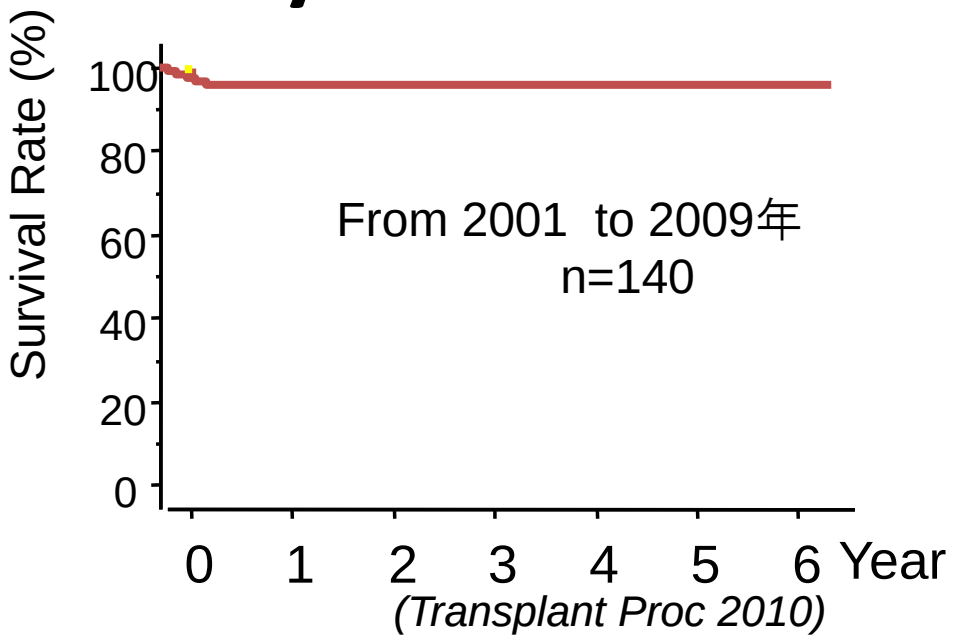
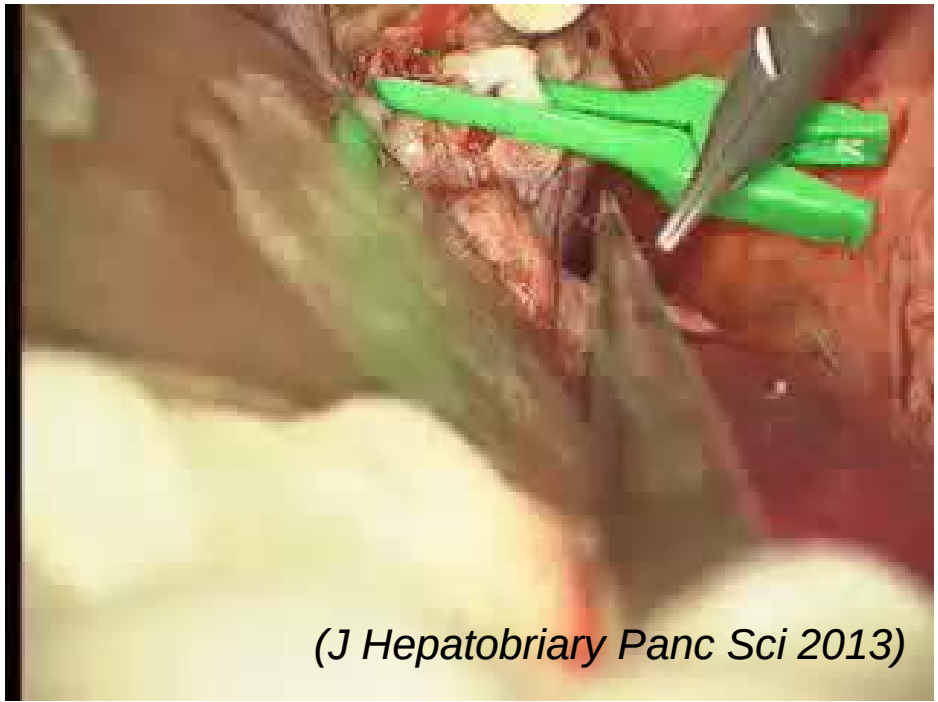
Firefly rat
Application for Drug Screening and Stem Cell Research

Eiji Kobayashi, MD, PhD
Department of Organ Fabrication,
Keio University School of Medicine, Japan

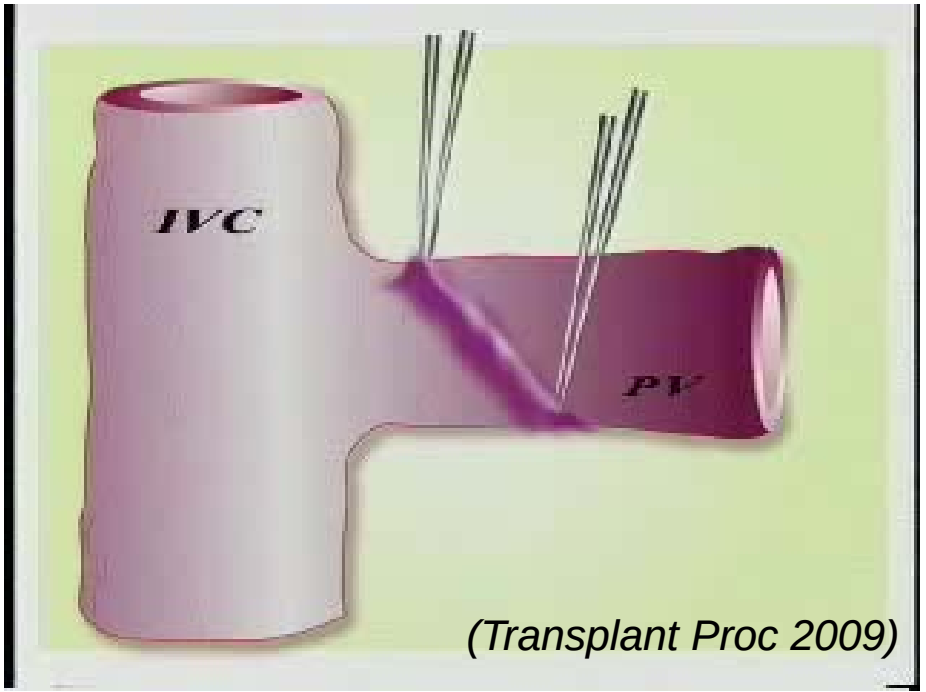
My clinical carrier



(Transplant Proc 2010)



(J Hepatobriary Panc Sci 2013)

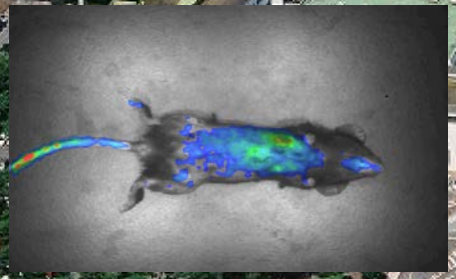


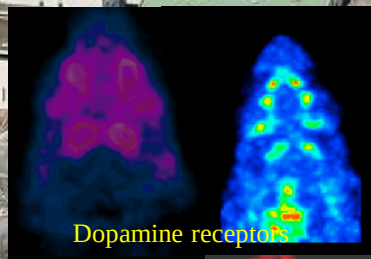
(Transplant Proc 2009)

