafficking

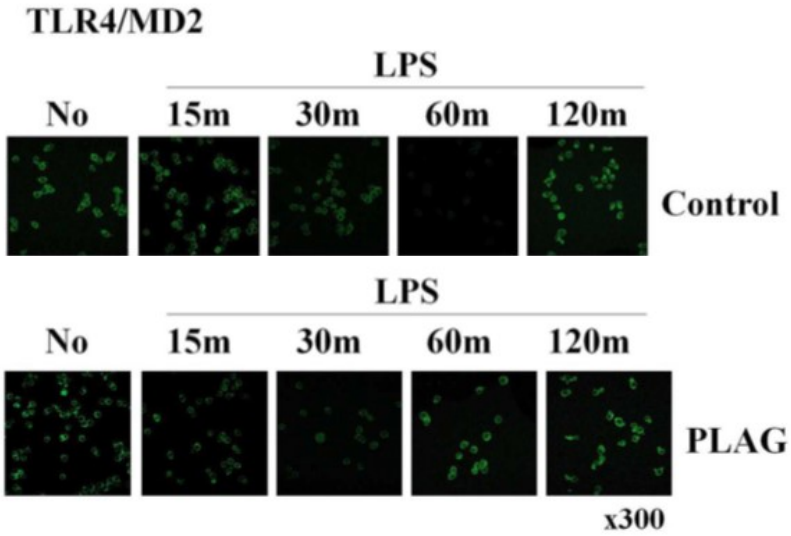


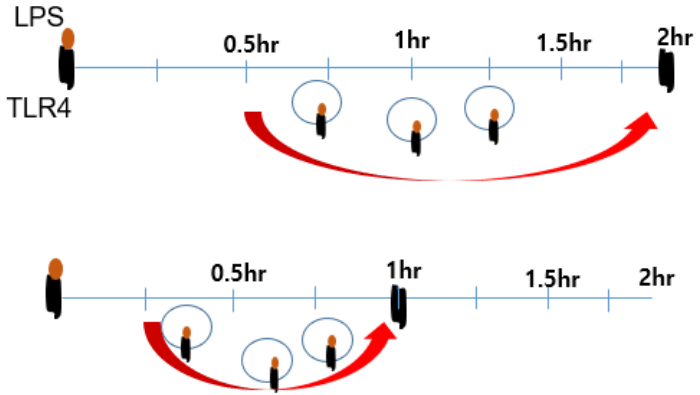




1. EC-18 forms vesicles
2. EC-18 is recognized by GPCRs as component of vesicle membrane
3. EC-18 stimulates GPCRs
4. Activated GPCR expresses CD14
5. CD14 accelerates TLR4 internalization and membrane return
6. Endosome dependent signaling is shortened by EC-18
7. Chemokine expression is down regulated

Early Internalization & Recycling Back to the Plasma Membrane of TLR4 by PLAG



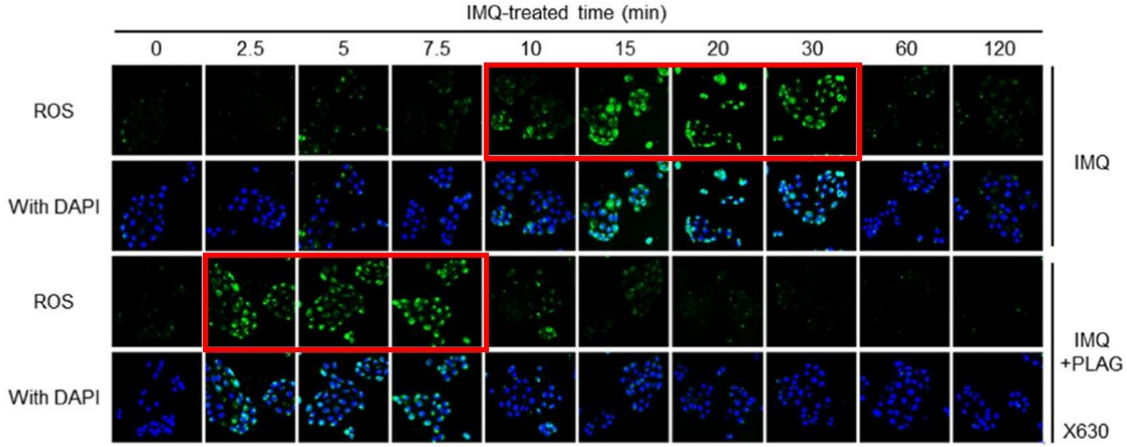


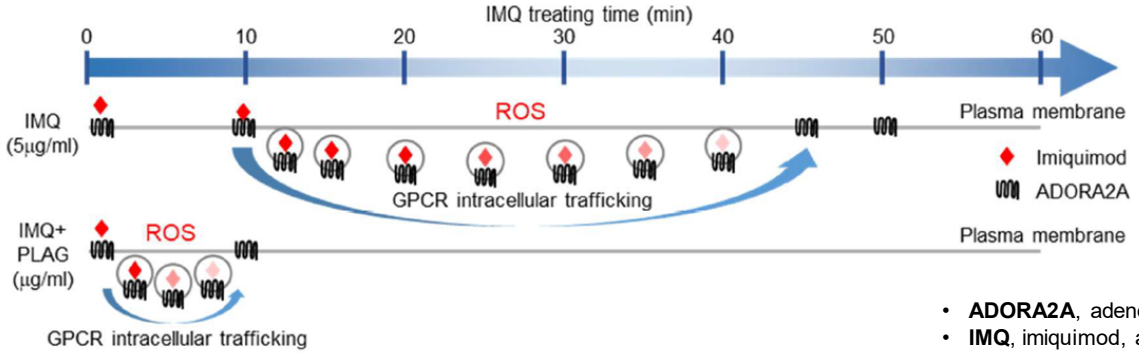
PLAG induces an earlier internalization of TLR4 in murine macrophage cells treated with LPS.
PLAG also induces a more rapid return of the TLR4 to the plasma membrane than the control group

