



Histopathology and imaging analysis

-SMC's CRO services in pharmacological R&D-

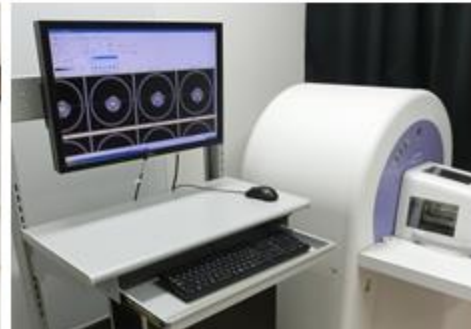
CONTENTS:

- *Company Profile (P2-)*
- *NASH Services (P4-)*
- *Basic Services (P12-)*

SMC Laboratories, Inc.

smccro-lab.com

- Founded in October 2006
- A privately-held non-clinical CRO based in Tokyo, Japan;
specialized in research on ***fibrosis*** and ***inflammation***
- CRO services
 - **Non-clinical pharmacology**
 - One of the leading CRO in liver research with Proprietary NASH-HCC (STAM™) Model
 - *In vivo* disease models for metabolic disorders, inflammation, fibrosis and tumor
 - **Histological imaging services**
 - Histological scoring: NAFLD activity score, fibrosis and inflammation scores etc.





■ SMC's CRO services

Non-clinical pharmacology study

- **High-performance** pharmacology services based on the company's specialty in fibrosis research
- **Various lineup** of disease models relating to fibrosis, inflammation, metabolic disorders and cancer
- **Strategic** study design proposed by experienced physicians and scientists

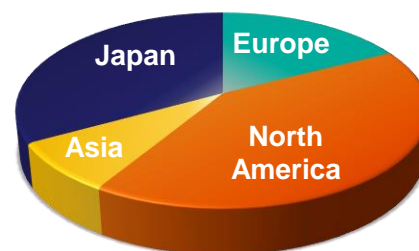
Histological imaging

- **In-house, high quality** analyses; proven skills in Histology
- **Elaborate and comprehensive** reports by Ph.D. holders
- **Professional** support for data publication/IND application

Performance

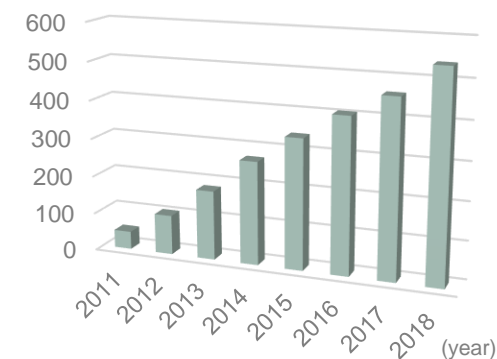
- **Over 600** clients worldwide
- **Over 110** peer-reviewed papers and presentations
- **15** successful CTA packages

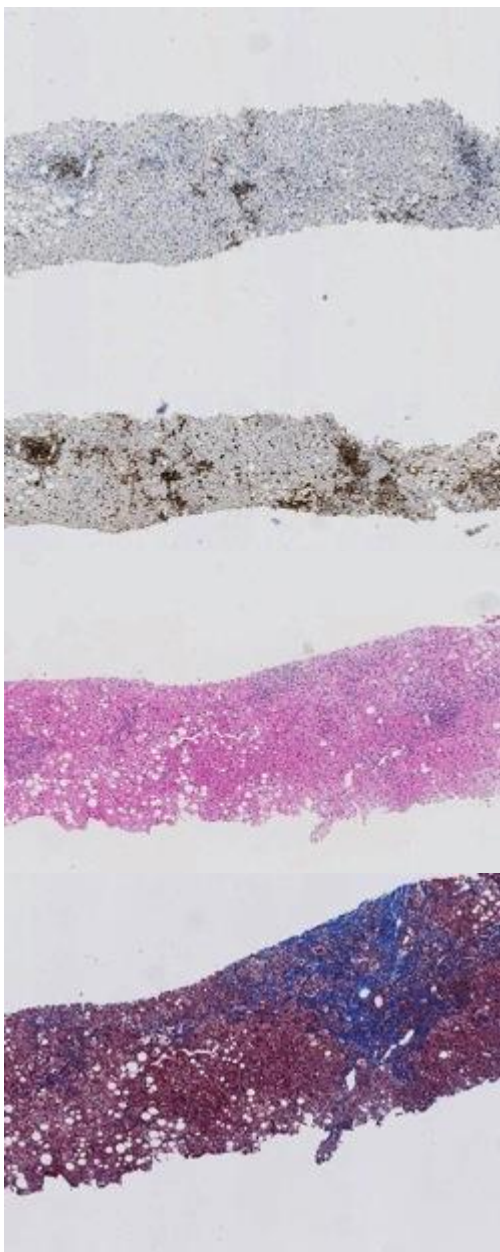
Region



(% of the customers)

Number of clients





■ Histopathological parameters are important endpoints in nonclinical as well as clinical studies in NASH and liver fibrosis.



SMC's outstanding performance in NASH/liver fibrosis research:

■ As a leading CRO in NASH, SMC has accumulated know-how by assessing over **600** pharmacology studies and **60,000** slides of NASH and related diseases in **mouse, rat and human**.

- judgment of NAS (especially, ballooning), assessment of pathological changes from disease control, discussion of clinical relevance... *and more!*

NAS; NAFLD Activity score



For...

■ Assessment the grade of disease (NASH, fibrosis) in not only nonclinical pharmacology studies but also clinical studies.

■ Development of animal models (including Tg and KO mice) for drug evaluation in the clients' own laboratory.

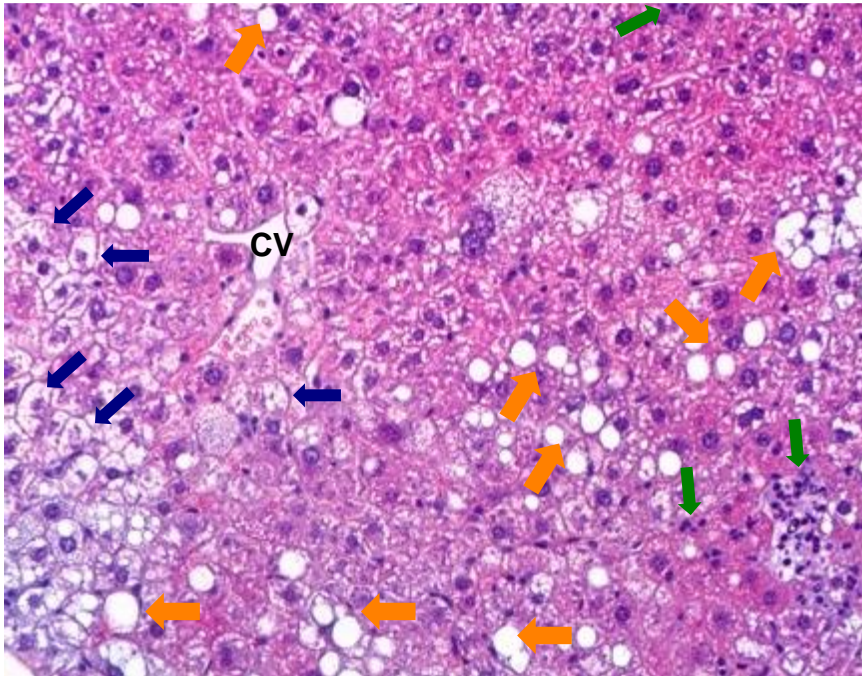
■ Achievement of high quality report/data package with objective evaluation.

NAS: “ballooning” or “pseudo-ballooning”?

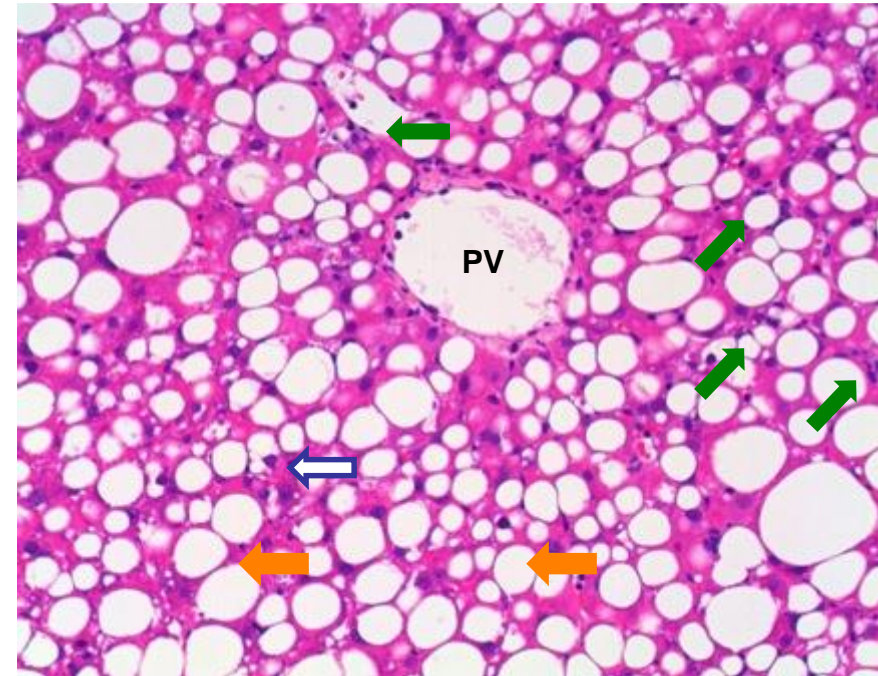


NAS (including ballooning, inflammation, steatosis) is an important parameter to evaluate the drug efficacy in NASH in both human and animal models.





In order to translate nonclinical results into clinical practice, it is essential to discriminate “true”- from “pseudo”-ballooning in disease models.



STAM™ NASH model mice



MCD mice

-  : steatosis (fat droplet)
-  : inflammation
-  : ballooning hepatocyte
-  : “pseudo”-ballooning hepatocyte

PV; Portal vein, CV; Central vein

An accurate scoring can evaluate the efficacy of the test substances on each components (steatosis, lobular inflammation, hepatocyte ballooning) of NAS.

Group	Steatosis				Lobular Inflammation				Hepatocyte Ballooning			NAS Mean (SD)	
	0	1	2	3	0	1	2	3	0	1	2		
	n												
V (early)	6	-	5	1	-	-	2	2	2	-	-	6	5.2 (1.0)
GM (early)	5	3	2	-	-	-	1	3	1	-	-	5	4.4 (1.1)
GR (early)	6	4	2	-	-	-	5	1	-	-	3	3	3.0 (0.9)
V (late)	4	1	2	1	-	-	2	2	1	-	-	5	5.3 (1.3)
GM (late)	5	1	2	2	-	-	2	2	1	-	-	5	5.0 (1.4)
GR (late)	6	3	3	-	-	-	3	2	1	-	3	3	3.7 (1.4)

Tabulation of scoring of steatosis, lobular inflammation, hepatocyte ballooning, and NAFLD activity score in liver sections performed as described in Methods on liver sections obtained from animal livers from the experiment described in Figure 1.

doi:10.1371/journal.pone.0083481.t001

Traber PG et al., PLoS One. 2013;8:e83481

Definition of NAS components

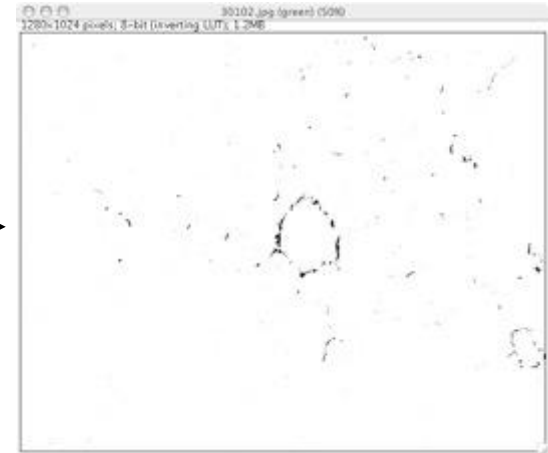
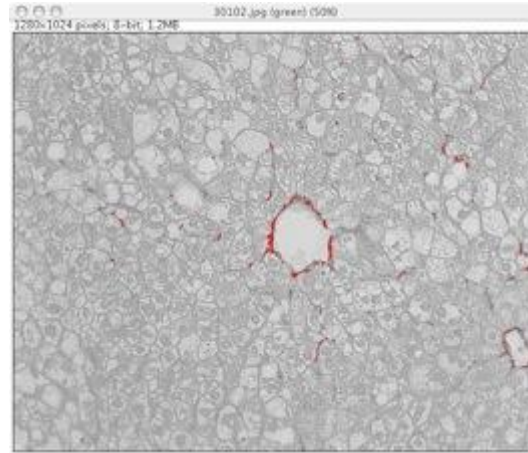
Item	score	Extent
Steatosis	0	<5%
	1	5-33%
	2	>33-66%
	3	>66%
Hepatocyte Ballooning	0	None
	1	Few balloon cells
	2	Many cells/prominent ballooning
Lobular Inflammation	0	No foci
	1	<2 foci/200x
	2	2-4 foci/200x
	3	>4 foci/200x