Fifteen Years Carrie

Experimental Animal
Division of Small Animal

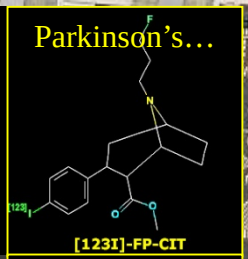
In vivo imaging Center

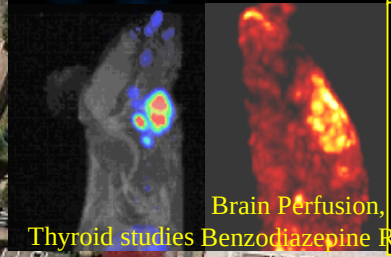




Dopamine receptors

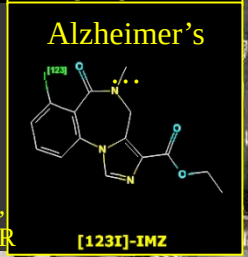
Parkinson's…



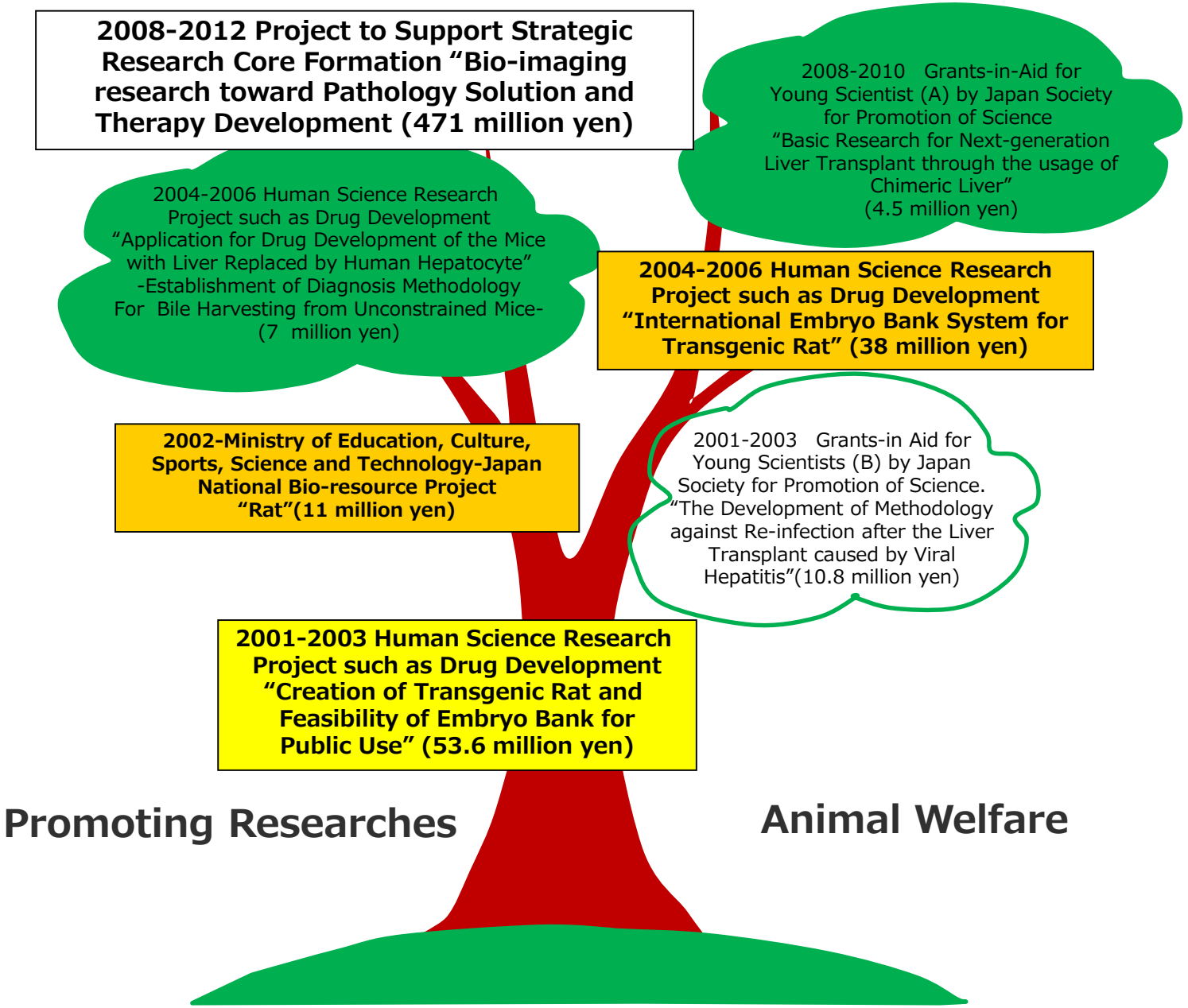


Brain Perfusion,
Thyroid studies Benzodiazepine R

Alzheimer's



Development of Experimental Animals through the Introduction of Bio-imaging Technologies



The trend of bioimaging for new drug development in Japan

国立研究開発法人量子科学技術研究開発機構
National Institutes for Quantum and Radiological Science and Technology

Alliance Business

The NIQRST conducts R & D alliance business cooperatively with several companies in specific fields "as groups" in order to solve the technical problems existing in the industry alone and to create innovation through the breakthrough of these problems. Traditionally, one-on-one collaborative research between individual companies and research institutions has been carried out numerous times. However, there have been few cases where multiple companies and research institutes (NIQRST) can become rivalry with each other on a one-to-one basis to deal with technical issues. This plan attracts attention as a new form of collaboration between industry and research institutes.

In FY2017, we will promote the alliance business in the following three fields.

1. Innovative Functional Polymer Material Alliance

2. Drug Discovery Alliance by Quantum Imaging "Brain and Mind"

Dr. Suhara Team leader with 14 companies

3. Drug Discovery Alliance of Quantum Imaging "Next Generation MRI / Contrast "

Dr. Aoki Team leader with 4 companies

(Translated by E Kobayashi)

Drug Discovery Alliance by Quantum Imaging "Brain and Mind"

Development of drugs for treating mental and neurological disorders has been stagnant for the past several years. It is mainly due to the inadequate clarification of the pathology of diseases, the absence of objective diagnostic methods and the identification of biomarkers that reflect the therapeutic effect. These result in the low success rate of drug development. Finally, the investment development cost will not be collected.

Dr. Suhara Team leader

In July 2017, the Science Council of Japan issued "Recommendations on Industry-Academia-Government Collaboration for Developing Therapeutic Methods for Mental and Neurological Diseases ". As the companies alone is difficult to develop the biomarker and the patient stratification technology etc., Public Private Partnerships (PPPs) are required to cooperate from the pre-competition phase across the researchers-the inter-company barriers.

Imaging biomarkers that can measure brain functions noninvasively can be evaluated with the same index from model animals to humans. Especially PET, which can directly image the site of action of drugs in humans, has been recognized to be important for drug evaluation.

(Translated by E Kobayashi)

Drug Discovery Alliance by Quantum Imaging "Brain and Mind“: Companies

astellas

Eisai

Otsuka

Otsuka-people creating new products for better health worldwide

ONO 小野薬品

SHIONOGI

Daiichi-Sankyo

大正製薬株式会社

大日本住友製薬

Takeda

田辺三菱製薬

すべての革新は患者さんのために

CHUGAI 中外製薬

ロシュ グループ

NOVARTIS

Pfizer

meiji

Meiji Seika ファルマ株式会社

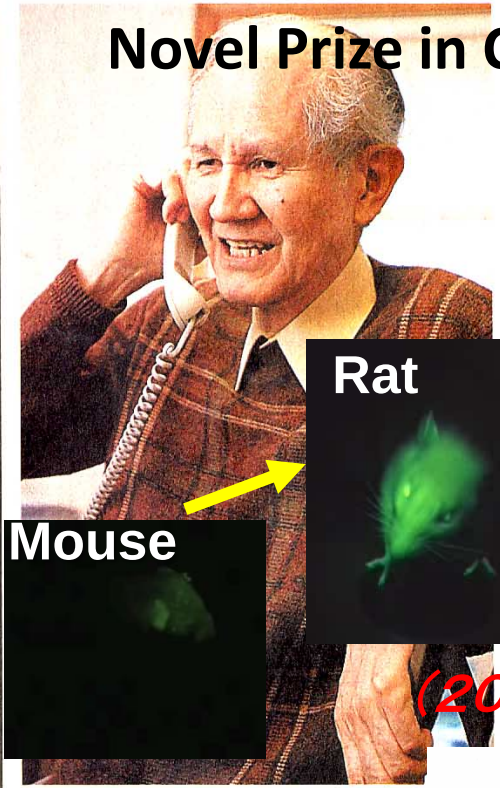
Summary of Imaging biomarkers in animals and human