Raw 264.7 cells: murine macrophage

Frontiers in Immunology 2019;10..article 2177

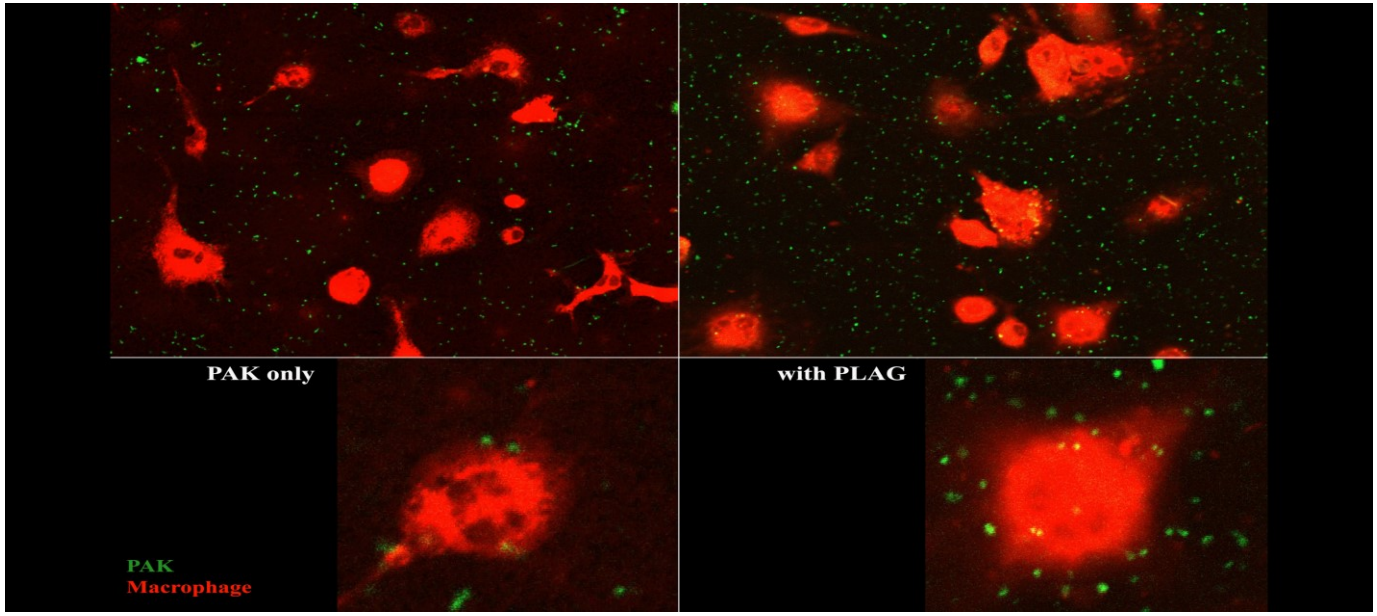
EC-18 Accelerates & Shortens the Process Time Interval of ROS Production





- **ADORA2A**, adenosine A_{2A} receptor
- **IMQ**, imiquimod, a nucleotide analog
- **PLAG = EC-18**

EC-18 (PLAG) Enhances Phagocytosis of Pseudomonas Aeruginosa by Macrophage



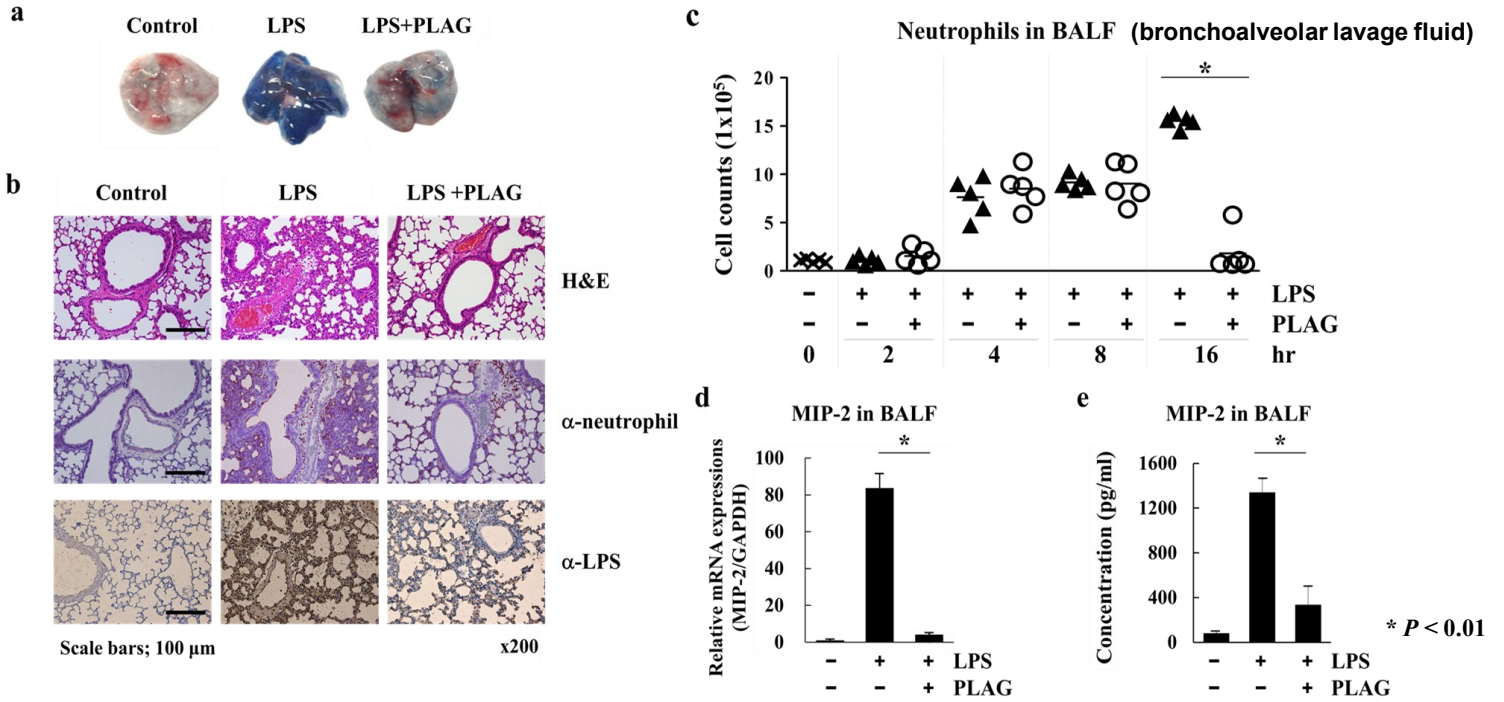
Pseudomonas aeruginosa **is an LPS-expressing Gram (-) bacteria, triggering TLR-4 induced phagocytic & inflammatory responses.**