Original image

Threshold setting
after extraction

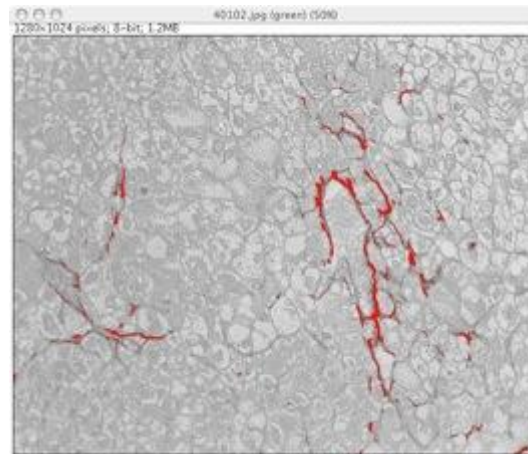
Image denoising

Steatosis



Fibrosis area: 0.3%

Steatohepatitis



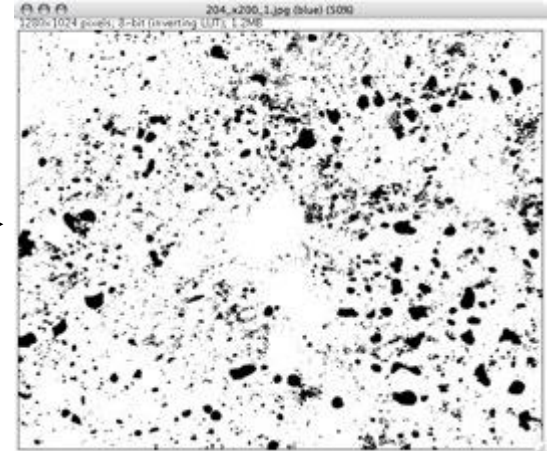
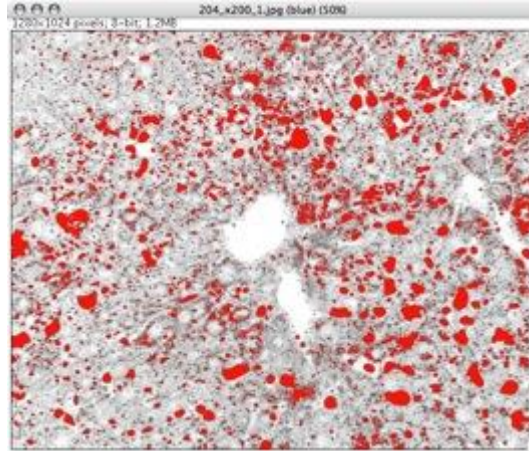
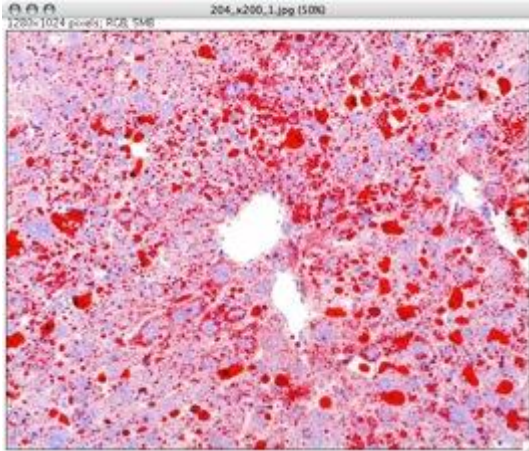
Fibrosis area: 1.0%

Original image

Threshold setting
after extraction

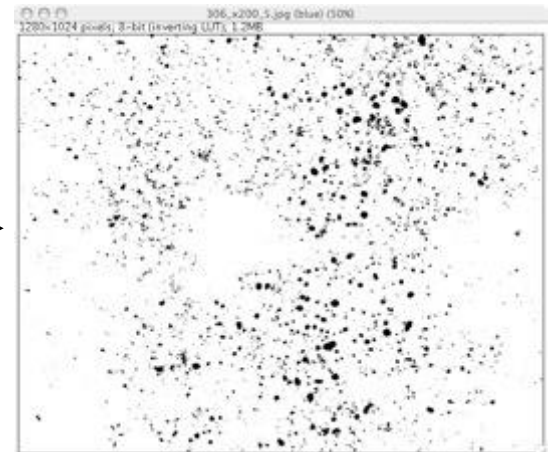
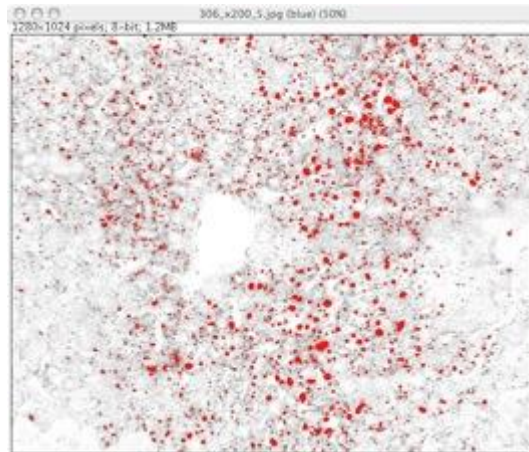
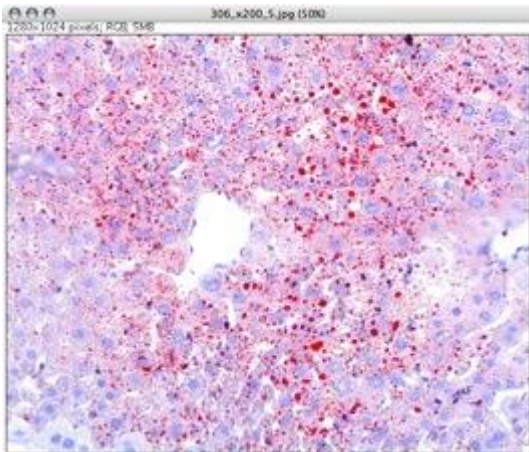
Image denoising

NASH-Vehicle



Fat deposition area:12.9%

NASH-Treatment



Fat deposition area:4.9%

The grade of inflammation (Method)

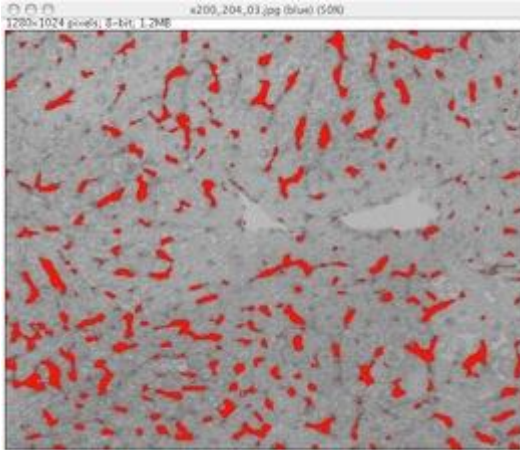
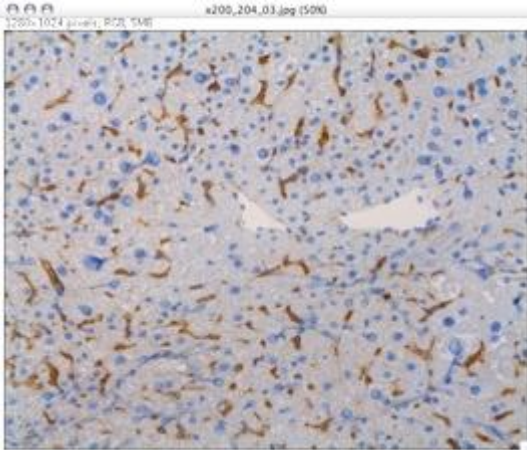


Original image

Threshold setting
after extraction

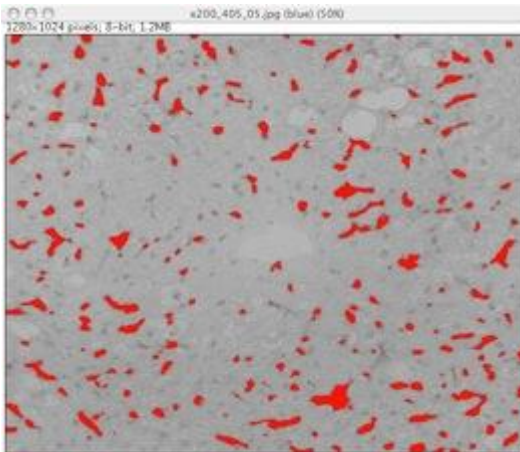
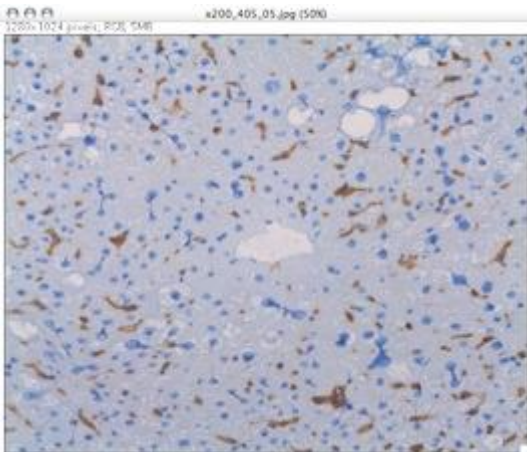
Image denoising

NASH-Vehicle



Inflammation area: 8.0%

NASH-Treatment



Inflammation area: 5.6%

Double/ Multiple staining:

❑ M1(F4/80 + CD16/32) / M2(F4/80 + CD206) ratio.

❑ Characterization of cytokine-producing cells.

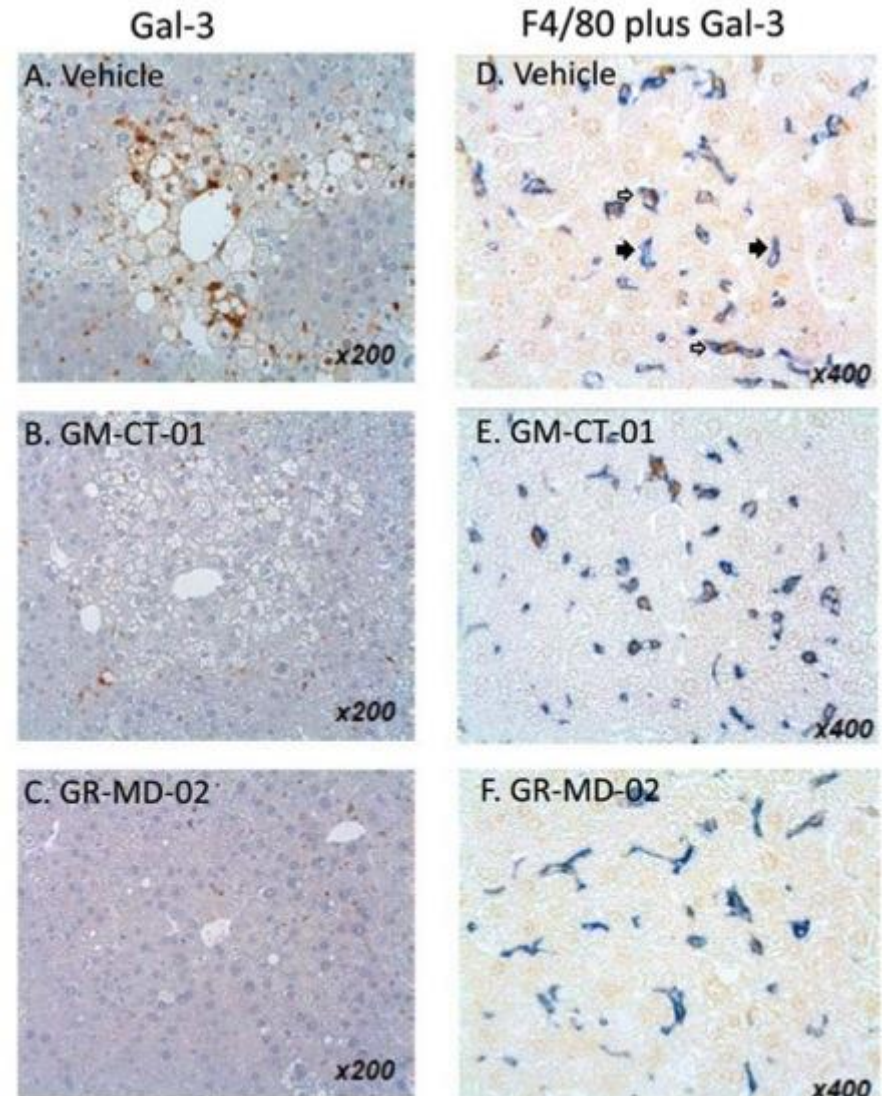
❑ Identification of target molecule-expressing cells (*see the right picture*).

❑ Investigation of proliferative cell types (insulin + BrdU, CD markers + BrdU or Ki-67).

❑ Cell-cell interaction (inflammatory cells + fibroblast, inflammatory cells + parenchymal cells).

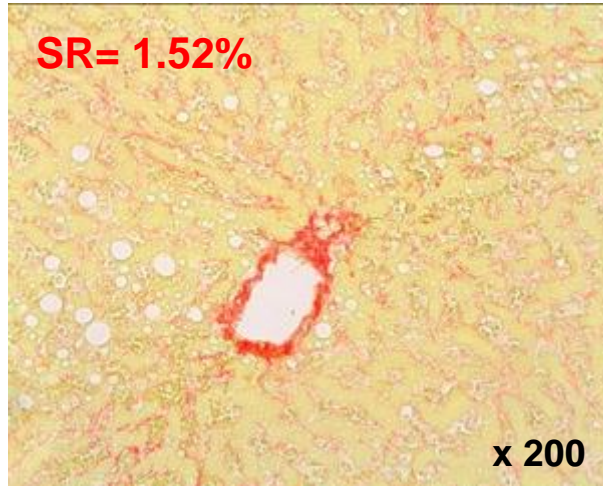
❑ Optimization of staining method with your developed antibodies and existing antibodies.

- Please ask us!