Methods	Human	Animals	Resolution(Time)	Application	Characters
PET	○	○	1～2mm; Min	Metabolic	High Sensitive/ Picomolar
CT	○	○	50～100 μm;Min	Anatomical	Poor Contrast
Echo	○	○	50 μm ; Min	Anatomical	Bad for Bone & Lung
MRI	○	○	80～100 μm;Sec～Min	Anatomical/ Metabolic	High Sensitive/ High Contrast
Fluorescence	×	○	1～3mm;Sec～Min	Molecular	Sharp/Shallow
Bioluminescence	×	○	1～10mm; Sec～Min	Molecular	Dim/Deep

(Kobayashi E 2001)

Osamu Shimomura

Novel Prize in Chemistry 2008



Mouse

Rat

(2001)

Rabbit

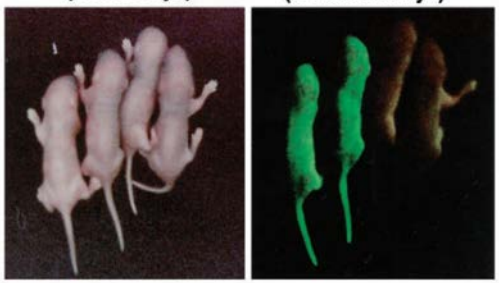
(2006)

Pig

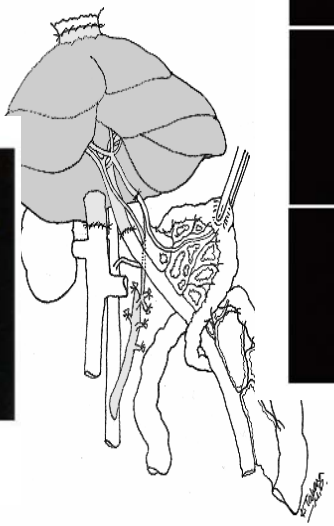
(2007)

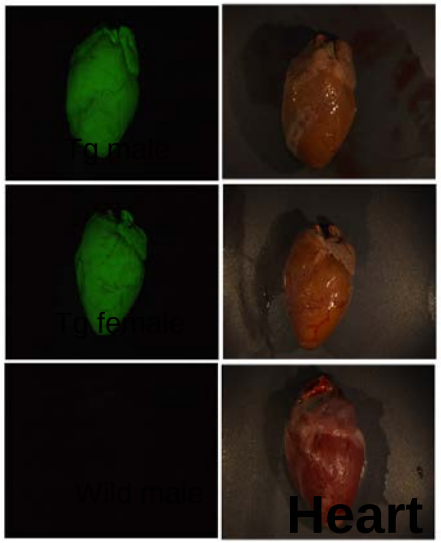
(2000)

(visible rays) (ultraviolet rays)



GFP (+) (+) (-) (-)
F1γ (+) (+) (-) (-)



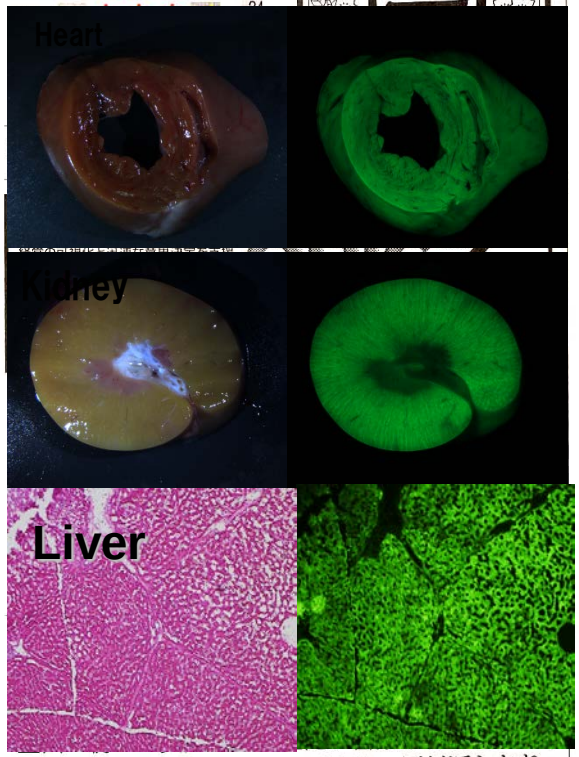


Tg male

Tg female

Wild male

Heart



Heart

Kidney

Liver

千葉 東京

8日、米マサチューセッツ州の自宅…ベル化学賞受賞の連絡を受けた後…話で喜びを語る下村脩さん＝AP

…賞を米ウッ…氏の計3人…で、緑色蛍…の研究で、…に使われて…

…ウェーデンの王立科学アカデ…洋…物学研究所・元上席研究員の下…表した。下村さんは、オワンクラ…質（GFP）を分離し、その構造を…く…子にくっつけて追跡する便利…