PAK: *Pseudomonas aeruginosa* strain k THP-1 (human macrophage)

PLAG Resolves LPS-induced Acute Lung Injury (ALI) through Regulation of Neutrophil Infiltration

doi.org/10.3389/fimmu.2019.02177

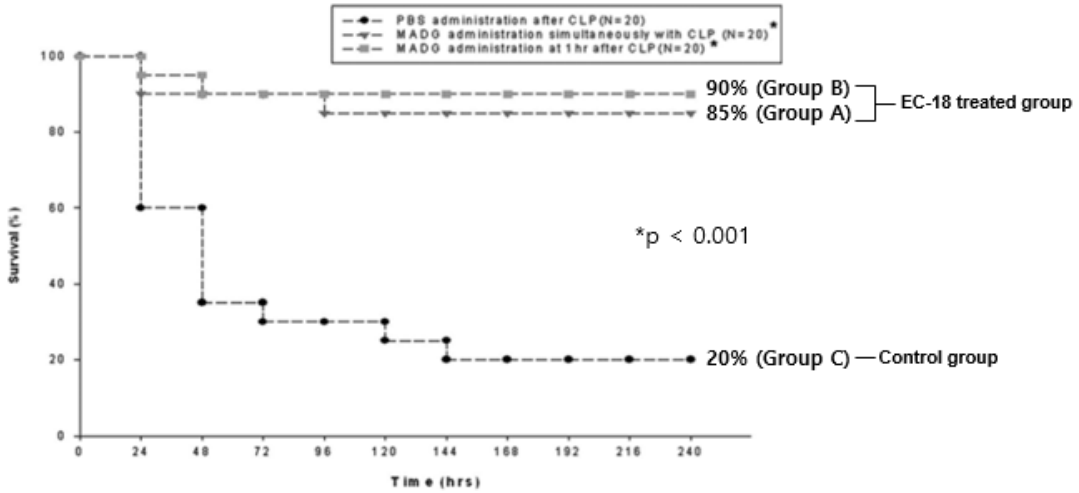
- **LPS:** 25 mg/kg intranasal administration
- **EC-18**: 250 mg/kg oral administration



PLAG/LPS co-treated animals exhibited the earlier return to baseline levels in the neutrophil number of BALF after 16 hr. The secreted level or mRNA of MIP-2 was also significantly increased in BALF following LPS administration, and markedly decreased in PLAG co-treated mice.

Oral Administration of EC-18 Improves Survival in a Murine Model of Abdominal Sepsis

Efficacy of EC-18 in CLP (cecal ligation & puncture)-induced sepsis mouse model



EC-18 improves survival in murine sepsis model via down-regulation of pro-inflammatory cytokines production contributing to earlier return to immune homeostasis