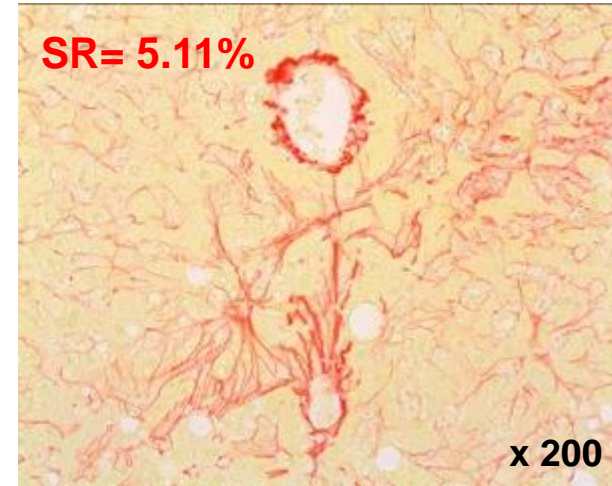
*Both human and animal samples can be evaluated by the same and standard methods.
Useful for discussion of clinical relevance.*

Steatosis

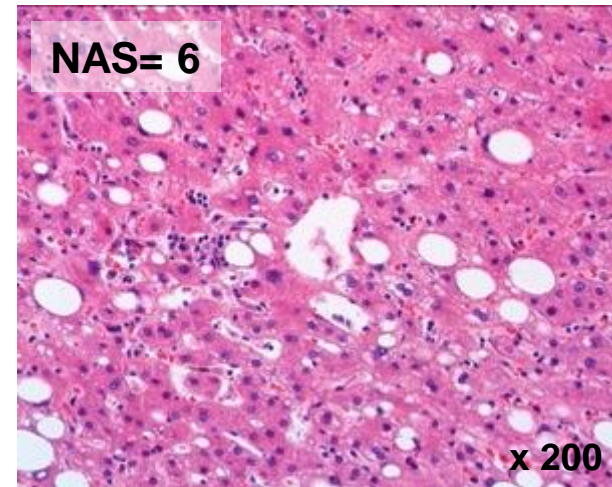
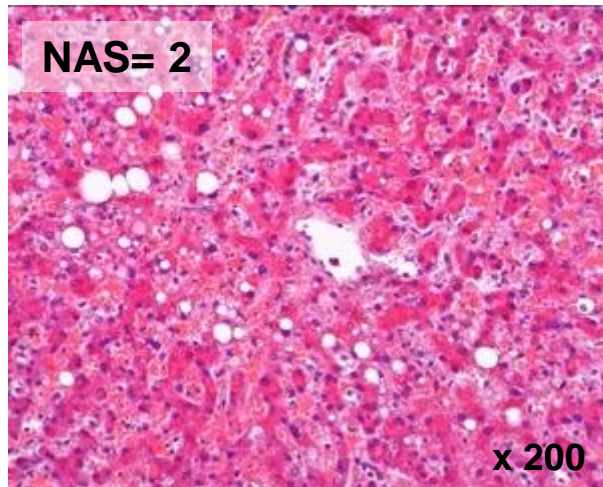


SR positive area

Steatohepatitis



NAFLD
Activity Score



Analysis of Collagen proportionate are (CPA) is also available!

Processing & Embedding :

- Embedding of human and animal tissues in paraffin (FFPE blocks) from fixed tissues.
- Embedding of human and animal tissues in OCT (frozen OCT embedded tissues) from fixed tissues.
- Instruction of fresh frozen block preparation for frozen sections.

Sectioning:

- Cutting of sections (4-8 μm thick) from paraffin and O.C.T. embedded blocks.
- Preparation of serial thin sections.

Routine staining:

- HE, Sirius red, Masson trichrome, PAS, Oil red, etc.

Immunohistochemistry (IHC): Inflammation-related molecules, fibrosis-related molecules etc.

- Functional Immunohistology (Double/multiple staining).
- New antibody staining protocol optimization & validation.

Imaging analysis:

- Area, length, diameter, stained cell count, percentage positive area, shape etc.
- Proliferation, Apoptosis, pathological grading etc.

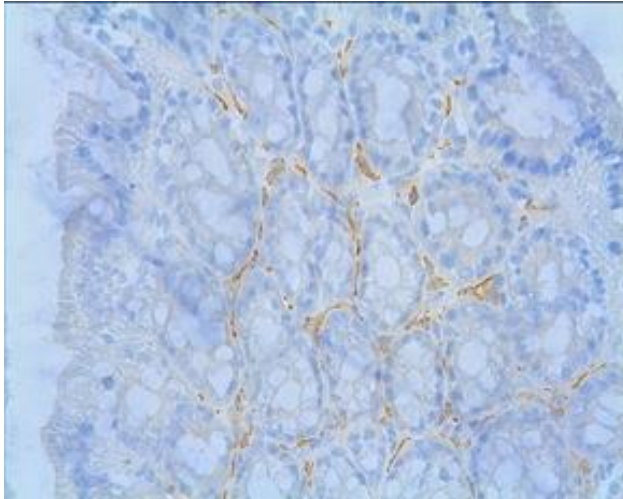
Interpretation of results:

- Discussion of the histological findings in view of pharmacology.

Reporting:

Providing optimal disease/tissue-specific analytical methods to evaluate the grade of fibrosis in accordance with the types of fibrotic/inflammatory diseases.

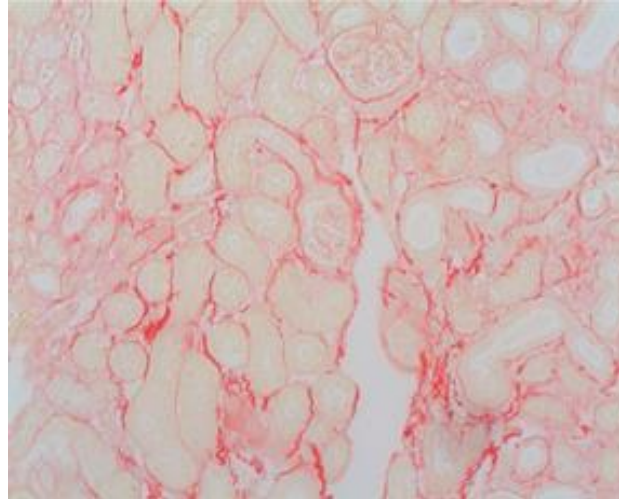
The number of positive cells



Model:
DSS-induced colitis model
Organ: Colon

Calculating the α -SMA positive cells per field

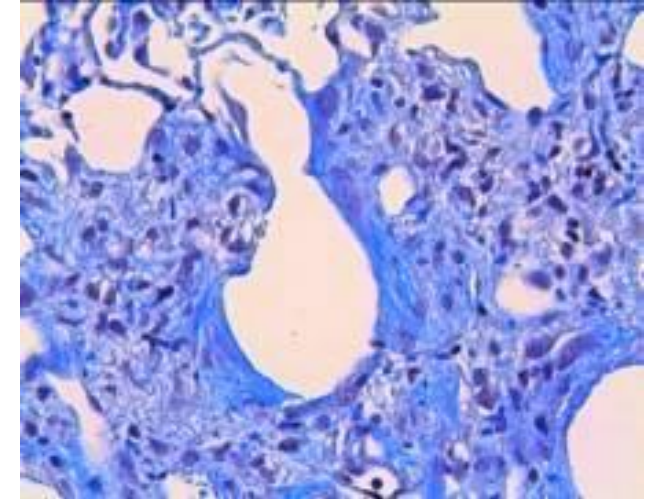
Positive area



Model:
UUO-induced renal fibrosis model
Organ: Kidney

Calculating the Sirius red positive area per field

Qualitative score



Model:
BLM-induced lung fibrosis model
Organ: Lung

Assigning a numerical scale of the amount of fibrotic area (i.e., Ashcroft score)

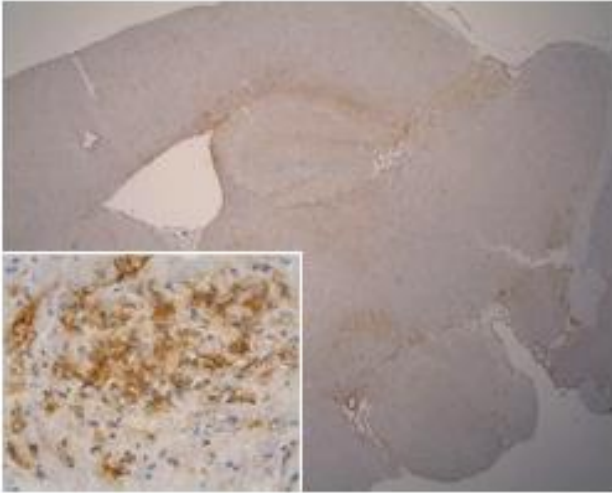
Inflammation in various disease models



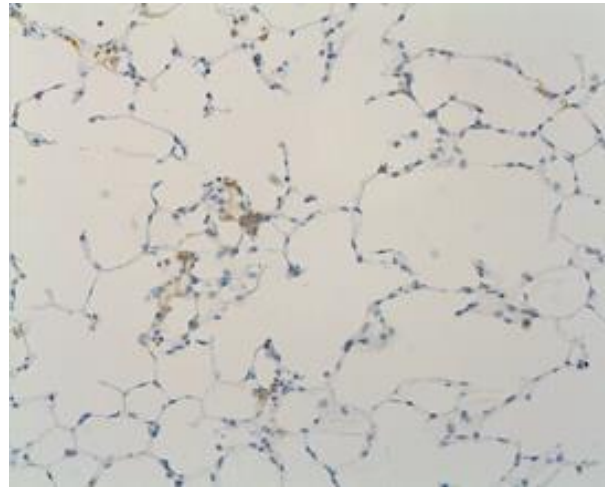
Evaluation of the grade of inflammation in various tissues in various disease models by using F4/80 as a macrophage markers.