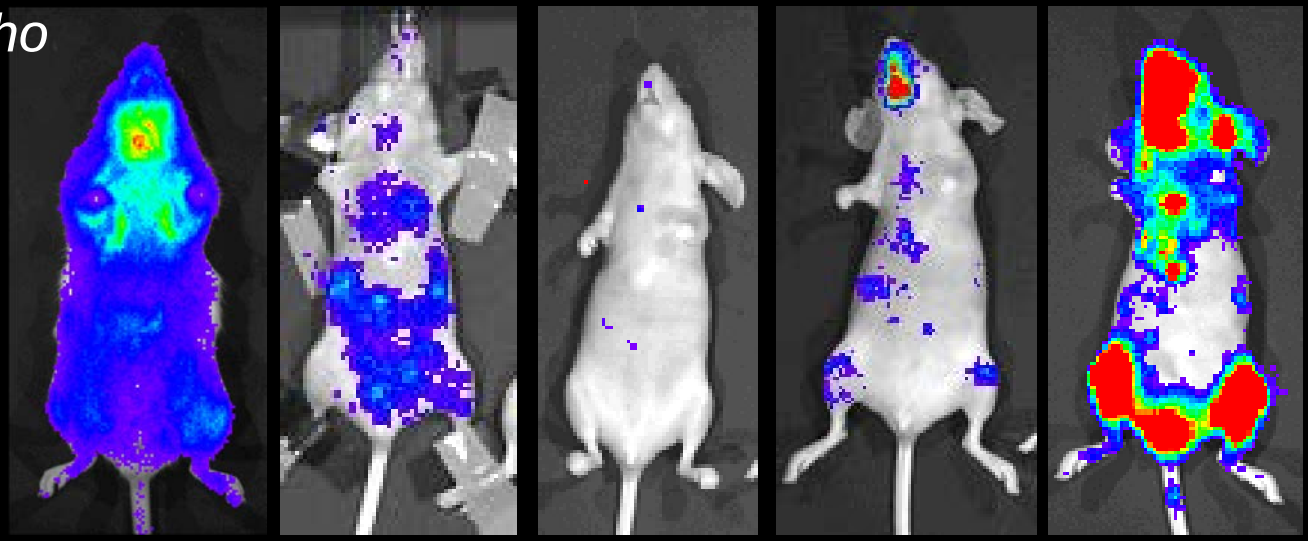
…かの県警に広がることには否定的。反対派もおり議論を呼びそうだ。 35面

…検討する。

Bioluminesence system after intracardiac injection of the human cancer lines

High Performance Echo Vevo 770

MDA-231

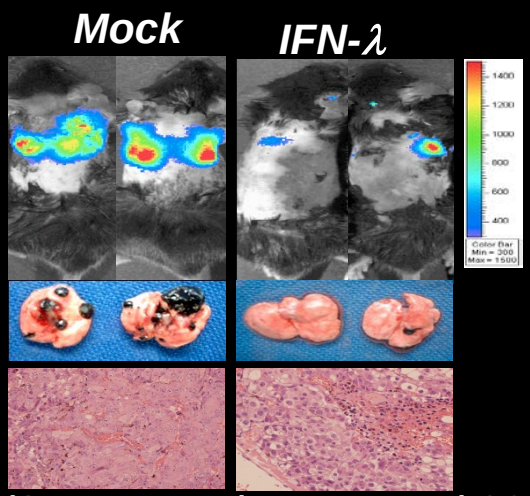


day0 *day1* *day12* *day28* *day42*

Days after tumor injection

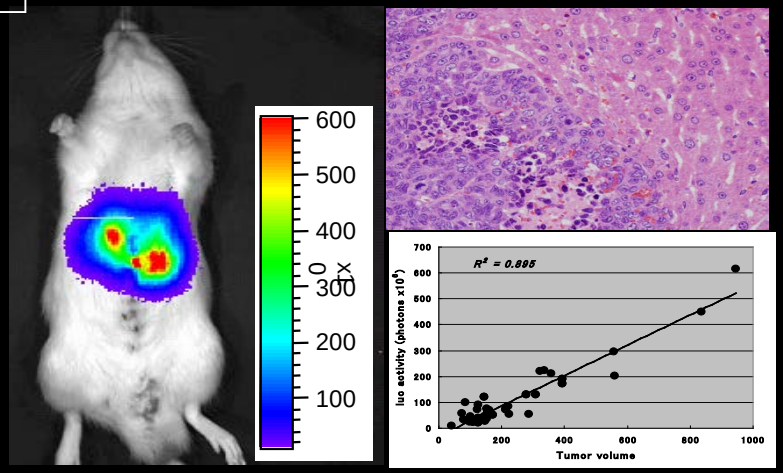
Many anti-cancer drugs tested by this technology

Mouse Model



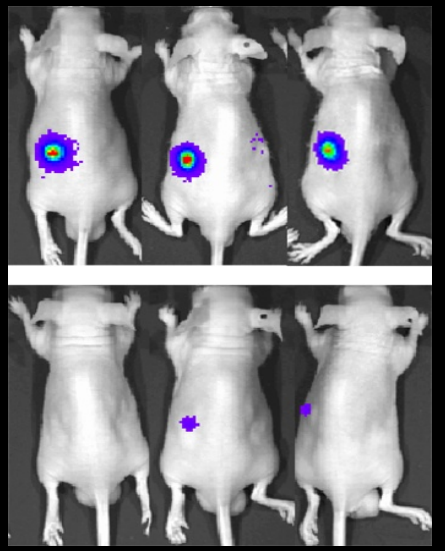
(Sato A, et al. J Immunol 2006)

Rat Model



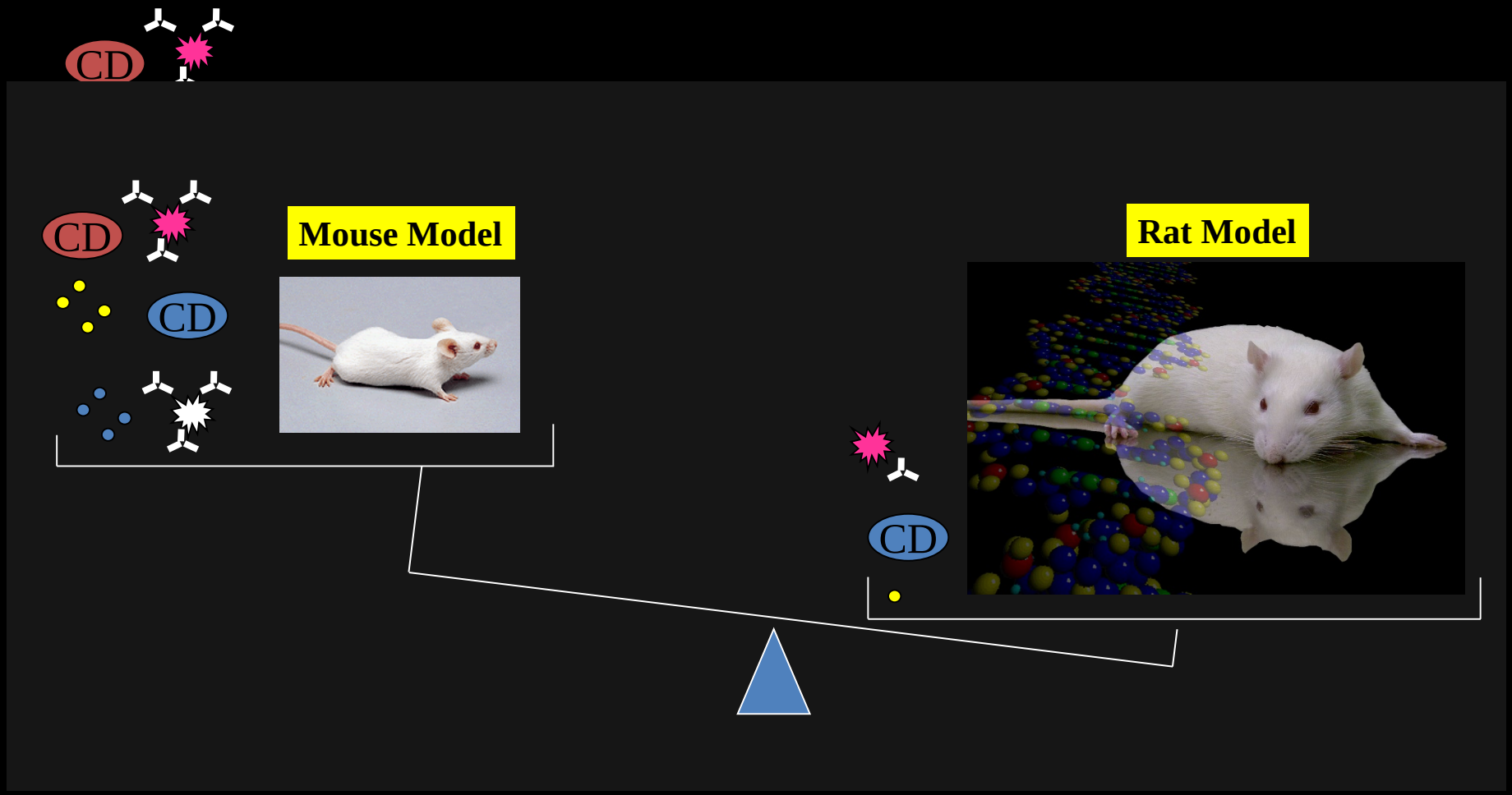
(Ohsawa et al. Transplantation 2006)

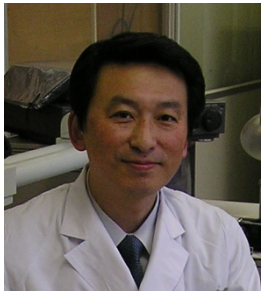
Xeno (Human to nu/nu) Model



(Okada T, et al. Mol Ther 2006)