J Trauma 2010; 68: 62-68

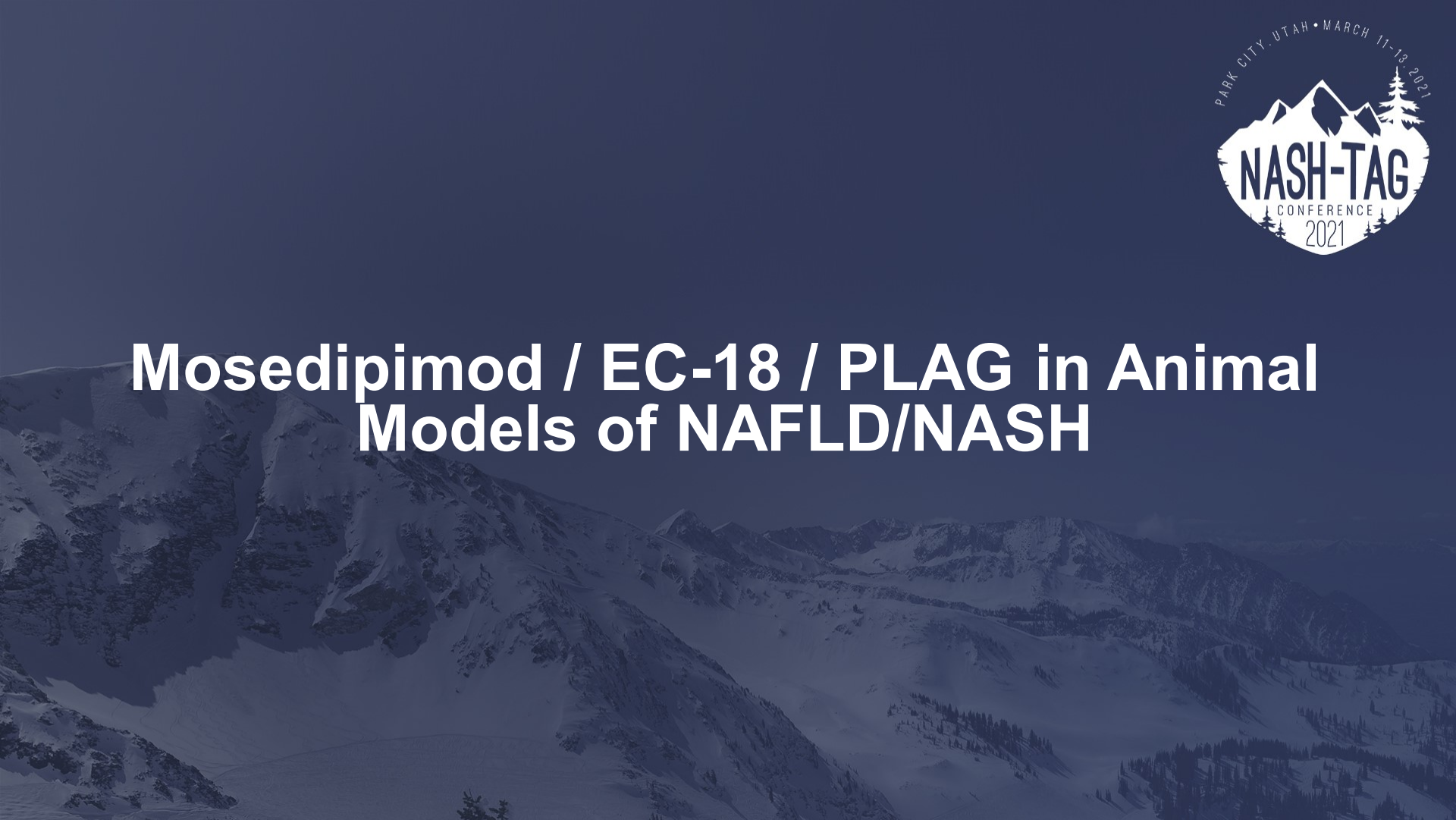
MOA of EC-18

1. ***Molecular Target***: a GPCR (G protein-Coupled Receptor)

2. ***Proof of Concept***: Rapid removal of DAMPs/PAMPs contributing to faster resolution of inflammation

3. ***Primary Action Mechanism***: Acceleration of intracellular trafficking by TLR4 (internalization, movement to endosome and recycling back to the cell surface) of PRR

4. ***Secondary Action Mechanism***: Acceleration of resolution through stopping further neutrophil recruitment resulting in termination of necroptosis signaling and enhancing the efferocytosis of macrophage



Mosedipimod / EC-18 / PLAG in Animal Models of NAFLD/NASH

In vivo Study

1. STAM™ Mouse Model

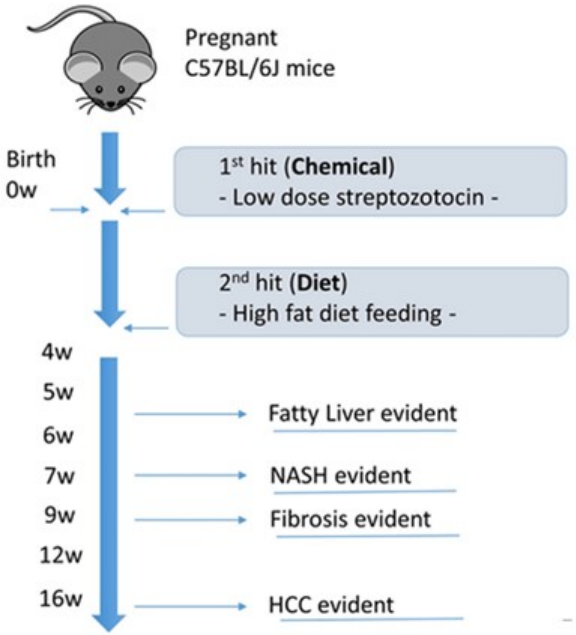
- Steatosis/Steatohepatitis/Fibrosis
- Low-dose STZ (Streptozotocin)/HFD (high-fat diet)-induced NASH & insulin resistance mouse model
- Non-genetic C57BL/6 mice

2. High-Fat High-Fructose (HFHF) Diet-fed Mouse Model

- Steatosis/Steatohepatitis
- Obesity and Type 2 diabetes-induced NASH
- ICR (Institute of Cancer Research) mice

*STAM™ and HFHF-fed mouse models may replicate key features of NASH in humans.

NASH Mice Model (STAM™, SMC Lab, Tokyo)



- Diabetes mellitus was achieved by the i.p. administration of low-dose (200 μg) streptozotocin (STZ) shortly after birth, which results in a chemical inflammation of the pancreatic islets.

- Following neonatal STZ administration, a 60% high-fat-diet feeding starts from 4 weeks to 9 weeks of age. **Daily oral EC-18 was started at 6 weeks** and continued **until 9 weeks** till the mice were sacrificed.

Test Compounds

EC-18 (PLAG) groups (n=8 per group)

- 30, 100, and 250* mg/kg per day (three doses)

OCA** (obeticholic acid) group (n=8)

- 30 mg/kg per day
- Farnesoid X Receptor (FXR) agonist

MGL-3196** group (n=8)

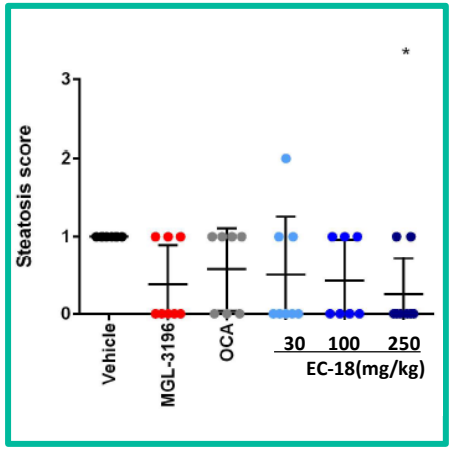
- 3 mg/kg per day
- Thyroid hormone receptor (THR) β-selective agonist

*: Considering the body surface area, the human [80 kg] equivalent dose is 1,600 mg per day.
EC-18 dose used in phase 2 clinical trial of chemoradiation-induced oral mucositis currently ongoing in the US is 2,000 mg per day.