F4/80

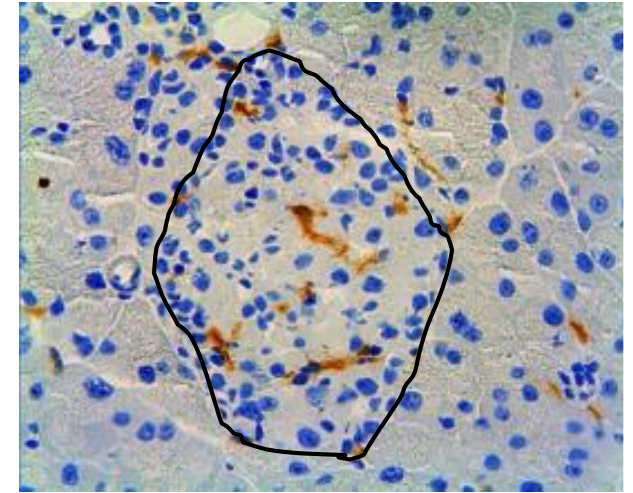
Brain



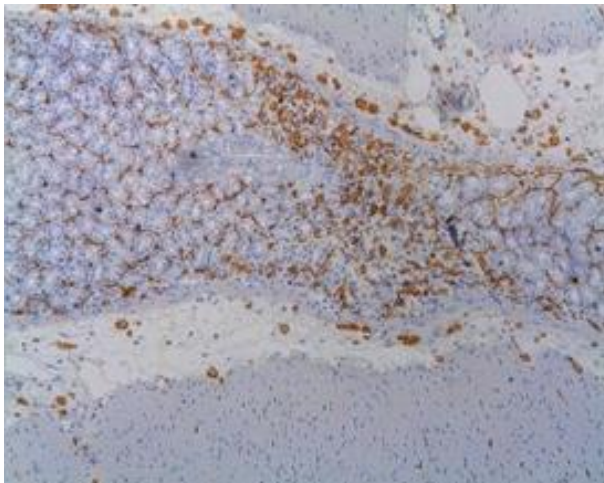
Lung



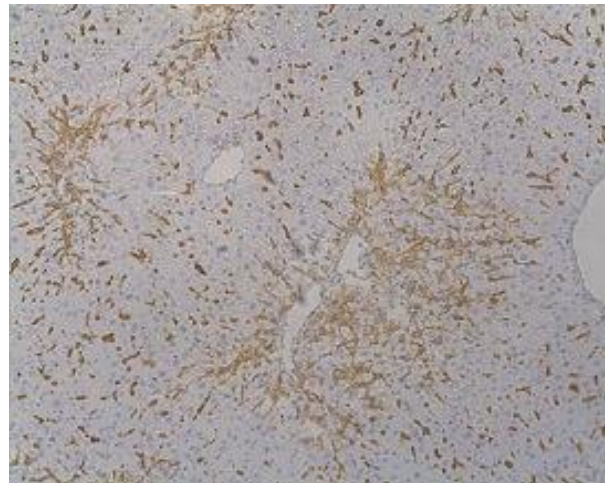
Pancreas



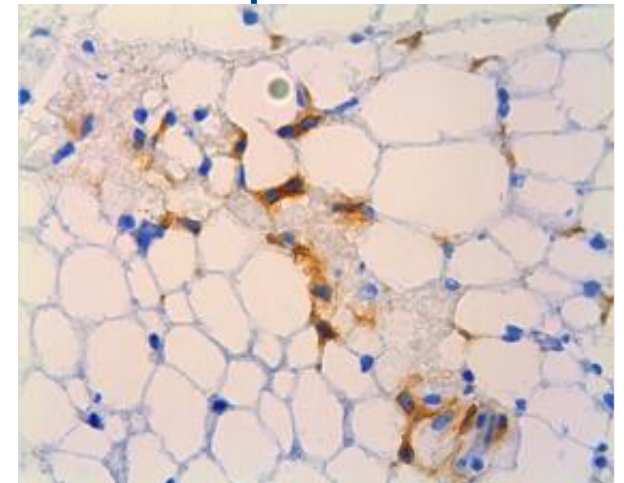
Gut



Liver

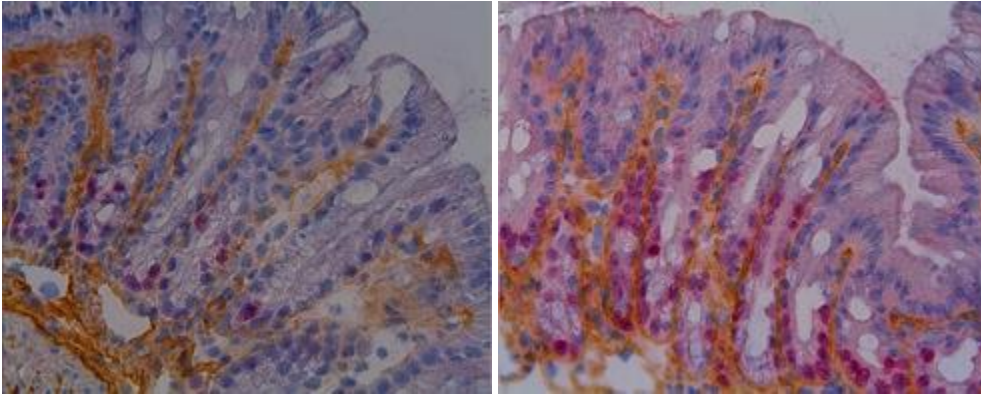


Adipose tissue



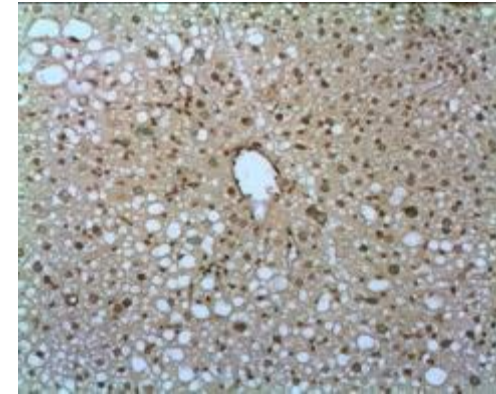
Characterization, distribution and quantitative analysis of proliferative/apoptotic cells.

Gut



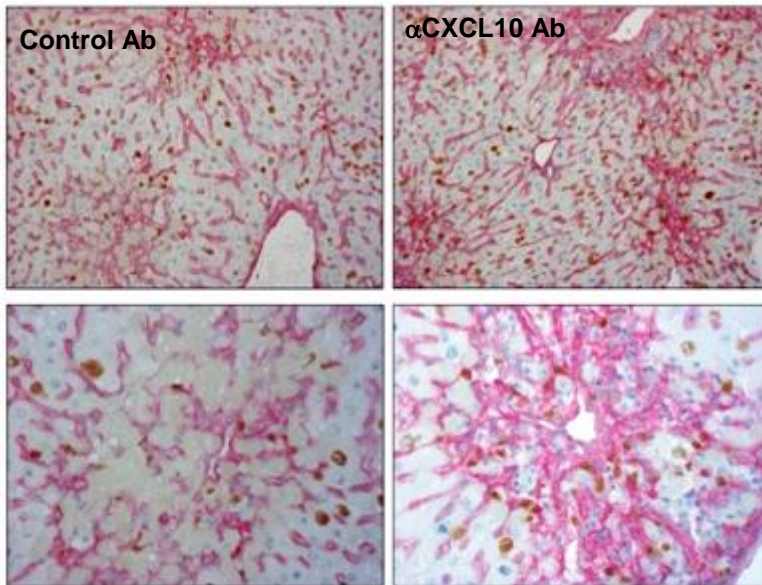
Fibroblast + Ki-67

Liver

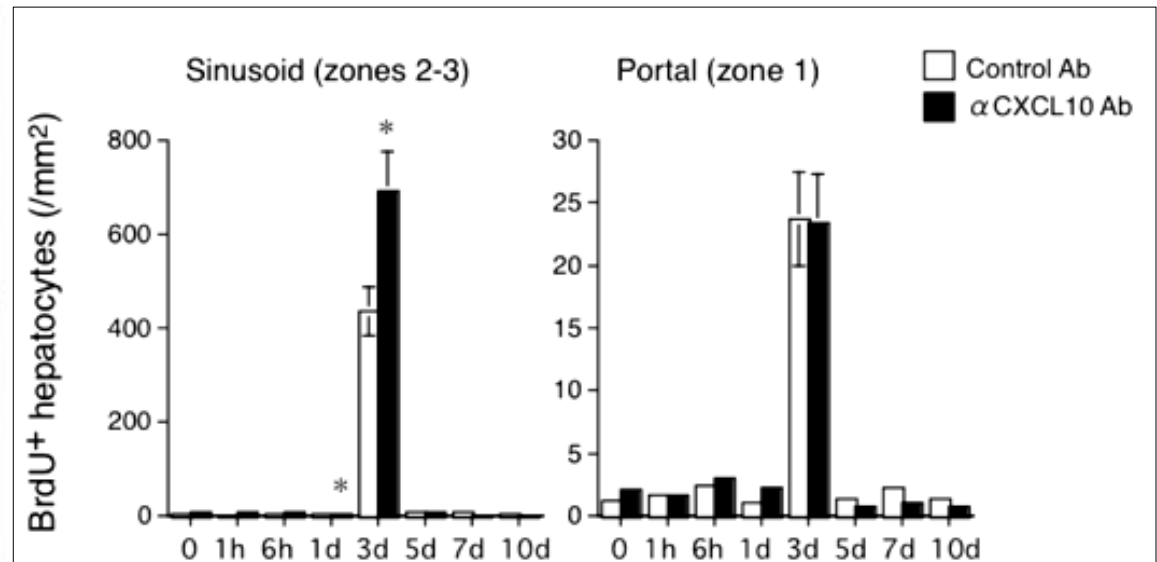


TUNEL + Nucleus

Liver



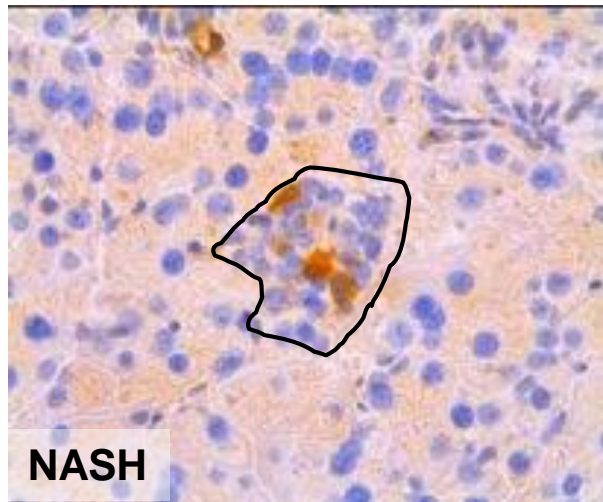
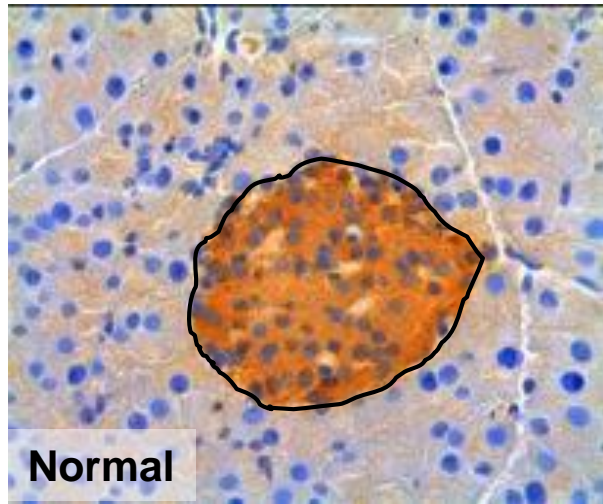
BrdU + Type IV collagen



Yoneyama H et al., Med Mol Morphol. 2007;40:191-7

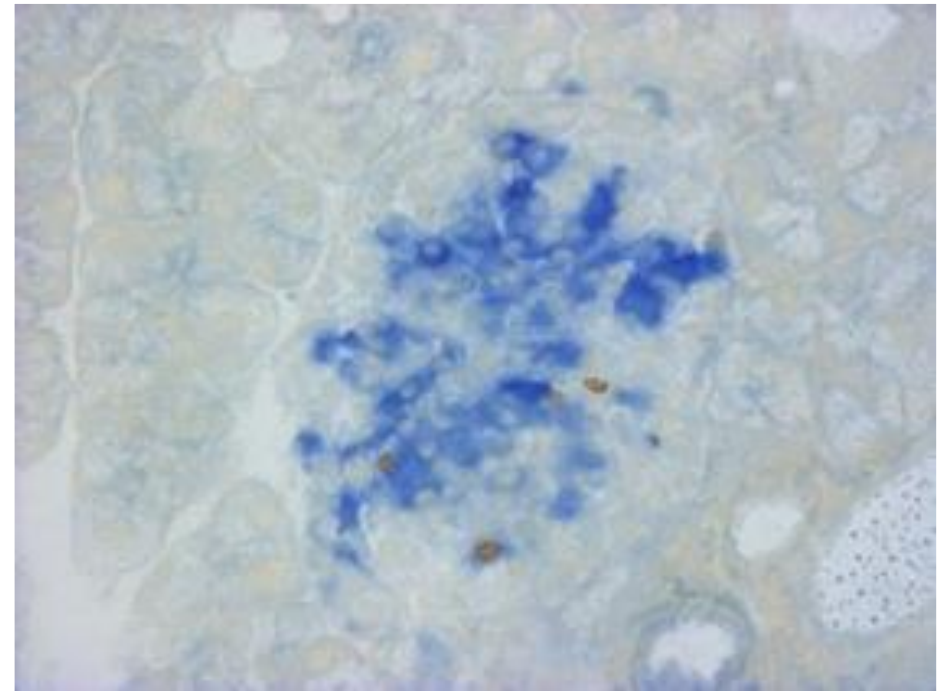
Insulin positive area, the number of insulin positive cells/proliferating β cells.

The number of insulin positive cells



Insulin

The number of BrdU+ β cells (after β cell injury by STZ)

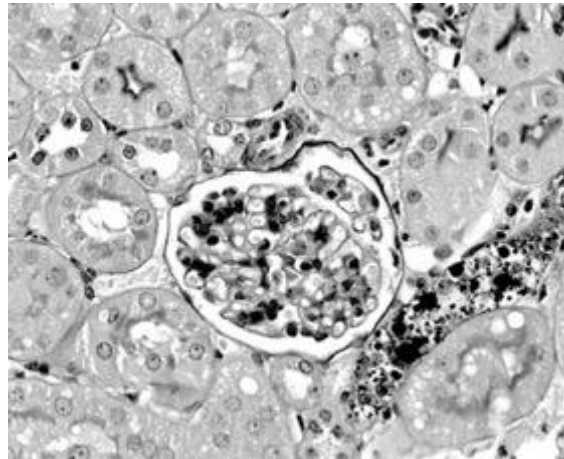
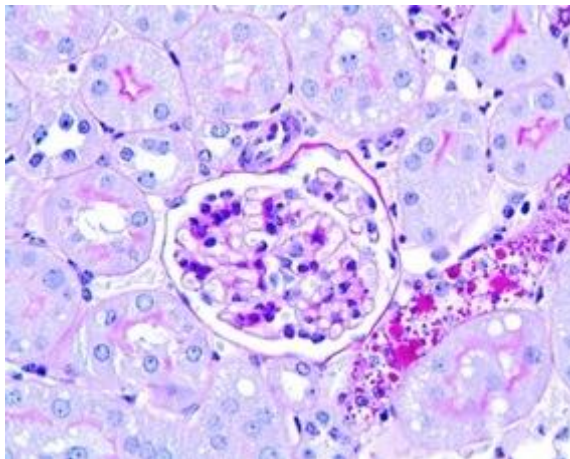
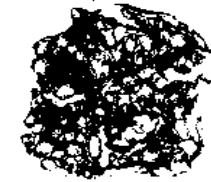
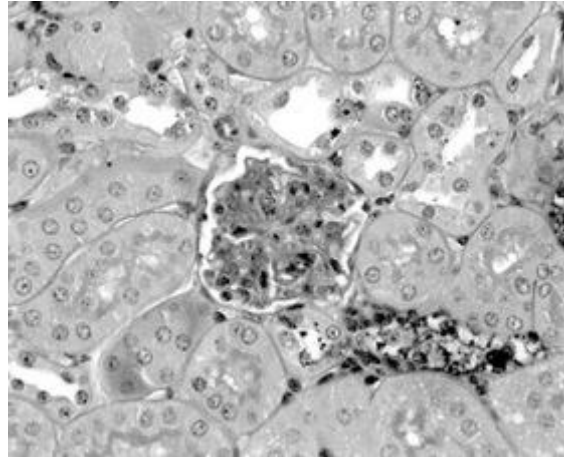
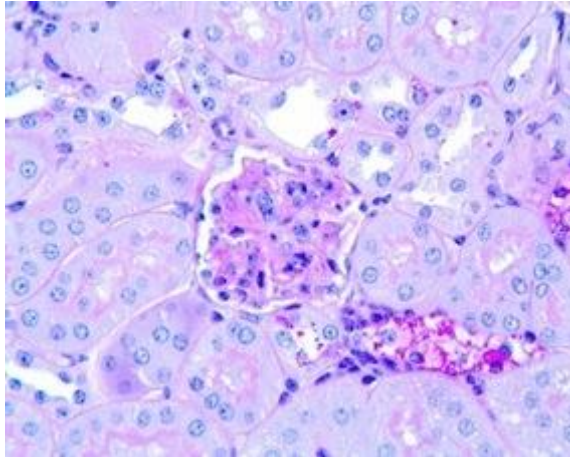