Comparison of Animal Model between Rat and Mouse





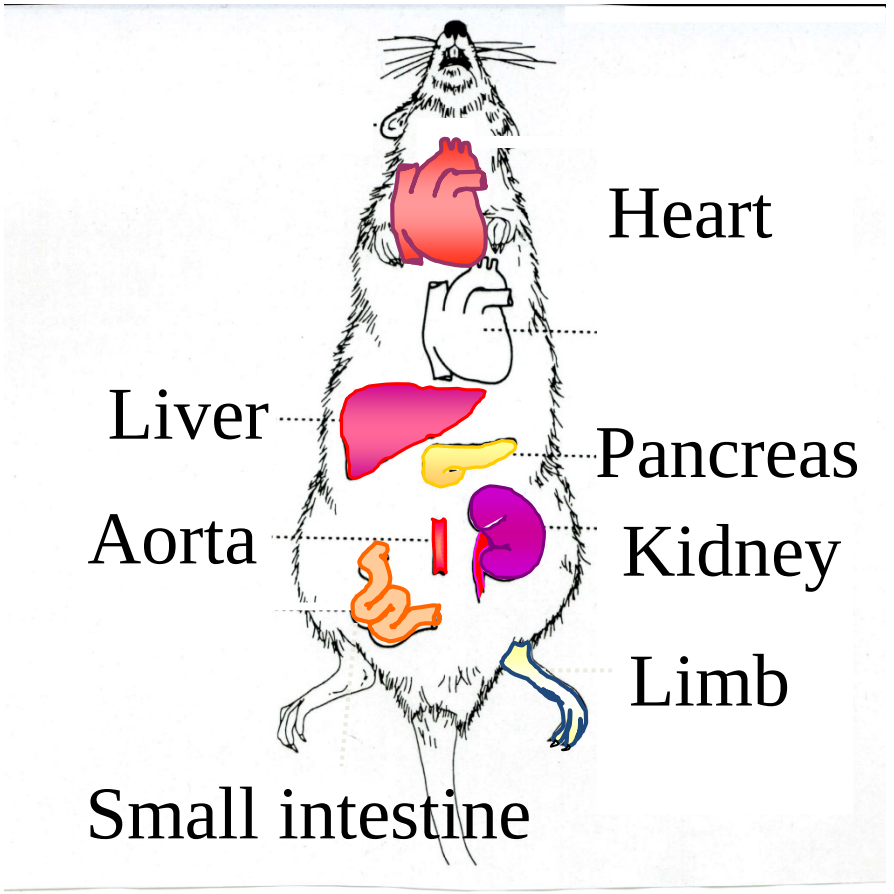
Professor Eiji Kobayashi

1982 Graduation from Jichi Med Sch
2001 Professor, CMM and Dep Surgery
2003 Director, CEM , Jichi Medical Univ

President-Elect; International Society for Experimental Microsurgery (ISEM)
Editorial; Transplantation, Microsurgery

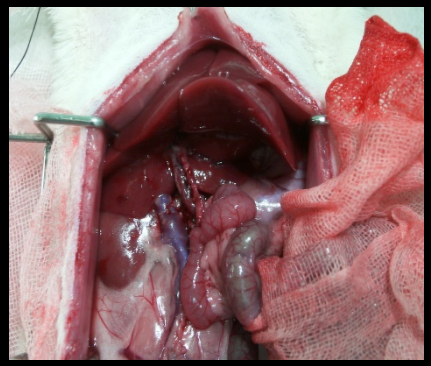
In his Labo, you can muster excellent technique.

Almost all organs can be transplanted in rats

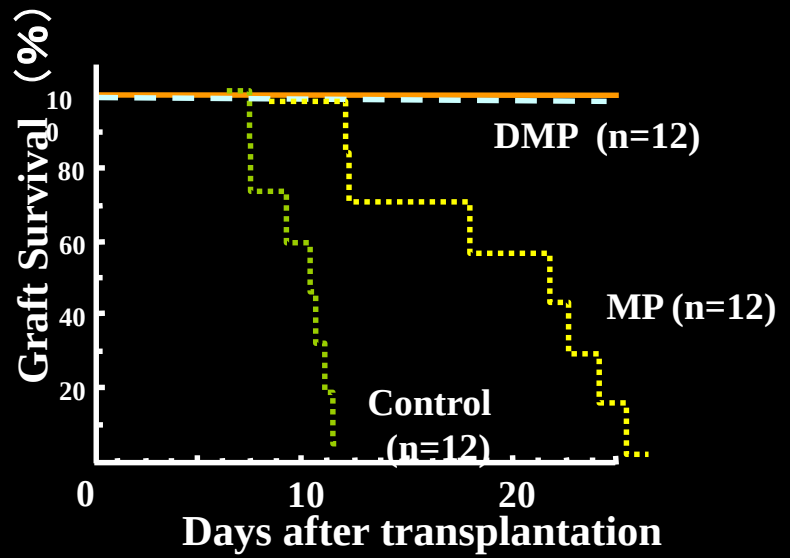


In vivo Screening System for Immunosuppressive Drugs

Rat OLT Model

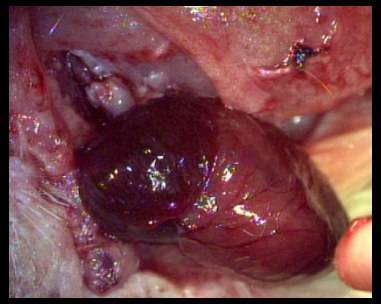


(Kobayashi E Microsurgery 1994)

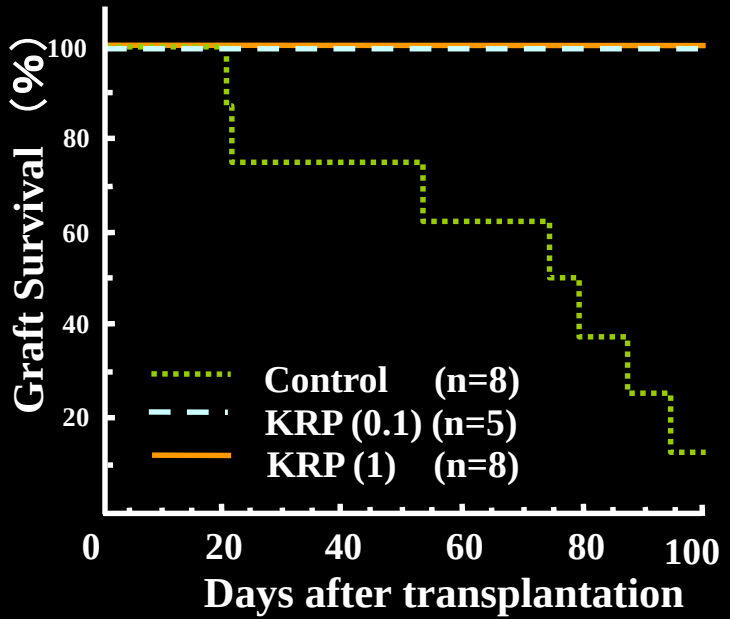


(Chimalakonda AP, et al. Transplantation 2005)

Rat HHT Model



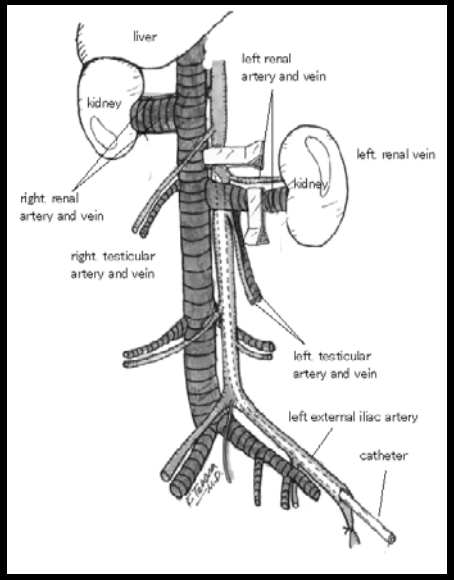
(Xu, et al Microsurgery 1999)



(Shimizu H, et al. Circulation 2005)

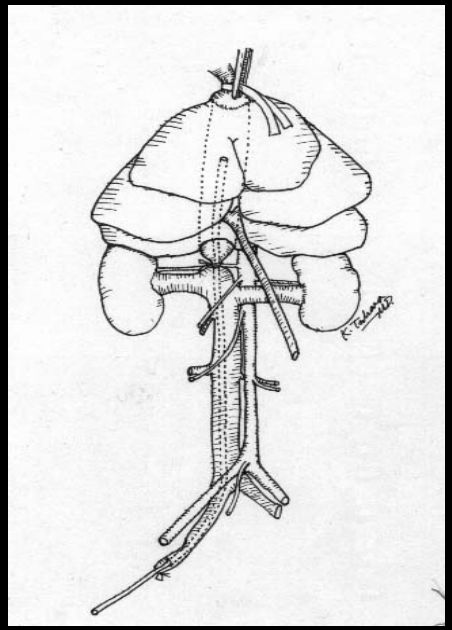
We can apply the catheter technique !