**: Drug candidate for NASH in phase 3 stage of the clinical trial

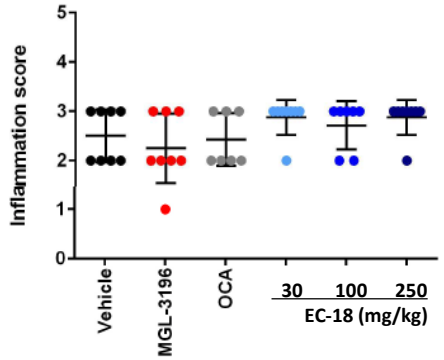
Individual Scores of NAS

A Steatosis score

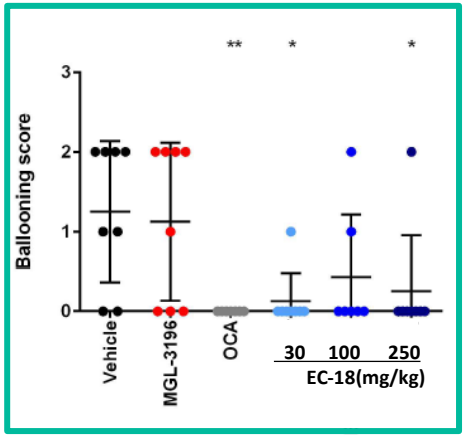


*; p<0.05 (vs Vehicle)

B Inflammation score



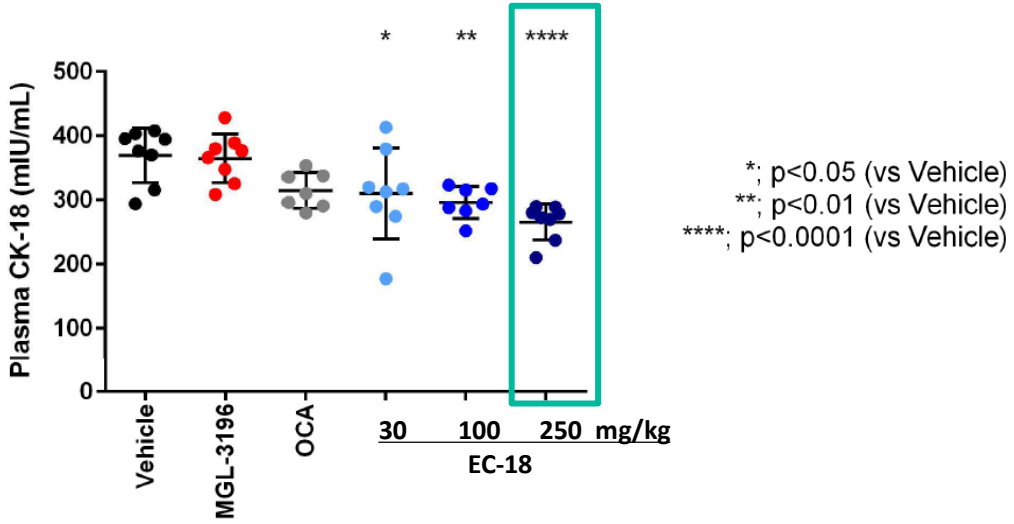
C Ballooning score



*; p<0.05 (vs Vehicle)
**; p<0.01 (vs Vehicle)

EC-18 treatment improved steatosis & hepatic ballooning leading to overall reduction in NAS score.

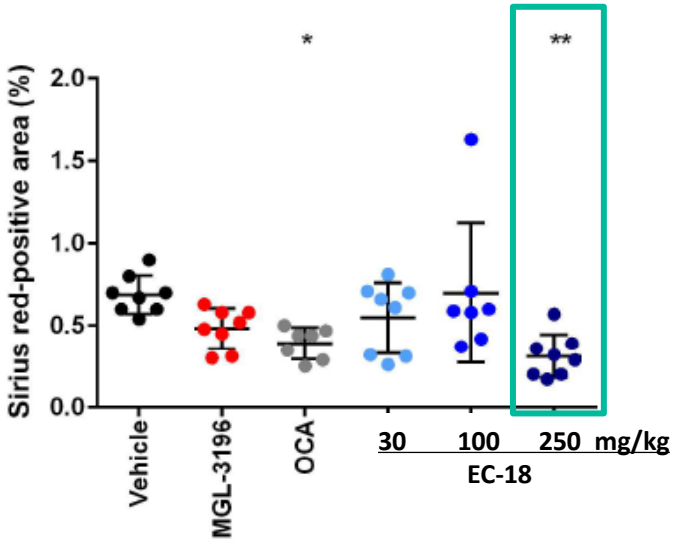
Comparison of CK-18 Fragments in Plasma



CK-18: Plasma caspase-generated cytokeratin-18 fragments

Plasma CK-18 level has a high specificity for NASH & fibrosis and is used as a noninvasive biomarker for monitoring disease progression and response to therapy. CK-18 levels are a marker of hepatocyte apoptosis. Only EC-18 significantly reduced plasma CK-18 compared to vehicle, in a dose-dependent manner.

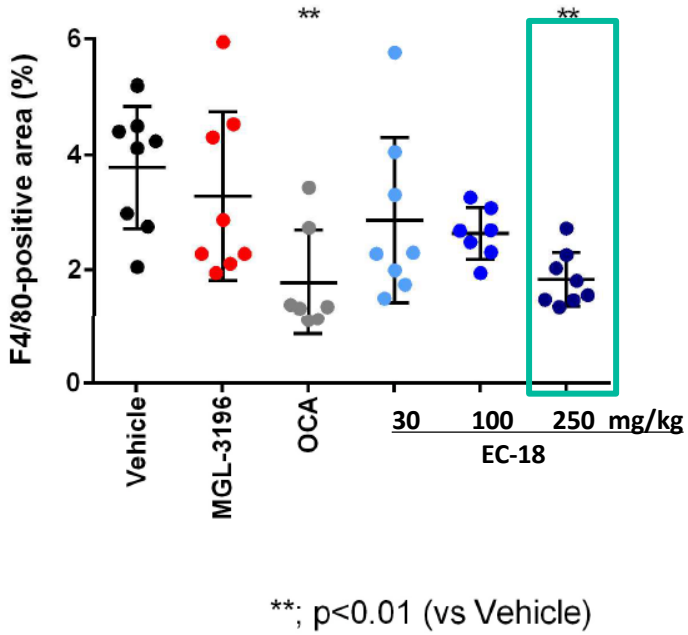
Hepatic Fibrosis



*; p<0.05 (vs Vehicle)
**; p<0.01 (vs Vehicle)

EC-18 significantly decreased CSA

Hepatic Macrophage Infiltration Assessed by F4/80-positive Area



F4/80 (macrophage marker): F4/80 is used to identify hepatic macrophages in liver sections.