Insulin + BrdU

PAS staining

Threshold setting
after extraction

PAS-positive area in the tuft
(excluding the nuclear region)



Mesangial matrix area = PAS-positive area/tuft area

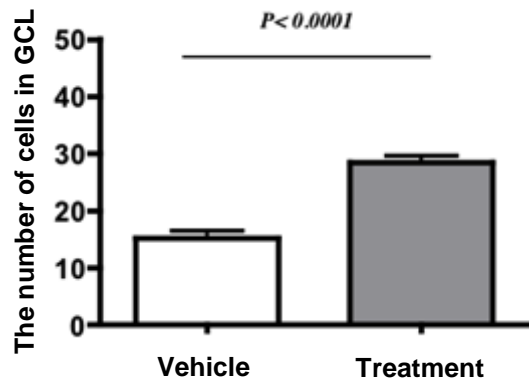
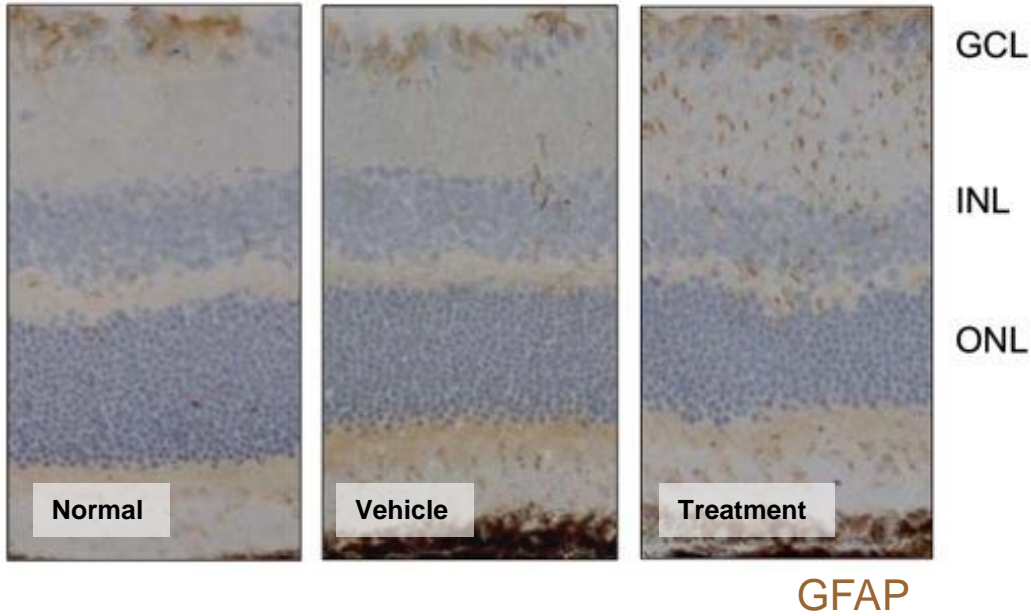
Okada S et al., Diabetes, 2003;52:2586-93

Target cell counts based on anatomical compartments



Quantitative analysis considering the distribution of cells of interests based on anatomical and functional compartment.

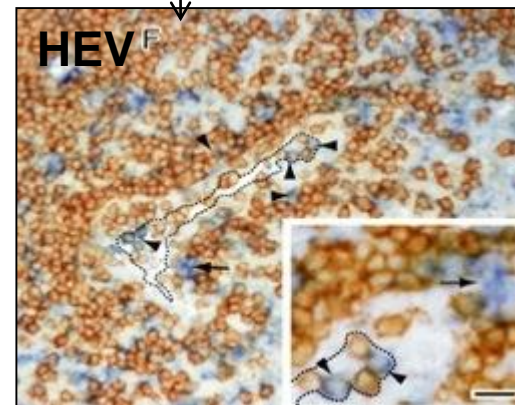
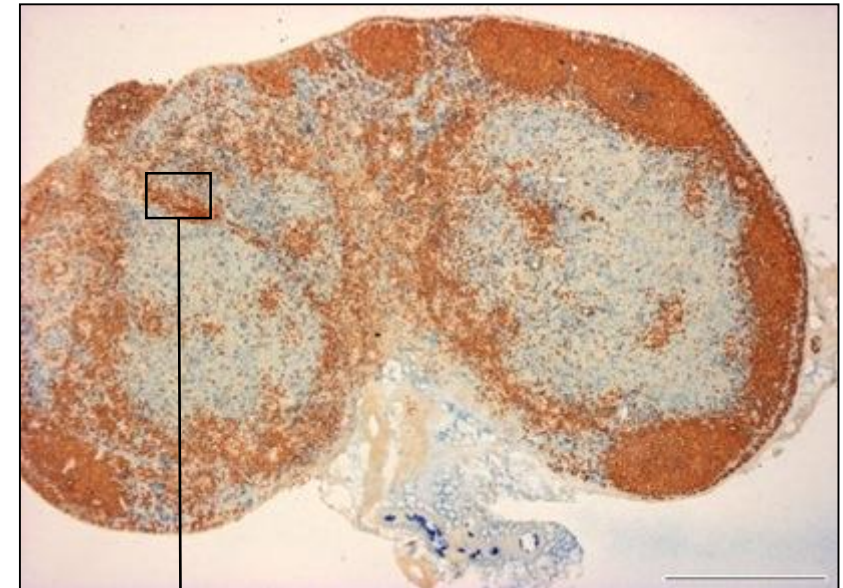
Ophthalmology: Retina



GCL: Ganglion Cell Layer
INL: Inner Nuclear Layer
ONL: Outer Nuclear Layer

The number of target cells in GCL

Immunology: Lymph node



B220 + CD11C

HEV: High Endothelial Venule

The number of target cells in HEV

■ STAM™: Premium preclinical platform for NASH-HCC

1. Pharmacology study

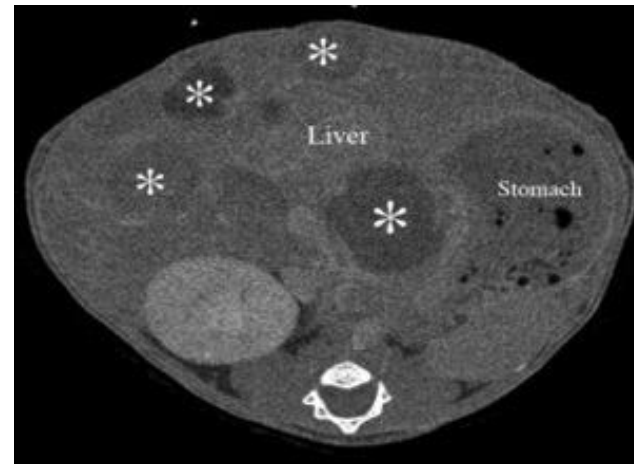
- For efficacy evaluation of drug candidates/existing drugs
- **Histological review** with in-depth knowledge of inflammation and fibrosis

2. Delivery of STAM™ Mice samples

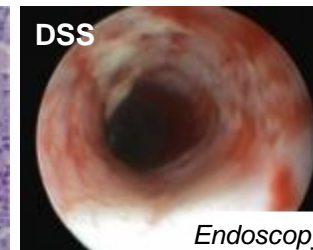
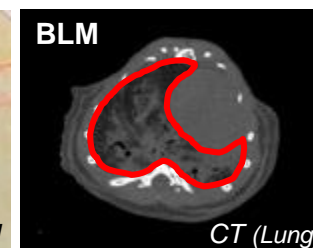
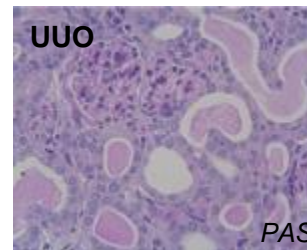
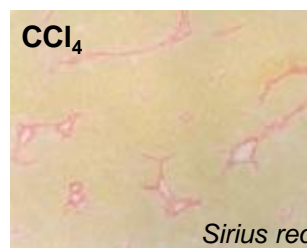
- For target (biomarker) discovery and validation
- Provided as tissue and/or plasma (serum) samples

■ Conventional disease models

- **Fatty liver/NAFLD:** ob/ob model, MCD model
- **Diabetes mellitus :** nSTZ model
- **Liver fibrosis:** CCl₄ model, BDL model
- **Acute liver failure:** CCl₄ model, Concanavalin A model
D-gal/LPS model, TAA model
- **Pulmonary fibrosis:** BLM-induced lung fibrosis model
- **Skin fibrosis:** BLM-induced skin fibrosis model
- **Renal diseases:** UUO-induced renal fibrosis model
Adriamycin-induced nephropathy model
- **IBD:** DSS-induced colitis model
- **COPD:** PPE-induced emphysema model
- **Alzheimer's disease:** icv-STZ model
- **Cancer:** DEN- CCl₄ liver cancer model
Xenograft tumor model



CT-image of HCC (STAM™)



■ Facility

- Accreditation by MEXT*
- Sponsor audit (QAU)
- Animal welfare audit by global pharmaceuticals

■ SPF-grade animal room:

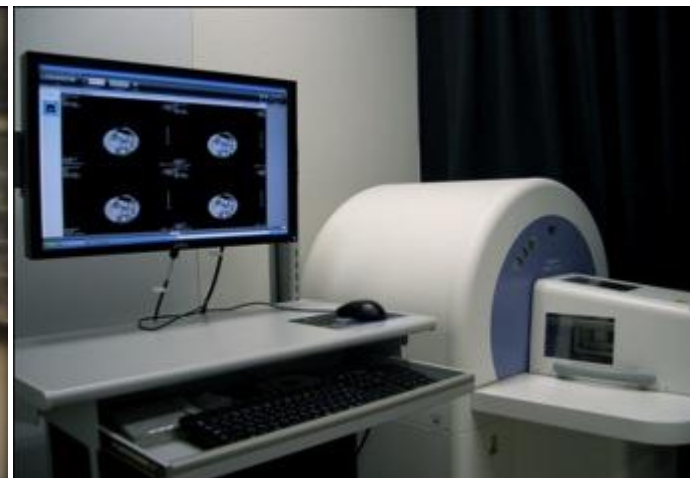
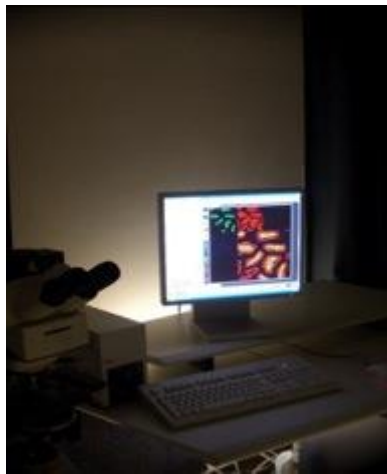
- 2080 mice

■ CRO science team:

- 10 full-time researchers
- 5 visiting scientists (MD, PhD)
- 3 external pathologists

■ Equipment:

- CT system (In vivo)
- Endoscopy (In vivo)
- Confocal microscopy
- Dry-chemistry analyzer
- Real-time PCR
- Multi-mode microplate reader and more...