AAV vectors



(Takeda et al. Nephron Exp Nephrol 2004)

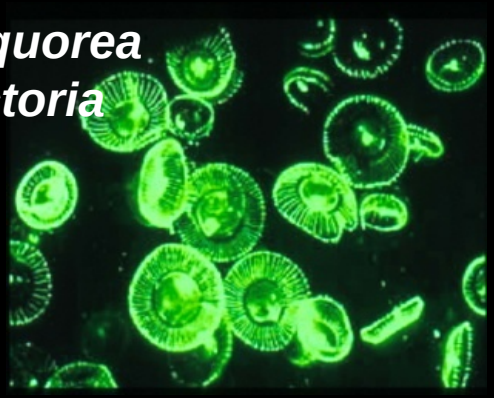
Naked DNA
siRNA

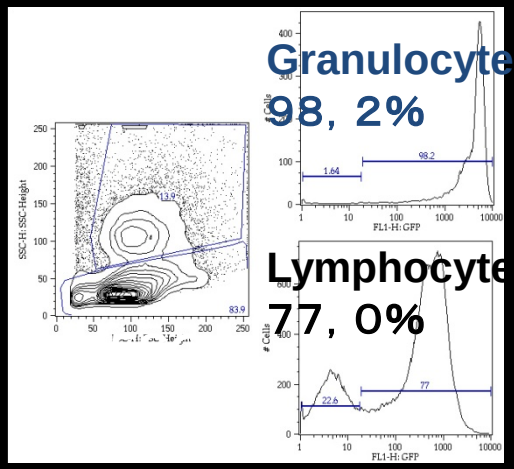


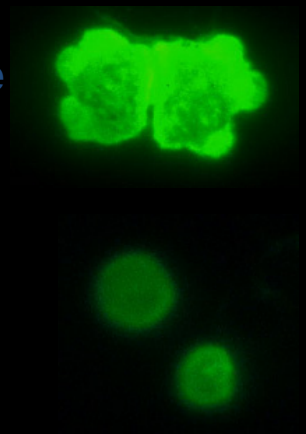
(Inoue S, et al. Transplantation 2004)

Benefits in bio-imaging system using ‘fluorescence’

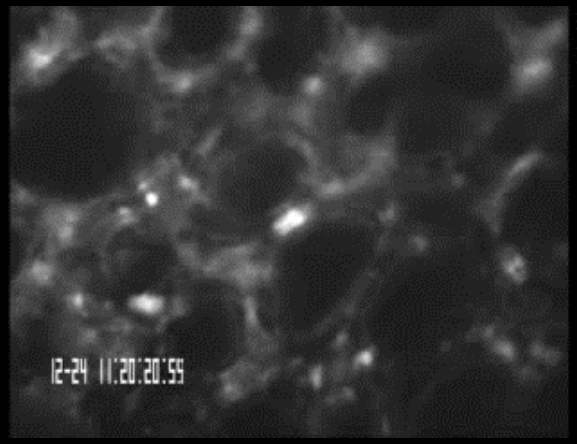
Aequorea victoria







(Sakuma Y, et al.Transplant Immunol 2004)



Lung; 25 min after LPS injection

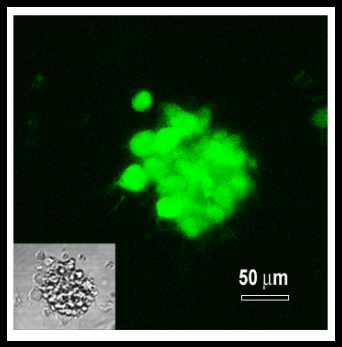
(Sato A, et al. Anesth Analg 2005)

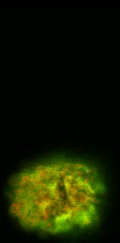
Transplanted lung immediately after re-perfusion

(Enomoto A, et al. Microsurgery 2007)