STAM™ Study Results

In Vivo Efficacy Study of EC-18 in STAM Model of Non-alcoholic Steatohepatitis

STAM™ model, Tokyo, Japan

	Vehicle (n=8)	MGL-3196 (3mpk) (n=8)	OCA (30mpk) (n=7)	EC-18 (250mpk) (n=8)
NAS score	4.8 ± 0.7	3.8 ± 1.4	3.0 ± 0.8**	**3.4 ± 1.2***
Plasma CK-18$	369.5 ± 42.3	365.0 ± 37.8	314.8 ± 27.9	**265.8 ± 27.9****
Inflammation area F4/80	3.8 ± 1.1	3.3 ± 1.5**	1.8 ± 0.9	**1.8 ± 0.5****
Fibrosis area (Sirius red staining, %)	0.69 ± 0.12	0.48 ± 0.12	0.39 ± 0.10*	**0.31 ± 0.13****

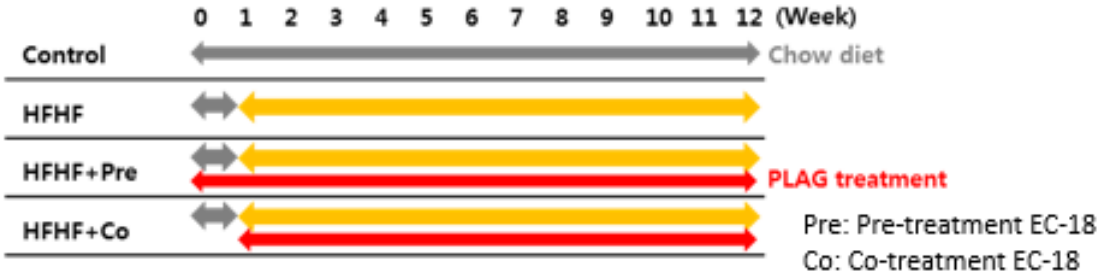
C57BL/6, male mice

$ Normal range: 220~260 mIU/mL

*; $p < 0.05$ (vs Vehicle), **; $p < 0.01$ (vs Vehicle), ****; $p < 0.0001$ (vs Vehicle)

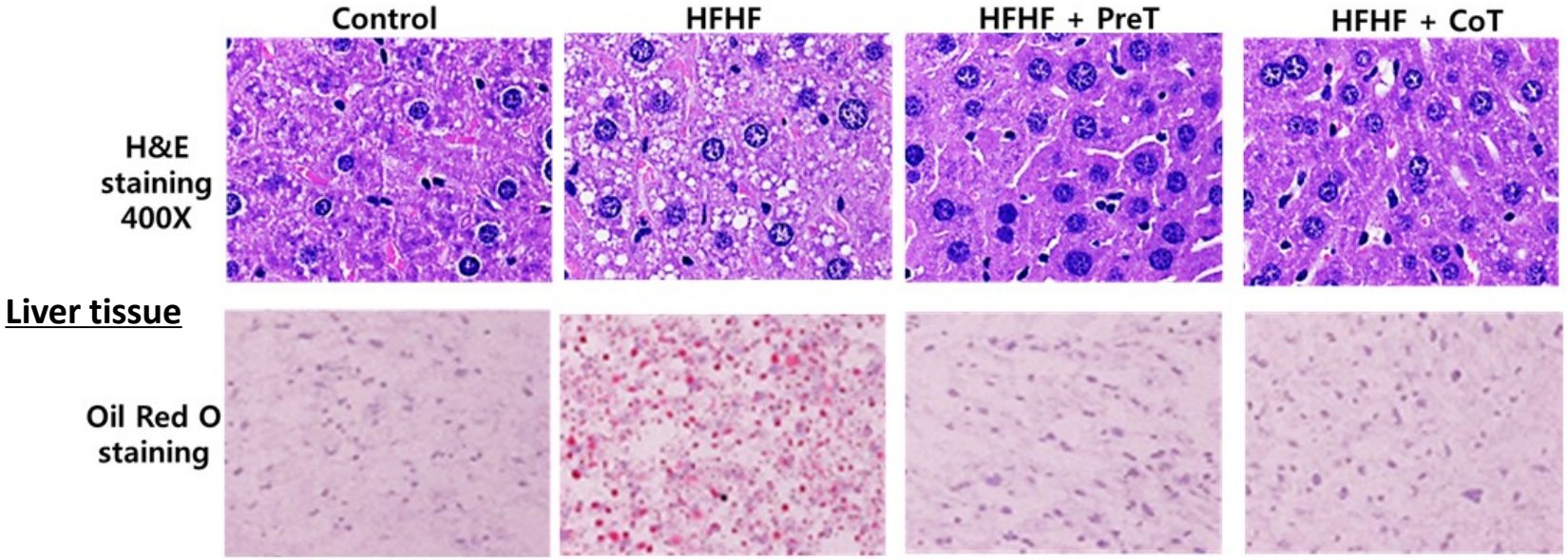
High-Fat High-Fructose (HFHF) Diet-fed Mice Model

- ICR mouse (male) aged 8 weeks
- High Fructose, High fat diet
- Treatment: EC-18 100 mg/kg/d P.O. pre & co
- Period: 12 weeks



HFHF diet: Fat 45%, carbohydrate 35%, protein 20%
Chow diet: 7% simple sugars, 3% Fat, 50% polysaccharide, 15% protein