*MEXT: Ministry of Education, Culture, Sports, Science and Technology



Publications

62. **Journal of Lipid Research**, “Insulin resistance dysregulates CYP7B1 leading to oxysterol accumulation: a pathway for NAFL to NASH transition” (*J Clin Exp Gastroenterol.*, DOI: 10.1194/jlr.RA120000924., 2020)
61. **Clinical and Experimental Gastroenterology**, “Changes in Function and Dynamics in Hepatic and Splenic Macrophages in Non-Alcoholic Fatty Liver Disease” (*Clin Exp Gastroenterol.*, DOI: 10.2147/CEG.S248635., 2020)
60. **Purinergic Signalling**, “Design and in vivo activity of A3 adenosine receptor agonist prodrugs” (*Purinergic Signal.*, DOI: 10.1007/s11302-020-09715-0., 2020)
59. **Cell Reports Medicine**, “Molecular Profiling Reveals a Common Metabolic Signature of Tissue Fibrosis” (*Cell Rep Med.*, DOI: DOI: 10.1016/j.xcrm.2020.100056., 2020)
58. **Journal of Biochemical and Molecular Toxicology**, “Omaveloxolone and TX63682 Are Hepatoprotective in the STAM™ Mouse Model of Nonalcoholic Steatohepatitis” (*J Biochem Mol Toxicol.*, DOI: 10.1002/jbt.22526., 2020)
57. **Journal of Medicinal Chemistry**, “6-Amino[1,2,5]oxadiazolo[3,4-b]pyrazin-5-ol Derivatives as Efficacious Mitochondrial Uncouplers in STAM Mouse Model of Nonalcoholic Steatohepatitis” (*J Med Chem.*, DOI: 10.1021/acs.jmedchem.0c00542., 2020)
56. **Scientific Reports**, “Novel Combinatorial Regimen of Garcinol and Curcuminoids for Non-alcoholic Steatohepatitis (NASH) in Mice” (*Sci Rep.*, DOI: 10.1038/s41598-020-64293-w)
55. **Journal of Medicinal Chemistry**, “[1,2,5]Oxadiazolo[3,4-b]pyrazin-5,6-diamine Derivatives as Mitochondrial Uncouplers for the Potential Treatment of Nonalcoholic Steatohepatitis” (*J Med Chem.*, DOI: 10.1021/acs.jmedchem.9b01440., 2020)
54. **Diabetology & Metabolic Syndrome**, “Intracellular toxic advanced glycation end-products (TAGE) in myoblasts may cause sarcopenia: Research article of a non-clinical study” (*Diabetol. Metab. Syndr.*, DOI: 10.21203/rs.2.23269/v1, 2020)
53. **European Radiology Experimental**, “Free fatty acid-based low-impedance liver image: a characteristic appearance in nonalcoholic steatohepatitis (NASH)” (*Eur Radiol Exp.*, 4: 3., 2020)
52. **Journal of medicinal chemistry**, “Nidufexor (LMB763), a Novel FXR Modulator for the Treatment of Nonalcoholic Steatohepatitis.” (*J Med Chem.*, DOI: 10.1021/acs.jmedchem.9b01621., 2020)
51. **Expert Opinion on Investigational Drugs**, “Pegbelfermin (BMS-986036): an investigational PEGylated fibroblast growth factor 21 analogue for the treatment of nonalcoholic steatohepatitis.” (*Expert Opin Investig Drugs.* 3:1-9., 2020)
50. **JHEP Reports**, “A Blocking Monoclonal Antibody to CCL24 Alleviates Liver Fibrosis and Inflammation in Experimental Models for Liver Damage” (*JHEP Reports*, 10.1016/j.jhepr.2019.100064, 2020)
49. **International Journal of Molecular Medicine**, “The A3 adenosine receptor agonist, namodenoson, ameliorates non-alcoholic steatohepatitis in mice.” (*Int J Mol Med*, 44(6):2256-2264, 2019)
48. **Science Translational Medicine**, “Targeting diacylglycerol acyltransferase 2 for the treatment of nonalcoholic steatohepatitis” (*Sci Transl Med.*, 11(520), 2019)
47. **Cells**, “Evaluation of NV556, a Novel Cyclophilin Inhibitor, as a Potential Antifibrotic Compound for Liver Fibrosis” (*Cells*, 8;8(11), 2019)
46. **International Journal of Molecular Sciences**, “The SGLT2 Inhibitor Canagliflozin Prevents Carcinogenesis in a Mouse Model of Diabetes and Non-Alcoholic Steatohepatitis-Related Hepatocarcinogenesis: Association with SGLT2 Expression in Hepatocellular Carcinoma”(*Int. J. Mol. Sci.* , 20(20), 5237, 2019)
45. **Toxicological Sciences**, “Gene Expression and DNA Methylation Alterations in the Glycine N-Methyltransferase Gene in Diet-Induced Nonalcoholic Fatty Liver Disease-Associated Carcinogenesis.” (*Toxicol Sci.*, 170(2):273-282, 2019)
44. **Biochemistry**, “A Panel of Protein Kinase Chemosensors Distinguishes Different Types of Fatty Liver Disease” (*Biochemistry*, 58(37):3911-3917, 2019)
43. **Hepatology Communications**, “Tropifexor - Mediated Abrogation of Steatohepatitis and Fibrosis Is Associated With the Antioxidative Gene Expression Profile in Rodents” (*Hepatol Commun.*, 3(8): 1085-1097, 2019)
42. **International Journal of Gastroenterology**, “Characterization of EDP-305, a Highly Potent and Selective Farnesoid X Receptor Agonist, for the Treatment of Non-alcoholic Steatohepatitis” (*International Journal of Gastroenterology*, DOI: 10.11648/j.ijg.20190301.12, 2019)
41. **Experimental Animals**, “Analysis of amino acid profiles of blood over time and biomarkers associated with non-alcoholic steatohepatitis in STAM mice” (*Exp Anim.*, DOI: 10.1538/expanim.18-0152, 2019)
40. **Frontiers in Genetics**, “Gene Expression and DNA Methylation Alterations During Non-alcoholic Steatohepatitis-Associated Liver Carcinogenesis” (*Front Genet.*, 10:486, 2019)
39. **Journal of Cellular and Molecular Medicine**, “The lysyl oxidase like 2/3 enzymatic inhibitor, PXS-5153A, reduces crosslinks and ameliorates fibrosis” (*J Cell Mol Med.*, 23:1759-1770, 2019)
38. **Scientific Reports**, “Connectivity mapping of angiotensin-PPAR interactions involved in the amelioration of non-alcoholic steatohepatitis by Telmisartan” (*Sci Rep.*, 9(1):4003, 2019)
37. **NPJ Precision Oncology**, “Transcriptomic analysis of hepatocellular carcinoma reveals molecular features of disease progression and tumor immune biology” (*NPJ Precis Oncol.*, DOI: 10.1038/s41698-018-0068-8, 2018)
36. **Cellular and Molecular Gastroenterology and Hepatology**, “Dipeptidyl Peptidase 4 inhibitors Reduce Hepatocellular Carcinoma by Activating Lymphocyte Chemotaxis in Mice” (*CMGH*, DOI: 10.1016/j.jcmgh.2018.08.008, 2018)
35. **Glycoconjugate Journal**, “Identification of unique glycoisoforms of vitamin D-binding protein and haptoglobin as biomarker candidates in hepatocarcinogenesis of STAM mice” (*Glycoconjug J.*, 35(5):467-476, 2018)
34. **Proc Natl Acad Sci U S A**, “Integrative genomic analysis of mouse and human hepatocellular carcinoma” (*Proc Natl Acad Sci U S A*, DOI: 10.1073/pnas.1811029115, 2018)
33. **Liver Cancer**, “Effects of a DPP4 Inhibitor on Progression of NASH-related HCC and the p62/Keap1/Nrf2-Pentose Phosphate Pathway in a Mouse Model” (*Liver Cancer*, DOI: 10.1159/000491763, 2018)
32. **PLoS One**, “Gemcabene downregulates inflammatory, lipid-altering and cell-signaling genes in the STAM™ model of NASH” (*PLoS One*, 13(5): e0194568 , 2018)



■ Publications (continued)

31. **World Journal of Gastroenterology**, "Mouse models for investigating the underlying mechanisms of nonalcoholic steatohepatitis-derived hepatocellular carcinoma" (*World J Gastroenterol*, 24(18):1989-1994, 2018)
30. **The FASEB Journal**, "Epigenetically mediated inhibition of S-adenosylhomocysteine hydrolase and the associated dysregulation of 1-carbon metabolism in nonalcoholic steatohepatitis and hepatocellular carcinoma" (*FASEB J*, DOI:10.1096/fj.201700866R, 2017)
29. **Oncotarget**, "MicroRNA deregulation in nonalcoholic steatohepatitis-associated liver carcinogenesis" (*Oncotarget*, 8:88517-88528, 2017)
28. **Oncotarget**, "Peretinoin, an acyclic retinoid, suppresses steatohepatitis and tumorigenesis by activating autophagy in mice fed an atherogenic high-fat diet" (*Oncotarget*, 8:39978-39993, 2017)
27. **Physiological Research**, "Pathophysiological analysis of the progression of hepatic lesions in STAM mice." (*Physiological Research*, 66:791-799, 2017)
26. **Molecular Cancer Research**, "Inhibition of the cell death pathway in non-alcoholic steatohepatitis (NASH)-related hepatocarcinogenesis is associated with histone H4 lysine 16 deacetylation" (*Molecular Cancer Research*, DOI:10.1158/1541-7786.MCR-17-0109, 2017)
25. **Magnetic Resonance Imaging**, "The natural history of streptozotocin-stimulated non-alcoholic steatohepatitis mice followed by Gd-EOB-DTPA-enhanced MRI: Comparison with simple steatosis mice." (*Magn Reson Imaging*, 38:123-128, 2017)
24. **Journal of Pharmacology and Experimental Therapeutics**, "Selective Inhibition of Autotaxin Is Efficacious in Mouse Models of Liver Fibrosis" (*J Pharmacol Exp Ther*, 360:1-13, 2017)
23. **Oncotarget**, "Distinctly altered gut microbiota in the progression of liver disease" (*Oncotarget*, 7:19355-19366, 2016)
22. **Diabetology & Metabolic Syndrome**, "Empagliflozin (an SGLT2 inhibitor), alone or in combination with linagliptin (a DPP-4 inhibitor), prevents steatohepatitis in a novel mouse model of non-alcoholic steatohepatitis and diabetes" (*Diabetology & Metabolic Syndrome*, 8:45, 2016)
21. **Journal of Immunology, Infection & Inflammatory Diseases**, "Solithromycin Diminishes Steatohepatitis by Modulating Gluconeogenesis and Inhibits Tumor Growth in a Diabetic Mouse Model of Non-Alcoholic Steatohepatitis" (*J Immunol Infect Inflam Dis*, 1:004, 2016)
20. **PLoS One**, "Antifibrotic Effects of the Dual CCR2/CCR5 Antagonist Cenicriviroc in Animal Models of Liver and Kidney Fibrosis" (*PLoS One*, 11:e0158156, 2016)
19. **Cell Reports**, "Cancer-Associated Fibroblasts Regulate Tumor-Initiating Cell Plasticity in Hepatocellular Carcinoma through c-Met/FRA1/HEY1 Signaling" (*Cell Press*, 15:1175-1189, 2016)
18. **International Journal of Medical Sciences**, "Palmitate-induced Regulation of PPAR γ via PGC1 α : a Mechanism for Lipid Accumulation in the Liver in Nonalcoholic Fatty Liver Disease" (*Int. J. Med. Sci*, 13:169-178, 2016)
17. **European Journal of Pharmacology**, "Lipid-lowering agents inhibit hepatic steatosis in a non-alcoholic steatohepatitis-derived hepatocellular carcinoma mouse model" (*Eur J Pharmacol*, 772:22-32, 2016)
16. **Scientific Reports**, "Characterization of hepatic lipid profiles in a mouse model with nonalcoholic steatohepatitis and subsequent fibrosis" (*Sci Rep.*, 12466, 2015)

15. **International Journal of Obesity**, "Low cytochrome oxidase 411 links mitochondrial dysfunction to obesity and type 2 diabetes in humans and mice" (*Int J Obes*, 39:1254-63, 2015)
14. **Proc Natl Acad Sci U S A**, "Immunomodulatory spherical nucleic acids" (*Proc Natl Acad Sci U S A*, 31;12:3892-7, 2015)
13. **Oncology Reports**, "Hepatic expression of the Sptlc3 subunit of serine palmitoyltransferase is associated with the development of hepatocellular carcinoma in a mouse model of nonalcoholic steatohepatitis" (*Oncol Rep*, 33:1657-66, 2015)
12. **Drug R D**, "In Vivo Efficacy Study of Milk Thistle Extract (ETHIS-094TM) in STAMTM Model of Nonalcoholic Steatohepatitis" (*Drugs R D*, 14:291-9, 2014)
11. **PLoS One**, "Photoacoustic Tomography of Human Hepatic Malignancies Using Intraoperative Indocyanine Green Fluorescence Imaging" (*PLoS One*, 9:e112667, 2014)
10. **Cancer Science**, "Silencing of microRNA-122 is an early event during hepatocarcinogenesis from non-alcoholic steatohepatitis" (*Cancer Sci*, 105:1254-60, 2014)
9. **Anticancer Research**, "Characterization of non-alcoholic steatohepatitis-derived hepatocellular carcinoma as a human stratification model in mice" (*Anticancer Res*, 34:4849-4856, 2014)
8. **PLoS One**, "L-carnitine prevents progression of non-alcoholic steatohepatitis in a mouse model with upregulation of mitochondrial pathway." (*PLoS One*, 9:e100627, 2014)
7. **Medical Molecular Morphology**, "Linagliptin alleviates hepatic steatosis and inflammation in a mouse model of non-alcoholic steatohepatitis" (*Med Mol Morph*, 47:137-149)
6. **PLoS One**, "Therapy of Experimental NASH and Fibrosis with Galectin Inhibitors" (*PLoS One*, 8:e83481, 2013)
5. **International Journal of Oncology**, "Identification of an H2-Kb or H2-Db restricted and glypican-3-derived cytotoxic T-lymphocyte epitope peptide" (*Int J Oncol*, 42:831-838, 2013)
4. **International Journal of Experimental Pathology**, "Inhibition of Glutamyl Cyclases alleviates CCL2-mediated inflammation of non-alcoholic fatty liver disease in mice" (*Int J Exp Pathol*, 94: 217-225, 2013)
3. **Medical Molecular Morphology**, "A murine model for non-alcoholic steatohepatitis showing evidence of association between diabetes and hepatocellular carcinoma" (*Med Mol Morph*, 46:141-152, 2013)
2. **Hepatology**, "Hydrogen-rich water prevents progression of non-alcoholic steatohepatitis and accompanying hepatocarcinogenesis in mice" (*Hepatology*, 56:912-921, 2012)
1. **Journal of Nutritional Science and Vitaminology**, "Effects of Dietary Supplementation with Betaine on a Nonalcoholic Steatohepatitis (NASH) Mouse Model" (*J Nutr Sci Vitaminol*, 58:371-375, 2012)

■ Presentations

85. **AASLD 2020**, "Selective Estrogen Receptor Modulator, OSU-ERB-12, Ameliorates Preclinical Models of Hepatic Fibrosis and NASH" [The Ohio State University](#)
84. **AASLD 2020**, "Preclinical development of small molecule drug discovery leads with novel moas for non-alcoholic steatohepatitis (NASH)" [twoXAR, Inc.](#)
83. **AASLD 2020**, "Oxysterol transformation of fatty liver to steatohepatitis is driven by insulin resistance" [Virginia Commonwealth University](#)