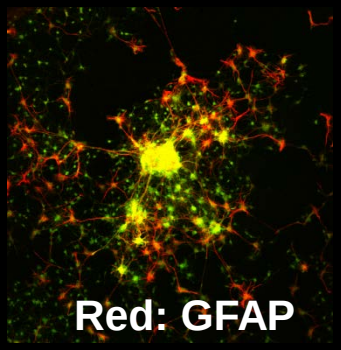
Differentiation from the fetal tissue

Neurosphere

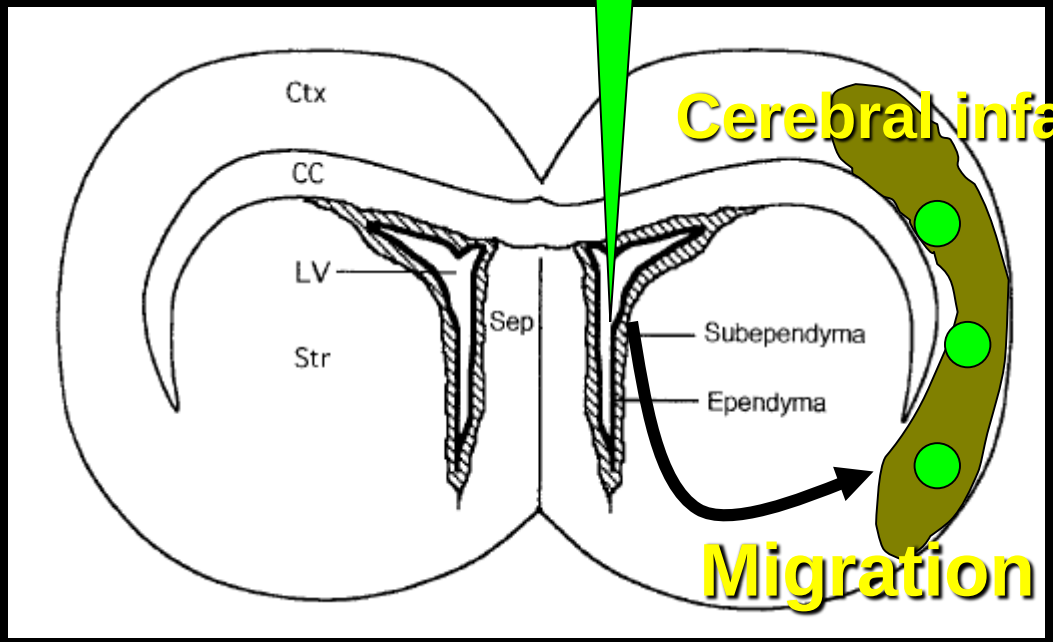




Red: Nestin

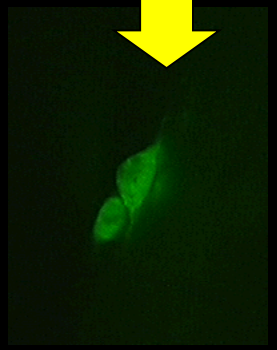


Red: GFAP



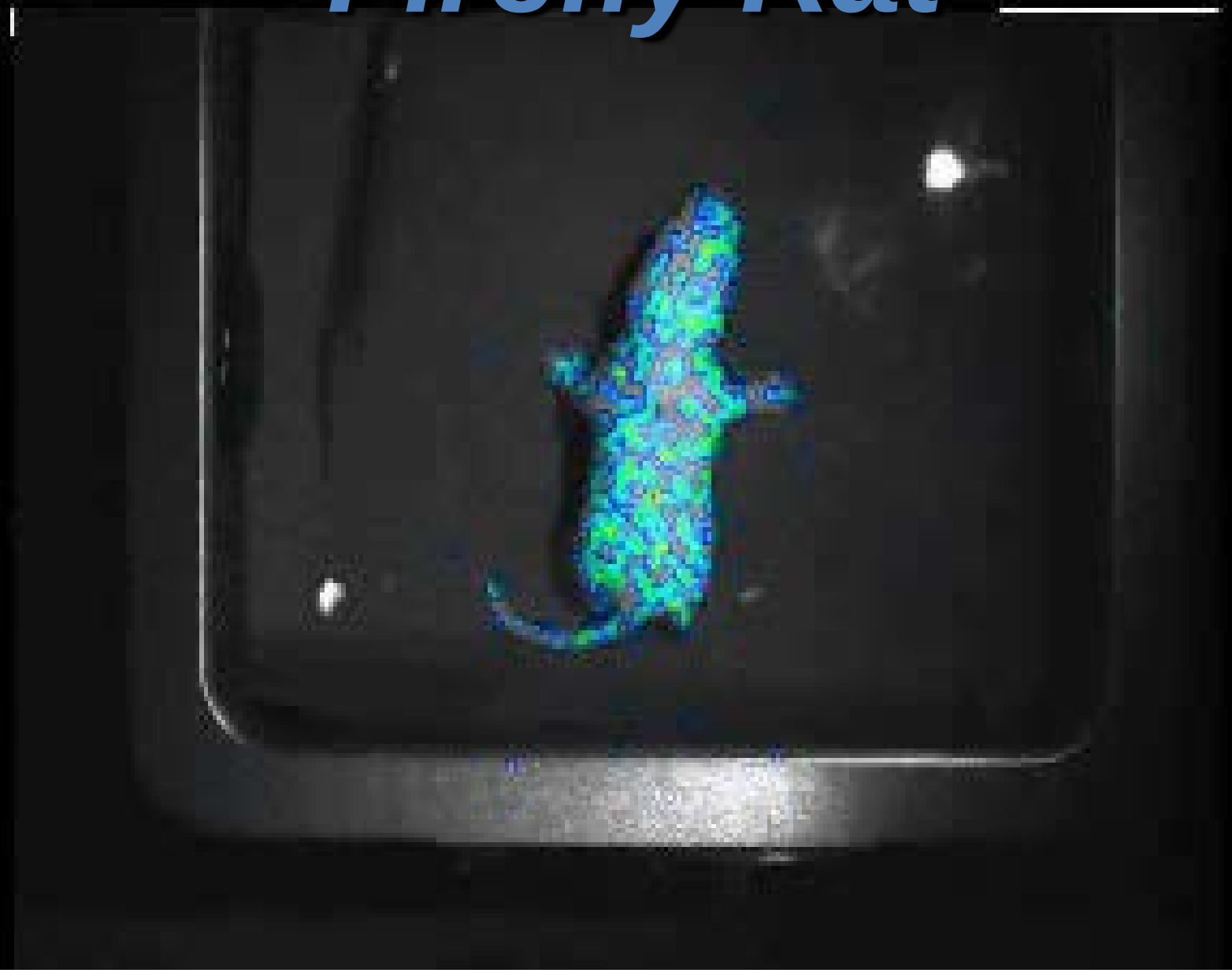
Cerebral infarction

Migration



(Inoue H, et al. BBRC 329:288,2005)

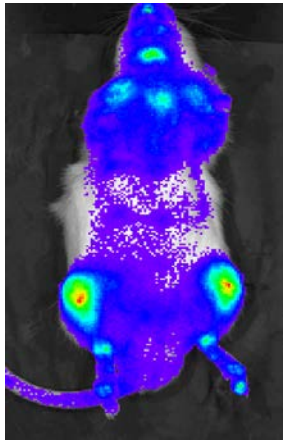
Firefly Rat



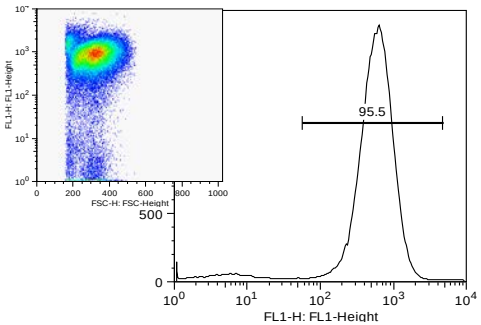
Double reporter Tg Rats

GFP LEW

Bone Marrow Tx

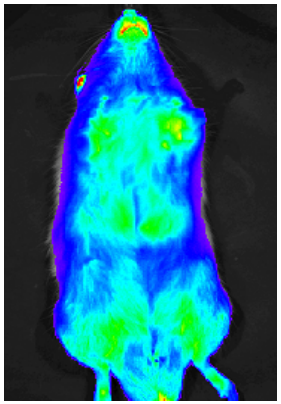


Luc detected by IVS system



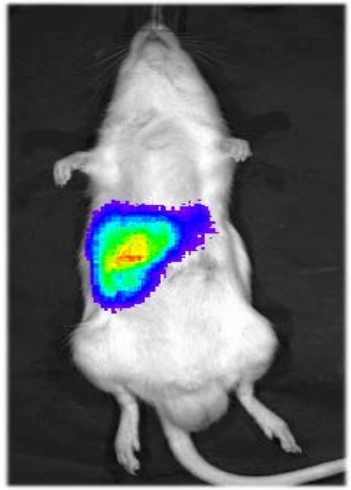
GFP detection by Facs scan

Luc LEW

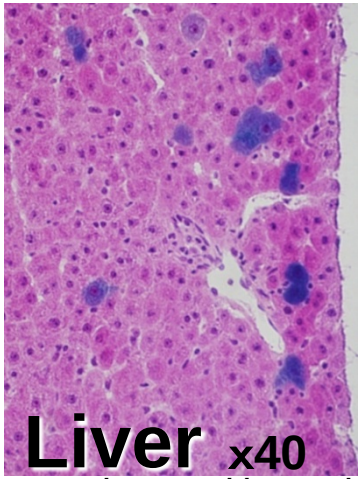


Hepatocyte Tx

LacZ LEW



Luc detected by IVS system

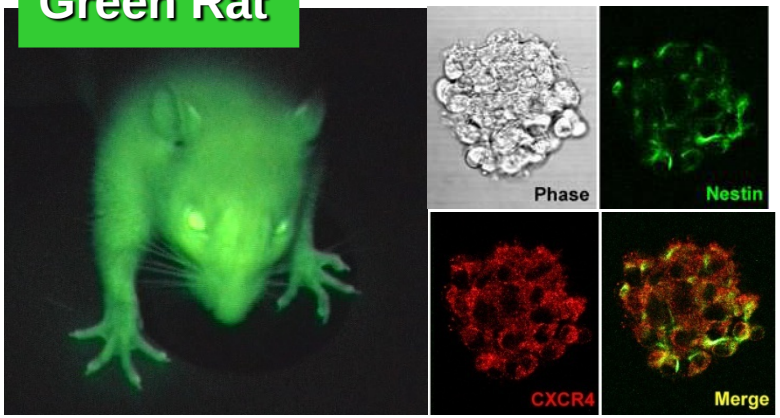


LacZ detected by pathrogy

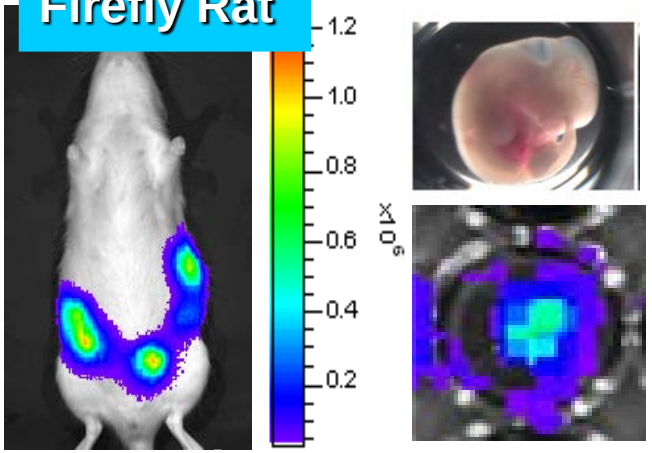
(Hakamata Y & Kobayashi E 2007)