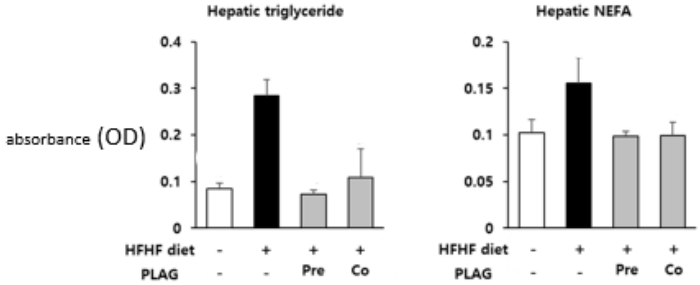
EC-18 Ameliorates Hepatic Steatosis



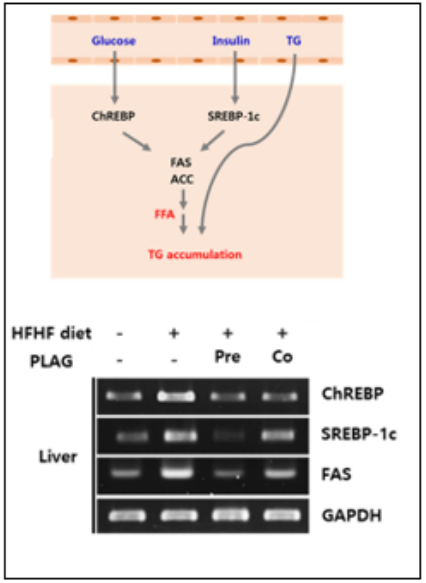
Lipid droplets (red dots) visualized by *Oil Red O staining*
Oil Red O staining determines intuitive *hepatic* steatotic phenotypes

Effects of EC-18 on *De Novo* Lipogenesis in Liver

NEFA (FFAs) have been considered to be responsible for lipotoxicity in NASH. NEFA are elevated in liver of mice in HFHF model of NASH. **EC-18 reduces hepatic NEFA and TG**.

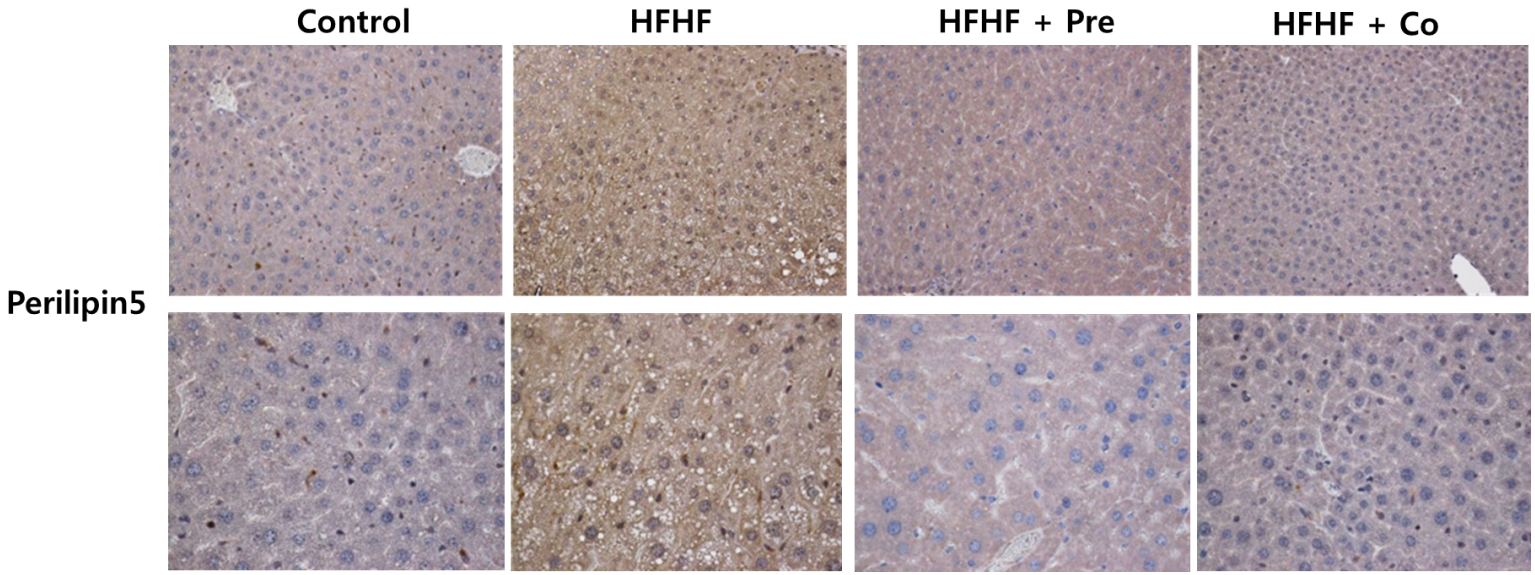


NEFA: Non-esterified fatty acid (FFA)
ChREBP: Carbohydrate-responsive element-binding protein
SREBP-1c: Sterol regulatory element-binding protein 1c
FAS: Fatty acid synthase
ACC: Acetyl-CoA carboxylase



SREBP-1c & ChREBP are transcriptional factors involved in de novo lipogenesis (DNL) in the liver. Long-chain saturated fatty acids (e.g. palmitic acid), which are the products of DNL, are cytotoxic to hepatocytes.