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82. **AASLD 2020**, “Iron loss triggers mitophagy through induction of mitochondrial ferritin” [Kawasaki Medical School](#)
81. **EASL the Digital International Liver Congress™ 2020**, “Iron loss-induced mitophagy via mitochondria ferritin suppresses NASH-related hepatocellular carcinoma” [Kawasaki Medical School](#)
80. **EASL the Digital International Liver Congress™ 2020**, “GPNMB modulates hepatic steatogenesis and liver cancer” [Heidelberg University](#)
79. **EASL the Digital International Liver Congress™ 2020**, “2-deoxy-D-glucose encapsulated PLGA nanoparticles suppress hepatocellular carcinoma through cytotoxic effect and activation of antitumor immunity” [Kawasaki Medical School](#)
78. **EASL the Digital International Liver Congress™ 2020**, “Novel autophagy inducer, a4368 improves non-alcoholic steatohepatitis in mice” [Autophagy Sciences](#)
77. **EASL the Digital International Liver Congress™ 2020**, “Therapeutic efficacy of the chitotriosidase inhibitors in STAM model of non-alcoholic steatohepatitis” [OncoArendi Therapeutics SA](#)
76. **AASLD 2019**, “Norursodeoxycholic acid (norUDCA) significantly ameliorates liver injury in the STAM mouse model for non-alcoholic steatohepatitis (NASH)” [Dr. Falk Pharma GmbH](#)
75. **AASLD 2019**, “EC-18, a novel immune resolution accelerator, improves NASH and liver fibrosis” [Institution” Enzychem Lifesciences Corporation](#)
74. **AASLD 2019**, “In vitro and in vivo characterization of EYP001 a novel, potent and selective FXR agonist now in a phase 2 clinical trial in NASH Institution” [Enyo Pharma SA](#)
73. **DDW 2019**, “Change of Gut Microbiome after Treatment with the Traditional Japanese Medicine Daisaikoto is Associated with Improved Liver Steatosis in a Non-alcoholic Fatty Liver Mouse Model” [TSUMURA & Co.](#)
72. **DDW 2019**, “Influence of the O-GlcNAc Modification in Hepatic Carcinogenesis by Non-alcoholic Fatty Liver Disease” [Osaka Medical College](#)
71. **EASL the International Liver Congress™ 2018**, “LXR inverse agonists reduce steatosis and fibrosis in the STAM mouse model but also improve insulin sensitivity in a high fat diet mouse clamp study” [Phenex Pharmaceuticals AG](#)
70. **3rd Annual World Preclinical Congress Europe 2018**, “LXR Inverse Agonists for the Treatment of NASH” [Phenex Pharmaceuticals AG](#)
69. **3rd Annual World Preclinical Congress Europe 2018**, “MTBL0036, a Promising, New Anti-NASH and Antifibrotic Candidate: MTBL0036 showed a decrease in NAFLD Activity score in the STAM model” [Metabolys, Inc.](#)
68. **AASLD 2018**, “AXA1125, a Novel Composition of Amino Acids Reprograms the Multifactorial Pathophysiology in NAFLD” [Axcella Health Inc.](#)
67. **AASLD 2018**, “Treatment of Hepatocellular Carcinoma Using 2-Deoxy-D-Glucose Encapsulated in PLGA Nanoparticles in Mice” [Kawasaki Medical School](#)
66. **AASLD 2018**, “Dipeptidyl Peptidase 4 Inhibitors Reduce the Progression of Hepatocellular Carcinoma By Activating T Cell and Natural Killer Cell Chemotaxis in Mice” [Kawasaki Medical School](#)
65. **AASLD 2018**, “Effects of a DPP4 Inhibitor on Progression of Nash-Related Hepatoma and DNA Synthesis Pathway Via p62/Keap1/Nrf2 in a Mouse Model: A Metabolomic Analysis” [Kurume University School of Medicine](#)
64. **AASLD 2018**, “Gemcabene Regulates Hepatic Genes Associated with Inflammation and Fibrosis with Impact on Non-Alcoholic Fatty Liver Disease” [Gemphire Therapeutics Inc.](#)
63. **AASLD 2018**, “CM101, a Novel CCL24 Blocking Antibody, Suppresses Hepatic Injury and Fibrosis In Experimental Models of Nash and Liver Fibrosis” [ChemomAb Ltd.](#)
62. **AASLD 2018**, “Unexpected Antidiabetic Effects Combined with Antifibrotic Activities of LXR Inverse Agonists in Mouse Models of NAFLD/Nash” [Phenex Pharmaceuticals AG](#)
61. **The 78th Scientific Sessions ADA, 2018**, “Canagliflozin, an SGLT2 Inhibitor, Prevents Development of Hepatocellular Carcinoma (HCC) from Nonalcoholic Steatohepatitis (NASH) in a Mouse Model of NASH-HCC Under Diabetic State” [Dokkyo Medical University](#)
60. **The 78th Scientific Sessions ADA, 2018**, “Combination of SGLT2 Inhibitor and Novel Selective PPARα Modulator, Tofogliflozin (Tofo) and Pemafibrate (Pema), Improves Survival Rate in STAM Mice as a Diabetic NASH Model” [Kowa Company Ltd.](#)
59. **EASL the International Liver Congress™ 2018**, “Interfering with local fibrotic platelet activation significantly inhibits fibrosis in multiple animal models: suggestions of the importance of the platelet-wound healing axis for fibrosis” [Symic Bio, Inc.](#)
58. **EASL the International Liver Congress™ 2018**, “BMS-986036, a PEGylated fibroblast growth factor 21 analogue, reduces fibrosis and PRO-C3 in a mouse model of non-alcoholic steatohepatitis” [Bristol-Myers Squibb Company](#)
57. **EASL the International Liver Congress™ 2018**, “LJN452 (tropifexor) attenuates steatohepatitis, inflammation, and fibrosis in dietary mouse models of nonalcoholic steatohepatitis” [Genomics Institute of the Novartis Research Foundation](#)
56. **EASL the International Liver Congress™ 2018**, “Clinical-grade human liver mesenchymal stem cells reduce NAS score and fibrosis progression in advanced stage NASH pre-clinical model through immunomodulation” [Promethera Biosciences LLC](#)
55. **First EASL NAFLD Summit 2017**, “Dual CCR2/5 antagonist decreases hepatic inflammation in acute liver injury and NASH metabolic animal models” [Pfizer Inc.](#)
54. **First EASL NAFLD Summit 2017**, “AXA1125, a novel defined amino acid composition (DAAC), improves NAFLD activity score (NAS) and reduces fibrosis in two rodent models of nonalcoholic steatohepatitis (NASH)” [Axcella Health, Inc.](#)
53. **AASLD 2017**, “The Anti-Fibrogenic and Liver Protective Effects of Namodenoson (CF102) in a Non-Alcoholic Steatohepatitis Model” [Can-Fite BioPharma Ltd.](#)
52. **AASLD 2017**, “DPP4 Inhibitor Suppressed Progression of NASH-related Hepatocellular Carcinoma with Inhibition of Metabolic Reprogramming in p62-Keap 1-Nrf2-pentose Phosphate Pathway in a Mouse Model: A Metabolomic Analysis” [Kurume University School of Medicine](#)
51. **AASLD 2017**, “CB4209 and CB4211 Reduce the NAFLD Activity Score in the STAM Model of NASH, Reduce Triglyceride Levels, and Induce Selective Fat Mass Loss in DIO Mice” [CohBar, Inc.](#)



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50. **AASLD 2017**, "Combination Treatment of LFN452 and Cenicriviroc Shows Additive Effects in a Diet-Induced NASH Model" *Genomics Institute of the Novartis Research Foundation/Allergan plc/Novartis Institutes for BioMedical Research, Inc.*
49. **AASLD 2017**, "Gemcabene Attenuates the NAFLD Activity and Fibrosis Scores, and Downregulates Hepatic Inflammatory Genes in the STAM™ Murine Model of NASH-HCC" *Gempire Therapeutics Inc.*
48. **DDW 2017**, "A HMG-CoA Reductase Inhibitor, Rosuvastatin, as a Potential Preventive Drug for The Development of Hepatocellular Carcinoma Associated With Non-alcoholic Fatty Liver Disease in Mice" *Osaka Medical College*
47. **EASL the International Liver Congress™ 2017**, "Anti-fibrotic effect of NV556, a sanglifhrin-based cyclophilin inhibitor, in a preclinical model of non-alcoholic steatohepatitis" *Neuro Vive Pharmaceutical AB*
46. **AACR 2017**, "Inhibition of gene expression during non-alcoholic steatohepatitis (NASH)-related hepatocarcinogenesis is mediated by histone H4 lysine 16 deacetylation" *FDA-National Center for Toxicological Research.*
45. **AACR 2017**, "Alterations in the chromatin accessibility in nonalcoholic steatohepatitis-associated hepatocellular carcinoma" *FDA-National Center for Toxicological Research*
44. **AACR 2017**, "Role of miRNAome deregulation in the pathogenesis of non-alcoholic steatohepatitis (NASH)-derived hepatocellular carcinoma" *FDA-National Center for Toxicological Research*
43. **AASLD 2017, Emerging Trends Conference: Emerging Trends in Non-Alcoholic Fatty Liver Disease**, "The Novel Antidiabetic Candidate MTBL0036 Greatly Diminishes The NAFLD Activity Score in The STAM Mouse Model of NASH" *Metabols Inc.*
42. **AASLD 2017, Emerging Trends Conference: Emerging Trends in Non-Alcoholic Fatty Liver Disease**, "DUR-928, An Endogenous Regulatory Molecule, Exhibits Anti-Inflammatory and Antifibrotic Activity in a Mouse Model of NASH" *DURECT Corporation*
41. **AASLD 2016**, "A Phase 2 study of BMS-986036 (Pegylated FGF21) in Obese Adults with Type 2 Diabetes and a High Prevalence of Fatty Liver" *Bristol-Myers Squibb Company*
40. **AASLD 2016**, "Effects of BMS-986036 (pegylated fibroblast growth factor 21) on hepatic steatosis and fibrosis in a mouse model of nonalcoholic steatohepatitis" *Bristol-Myers Squibb Company*
39. **DDW 2016**, "Inhibition of the Ileal Bile Acid Transporter (IBAT) by A4250 Reduces Hepatic Damage in a Mouse Model of Non-Alcoholic Steatohepatitis (NASH)" *Albireo AB*
38. **EASL the International Liver Congress™ 2016**, "DPP4 Inhibitor Suppresses Steatohepatitis and HCC Progression with Glucose Re-Programming in a Mouse Model of NASH" *Kurume University School of Medicine*
37. **HEP DART 2015**, "The Cyclophilin Inhibitor, CPI-431-32, is a Hepatitis B Oral Drug Candidate with Antiviral and Antifibrotic Activities" *Ciclofilin Pharmaceuticals Inc.*
36. **WDC 2015**, "Empagliflozin (an SGLT2 inhibitor), alone or in combination with linagliptin (a DPP-4 inhibitor), prevents steatohepatitis in a novel mouse model of non-alcoholic steatohepatitis and diabetes" *Dokkyo Medical University*
35. **AASLD 2015**, "Anti-Fibrotic Effect of Autotaxin and LPA1R Antagonists in a Rodent Model of NASH" *Bristol-Myers Squibb Company*
34. **AASLD 2015**, "Sitagliptin, a Dipeptidyl Peptidase 4 inhibitor, Suppressed Tumor Progression with Down-regulation of Nrf Nuclear Expression in a Mouse Model of Non-alcoholic Steatohepatitis-related Hepatocellular Carcinoma" *Kurume University School of Medicine*
33. **AASLD 2015**, "Reduction of Hepatic 27-Hydroxycholesterol in Steatohepatitis Model Mice with Insulin Resistance" *Tokyo Medical University Ibaraki Medical Center*
32. **AASLD 2015**, "Disturbance of regulatory T cells, MDSCs and NK cells is involved in NASH and mouse model of NASH" *Tohoku University Hospital.*
31. **AASLD 2015**, "Mechanism of Action of the Anti-NASH effects of Solithromycin in a Predictive NASH HCC Mouse Model" *Cempra Pharmaceuticals, Inc.*
30. **DDW 2015**, "Effects of Sitagliptin, a Dipeptidyl Peptidase 4 Inhibitor, on Tumor Progression and p62/SQSTM1 Subcellular Localization in a Mouse Model of Non-Alcoholic Steatohepatitis-Related Hepatocellular Carcinoma" *Kurume University*
29. **Keystone Symposia 2015**, "DGAT2 Inhibition Prevents NAFLD and Fibrosis in the STAM Mouse Model of NASH" *Pfizer Inc.*
28. **Keystone Symposia 2015**, "Oxidized-Phospholipid Small Molecule Inhibits Non-Alcoholic Steatohepatitis (NASH) and Liver Fibrosis" *Vascular Biogenics Ltd*
27. **AASLD 2014**, "L-carnitine prevents progression of non-alcoholic steatohepatitis in a mouse model with upregulation of mitochondrial pathway" *Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences*
26. **AASLD 2014**, "MN-001 (tipelukast), a novel, orally bioavailable drug, reduces fibrosis and inflammation and down-regulates TIMP-1, collagen Type 1 and LOXL2 mRNA overexpression in an advanced NASH (nonalcoholic steatohepatitis) model" *MediciNova, Inc.*
25. **ICLAF 2014**, "MN-001 (tipelukast), a nonselective phosphodiesterase, 5-lipoxygenase, leukotriene, phospholipase C and thromboxane A2 inhibitor, demonstrates anti-fibrotic effects in the bleomycin-induced idiopathic pulmonary fibrosis mouse model" *MediciNova, Inc.*
24. **ADA 2014**, "Liraglutide prevents steatohepatitis, liver fibrosis, and accompanying carcinogenesis in a diabetes and nonalcoholic steatohepatitis mouse model treated with STZ-HFD" *Saga University*
23. **ATS 2014**, "Solithromycin Reduces Inflammation In Mice Caused By Bleomycin-Induced Lung Injury" *Cempra, Inc.*
22. **DDW 2014**, "Anti-NASH Effects of Solithromycin in NASH-HCC Mouse Model" *Cempra, Inc.*
21. **AACR 2014**, "Clinicopathological characterization of non-alcoholic Steatohepatitis (NASH)-derived Hepatocellular carcinoma (HCC) as a patient stratification model in mice" *The Jikei University School of Medicine*
20. **Keystone Symposia 2014**, "The NADPH Oxidase (NOX) Inhibitor GKT137831 Alleviates Liver Inflammation and Fibrosis in a Mouse Model of Non-Alcoholic Steatohepatitis (NASH)" *Genkyotex S.A.*
19. **15th International Workshop on Co-morbidities and Adverse Drug Reactions in HIV**, "Anti-fibrotic and anti-inflammatory activity of the dual CCR2 and CCR5 antagonist cenicriviroc in a mouse model of NASH" *Tobira Therapeutics Inc.*
18. **AASLD 2013**, "Anti-fibrotic and anti-inflammatory activity of the dual CCR2 and CCR5 antagonist cenicriviroc in a mouse model of NASH" *Tobira Therapeutics Inc.*