EC-18 Prevents Steatosis via Downregulating Perilipin 5

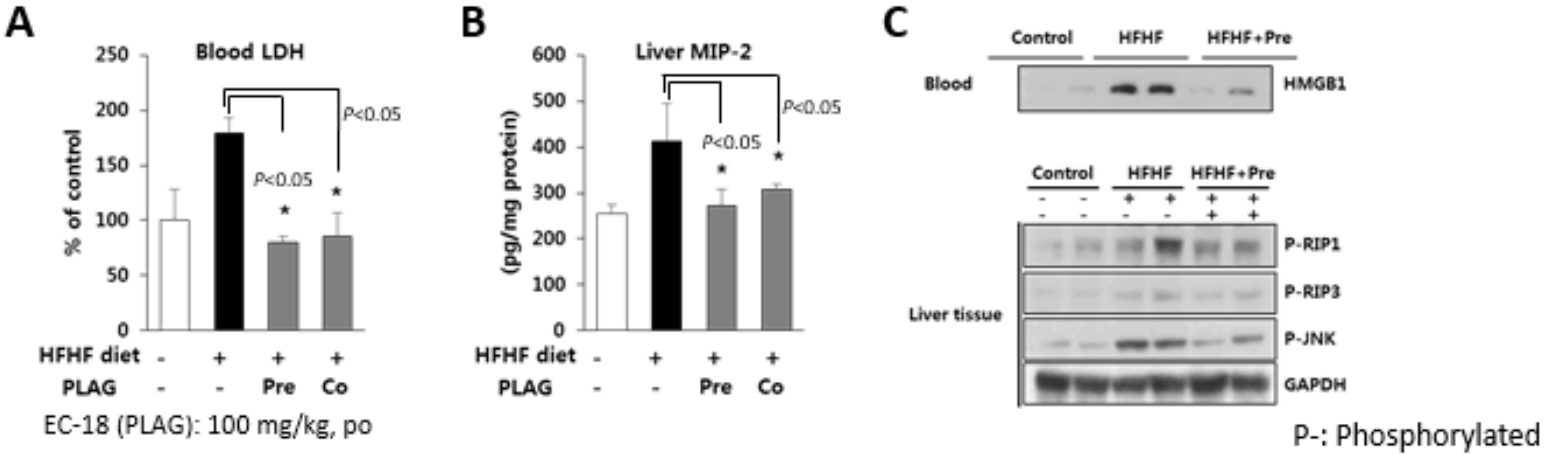


The IHC staining of perlipin5 is correlated with lipid accumulation in mouse liver

Perilipin5: Lipid-droplet binding protein which promotes hepatic steatosis

EC-18 Attenuates Necroptosis Signals & CXCL2 (MIP-2) Chemokine

Necroptotic cell death characterized by breakdown of cell membrane causes the release of intracellular contents, such as HMGB1 & LDH into extracellular space.

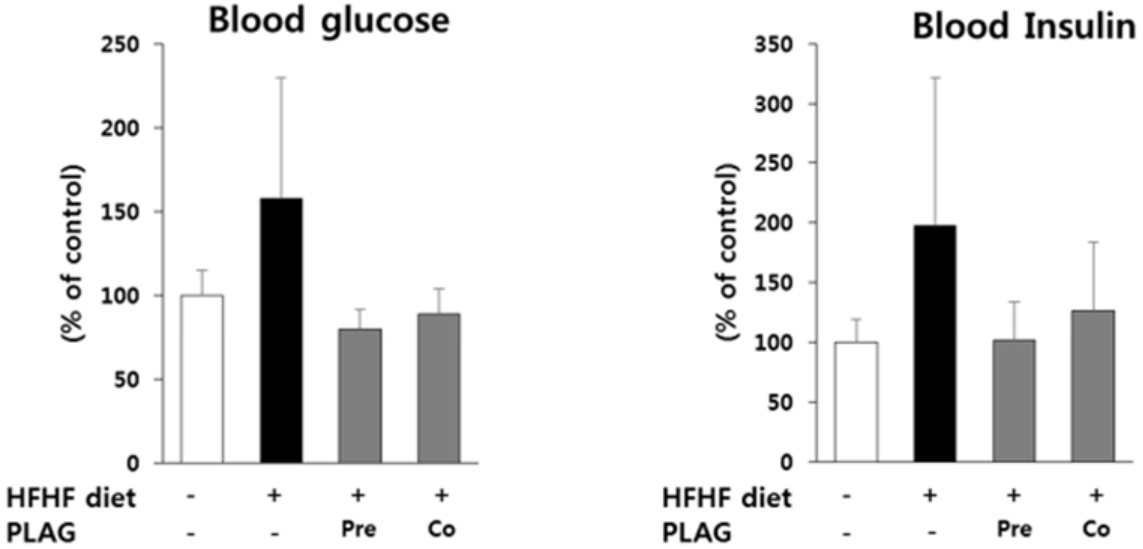


EC-18 reduces inflammatory signals through modulation of necroptosis

- RIPK: Receptor interacting protein kinase
- LDH: Lactate dehydrogenase : Measuring LDH release is a useful method for detecting necroptosis because the permeabilization of plasma membrane is a key signature of necrotic cell
- MIP-2: Macrophage inflammation protein 2
- JNK: c-Jun N-terminal kinase,
- HMGB1: High Mobility Group Box 1

EC-18 Improves Insulin Sensitivity

EC-18 (PLAG): 100 mg/kg, po



In the HFHF diet-fed mice model, EC-18 also lowered insulin levels as well as lowering blood glucose levels, suggesting that EC-18 reduced insulin resistance in insulin target tissues.

Conclusion

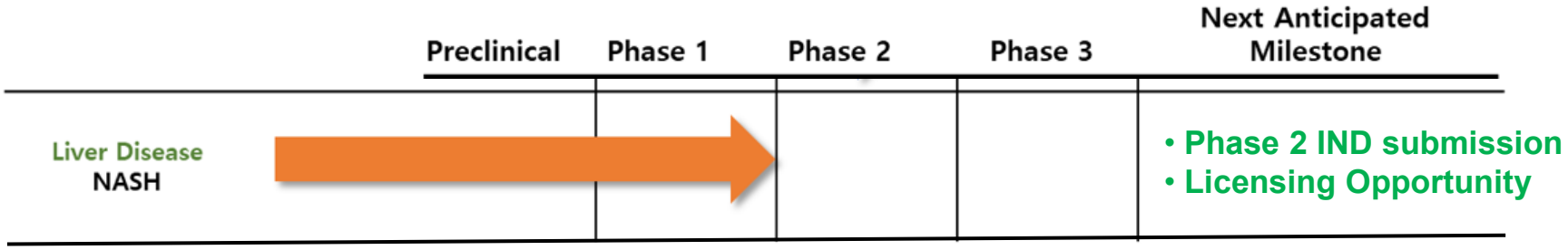
- **EC-18 may improve NASH and hepatic fibrosis via accelerating intracellular trafficking of TLR4 expressed in liver, adipose tissue, muscle, intestinal epithelial cells and circulating immune cells.**

- **This accelerated TLR4 endocytic trafficking contributes to swift removal of DAMPs/PAMPs leading to early termination of necroptosis signaling and avoiding recruitment of neutrophils for extended periods of time, resulting in faster resolution of inflammation without severe host damage and earlier return to homeostasis.**

- **IP protection, *in vitro* and *in vivo* data of EC-18 are very strong along with excellent safety and tolerability safety profile from Phase 1 clinical trials.**

- **EC-18 could be a promising therapeutic to resolve NASH, prevent progression to liver fibrosis, and reverse liver fibrosis as well.**

NASH Pipeline Development



2020 1Q