■ Presentations (continued)

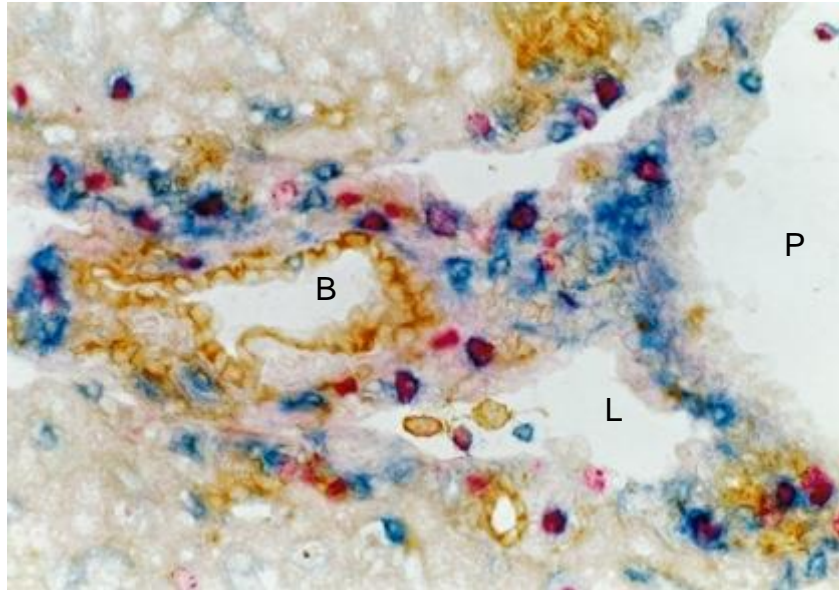
17. **AASLD 2013**, "L-carnitine prevents progression of non-alcoholic steatohepatitis with regulation of mitochondrial β -oxidation and redox system in NASH model Mice" Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences
16. **FASEB SRC 2013, Lysophospholipid and Other Related Mediators - From Bench to Clinic**, "ATX inhibition prevents progression of non-alcoholic steatohepatitis (NASH) in a hypoinsulinemic mouse model of progressive liver disease" F. Hoffmann-La Roche, Ltd
15. **DDW 2013**, "Vitamin E and L-Carnitine Prevents Progression of Non-Alcoholic Steatohepatitis With Regulation of Intestinal Inflammation Activation in NASH Model Mice" Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences
14. **DDW 2013**, "Rosuvastatin Prevents Liver Tumorigenesis in High-Fat Diet-Fed Mice" 2nd Department of Internal Medicine Osaka Medical College
13. **AASLD 2012**, "Comparative proteomic analysis of the liver in a murine model of non-alcoholic steatohepatitis" Third Department of Internal Medicine, Niigata University Medical School/Juntendo University School of Medicine
12. **AASLD 2012**, "Inhibition of endoplasmic reticulum stress by 4-phenylbutyrate prevents steatohepatitis progression and tumorigenesis in NASH-HCC model mice" Department of Gastroenterology
11. **AASLD 2012**, "Galectin-3 targeting drugs inhibit multiple pathological pathways leading to improvement of non-alcoholic steatohepatitis (NASH)" Galectin Therapeutics Inc.
10. **AASLD 2012**, "Hepatic gene expression of the SPTLC3 subunit of serine palmitoyltransferase is associated with the development of liver cancer in a NASH mouse model" Department of Human and Environmental Sciences, Kagoshima University Graduate School of Medicine and Dental Sciences
9. **The 72th Scientific Sessions ADA, 2012**, "Linagliptin is an Effective Therapeutic for Non-alcoholic Fatty Liver Disease (NAFLD) and Non-alcoholic Steatohepatitis (NASH)" Boehringer Ingelheim GmbH & Co. KG
8. **DDW 2012**, "A Novel Murine Model Recapitulates the Pathogenesis of Human Non-alcoholic steatohepatitis (NASH) and NASH-related Hepatocellular Carcinoma"
7. **DDW 2012**, "Effects of Telmisartan on a Murine Model of Non-alcoholic Steatohepatitis (NASH) and NASH-related Hepatocellular Carcinoma"
6. **DDW 2012**, "The Chemical Chaperon 4-Phenylbutyrate Inhibits Liver Fibrosis and Tumorigenesis in High-Fat Diet With N-acetyl- β -D-glucosaminidase Inhibitor-Induced NASH Model Mice" Department of Gastroenterology, Juntendo University School of Medicine
5. **EASL The International Liver Congress™ 2012 - 47th Annual Meeting of the European Association for the Study of the Liver**, "FXR agonists prevent steatosis, hepatocyte death and progression of NASH towards HCC in a hypoinsulinaemic mouse model of progressive liver disease" Phenex Pharmaceuticals AG
4. **AASLD 2011**, "The Dipeptidyl Peptidase-4 Inhibitor Linagliptin is an Effective Therapeutic for Metabolic Liver Disease in Several Rodent Models of Non-Alcoholic Fatty Liver Disease (NAFLD) and Non Alcoholic Steatohepatitis (NASH)" Boehringer Ingelheim GmbH & Co. KG

3. **EASL Special Conference - Liver Transplantation 2011**, "Improvement of steatosis, inflammation, and fibrosis in a mouse model of steatohepatitis following treatment with galectin inhibitor" Galectin Therapeutics Inc.
2. **EASL The International Liver Congress™ 2011 - 46th Annual Meeting of the European Association for the Study of the Liver**, "Novel FXR agonists with potent lipid lowering, insulin sensitising, anti-inflammatory and anti-fibrotic effects in mouse models of metabolic syndrome and NASH" Phenex Pharmaceuticals AG
1. **The 9th JSH SingleTopic Conference "NASH 2010"**, "Strong Anti-steatotic and Anti-fibrotic Effects of Novel FXR Agonists in a Murine NASH Model that Resembles Human NASH" Phenex Pharmaceuticals AG

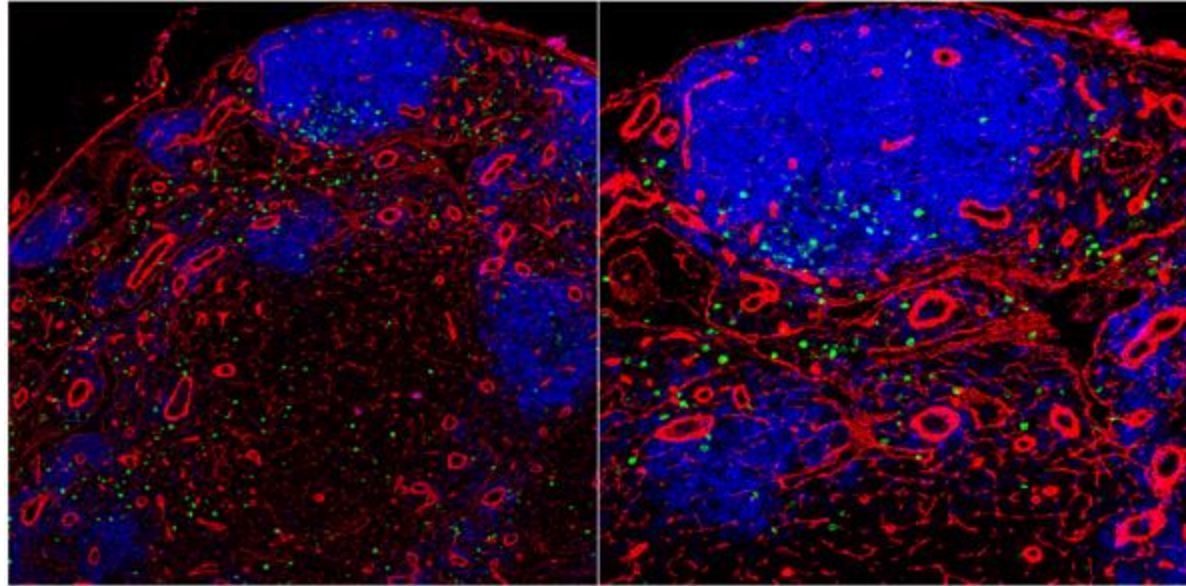
• List of presentations in domestic meeting is available only in Japanese version.

■ Patents

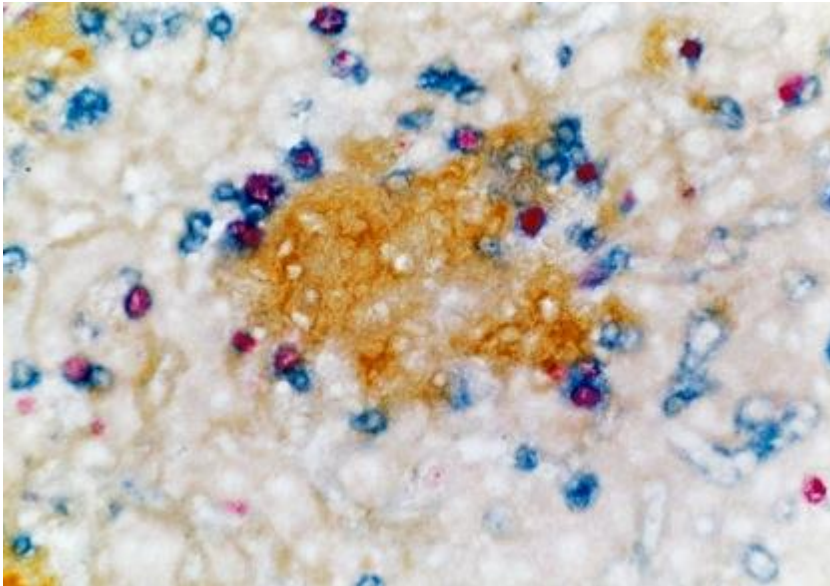
- International publication No.: WO2011/013247 Title of the invention: "Steatohepatitis-Liver Cancer Model Animal"
- Publication No. (JP) : 2009-178143 Title of the invention: "Steatohepatitis-Liver Cancer Model Animal (EN)"



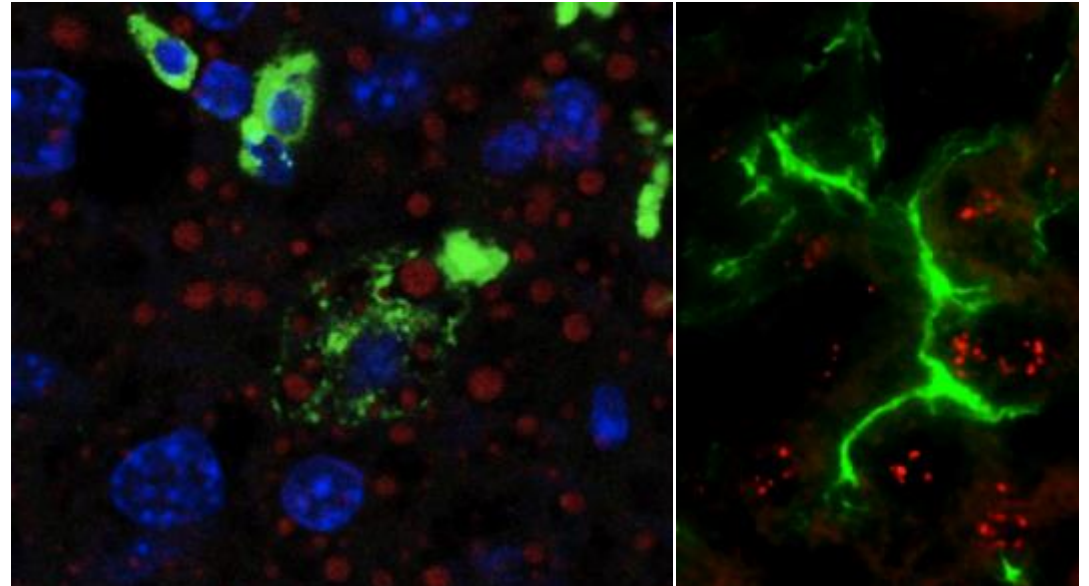
Dendritic cells efflux from the liver through lymph



Distribution of proliferating B cells during the second challenge of immune responses



Clustering between dendritic cells and proliferating T cells in the liver



Fat-laden Kupffer cells in NASH liver

Clustering between HSCs and dividing hepatocytes