A Powerful Tool for Translational Research
- Engineered Rats -

Green Rat



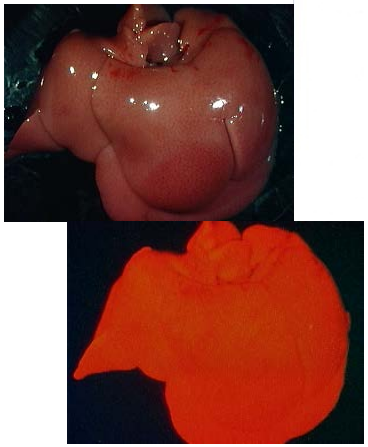
Firefly Rat



Blue Rat

Development

Red Rat



Gene Therapy

International Cooperation between DGMIF

Daily medy 2013년 02월 19일 화요일

대구첨복, 신약개발 동물실험 인프라 구축

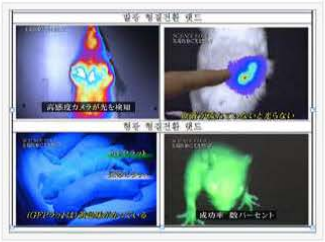
국내 최초 형광 형질전환 랫드 도입

2013.02.19 06:35 입력

대구경북첨단의료산업진흥재단이 글로벌 신약개발을 위한 동물실험 인프라를 구축했다.

대구경북첨단의료산업진흥재단은 오는 20일 국내 최초로 형광 형질전환 랫드를 일본 지치의과대학(고바야시 박사)으로부터 도입한다.

발광 형질전환 랫드는 특정 기질(Luciferin)과 반응하면 생체 내에서 빛을 발생시킬 수 있는 루시퍼라제(Luciferase)라는 유전자를 정상 랫드의 유전체에 인위적으로 삽입해 제작한 것이다.



형광 형질전환 랫드는 특정 파장대의 빛(excitation light, 여기광)을 비췄을 때, 형광을 낼 수 있는 GFP(Green Fluorescent Protein, 녹색형광단백) 유전자를 정상 랫드의 유전체에 인위적으로 삽입해 제작한 것이다.

특정 조건하에서 이들 동물자원이 발생시키는 빛은 광학영상 장비를 이용해 시각화가 가능하다.

이러한 광학영상시스템은 글로벌 연구소, 대학, 기업에서 신약개발을 위해 기본적으로 구축하는 추세이며, 대구경북첨단의료산업진흥재단 실험동물센터는 첨단 광학영상장비의 구축을 이미 완료한 상태이다.

이번 형광 형질전환 랫드 도입으로 대구경북첨단의료산업진흥재단 실험동물센터는 '신약 및 의료기기 개발을 위한 글로벌 수준의 동물실험지원시스템 구축'을 위한 초석을 다지게 됐다.

형광 형질전환 랫드 도입을 위해 김충용 실험동물센터장은 2011년 12월과 지난해 5월 두 차례에 걸쳐 일본 지치의과대학을 방문해 해당 동물자원(발광/형광 형질전환 랫드)을 도입하기 위한 구체적 협의를 진행했다.

지난해 6월 일본 지치의과대학과 자원 이전협약을 체결함으로써 도입을 위한 절차를 마치고, 지난 5일 국내 최초로 발광 형질전환 랫드를 들여왔고 오는 20일에는 형광 형질전환 랫드를 도입하게 됐다.

김충용 실험동물지원센터장은 "신약개발지원센터, 첨단의료기기개발지원센터뿐만 아니라 단지 내·외의 기업, 연구소, 대학 등을 효율적으로 지원하기 위해 우수 동물자원을 적극적으로 도입할 계획"이라고 밝혔다.

Dailymedi 김도경기자 kimdo@dailymedi.com

NO. 2012-37

DGMIF

LETTER OF COMMISSION

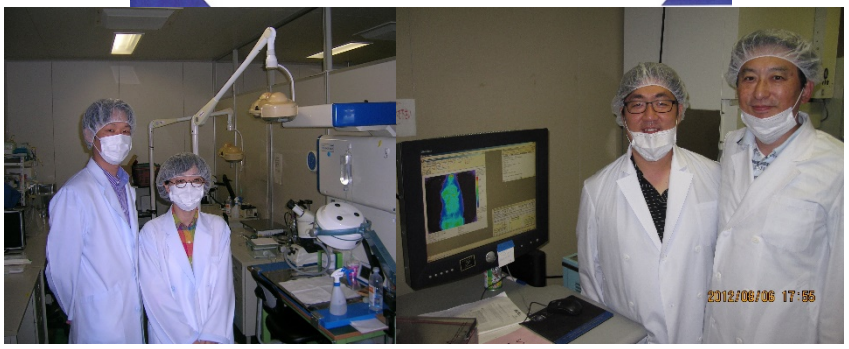
Eiji Kobayashi, M.D., Ph.D.
Visiting Professor
Jichi Medical University

Dr. Eiji Kobayashi is hereby commissioned to be *a Special Advisor to Daegu-Gyeongbuk Medical Innovation Foundation (DGMIF).*

Area: Laboratory Animal Center
Period of Commission: April 1, 2012~ March 31, 2014
Specialty: Bio-imaging Screening Method with Photogenic Human Tumor Cell Lines/Transgenic Rats

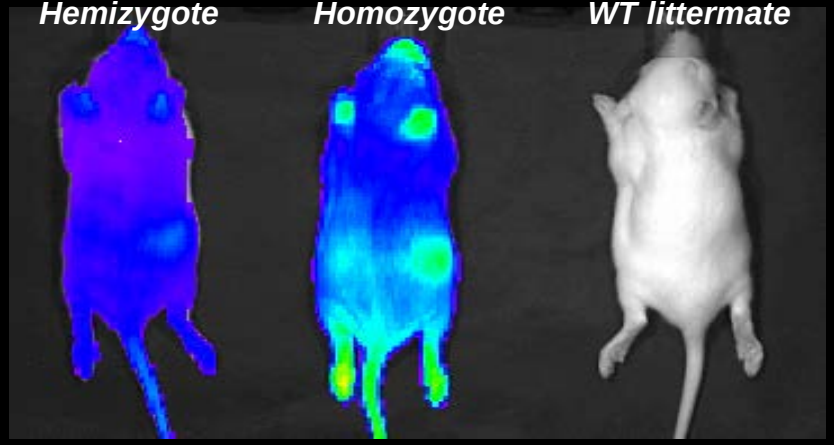
April 1st, 2012

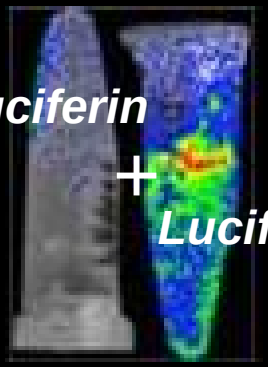
YouSeung Kim
Chairman
DGMIF
Republic of Korea



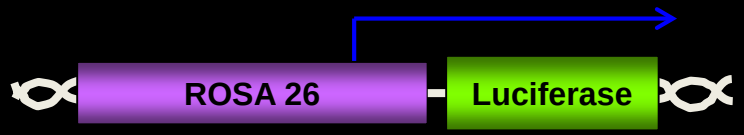
Photnus pyralis

Firefly Tg Rat



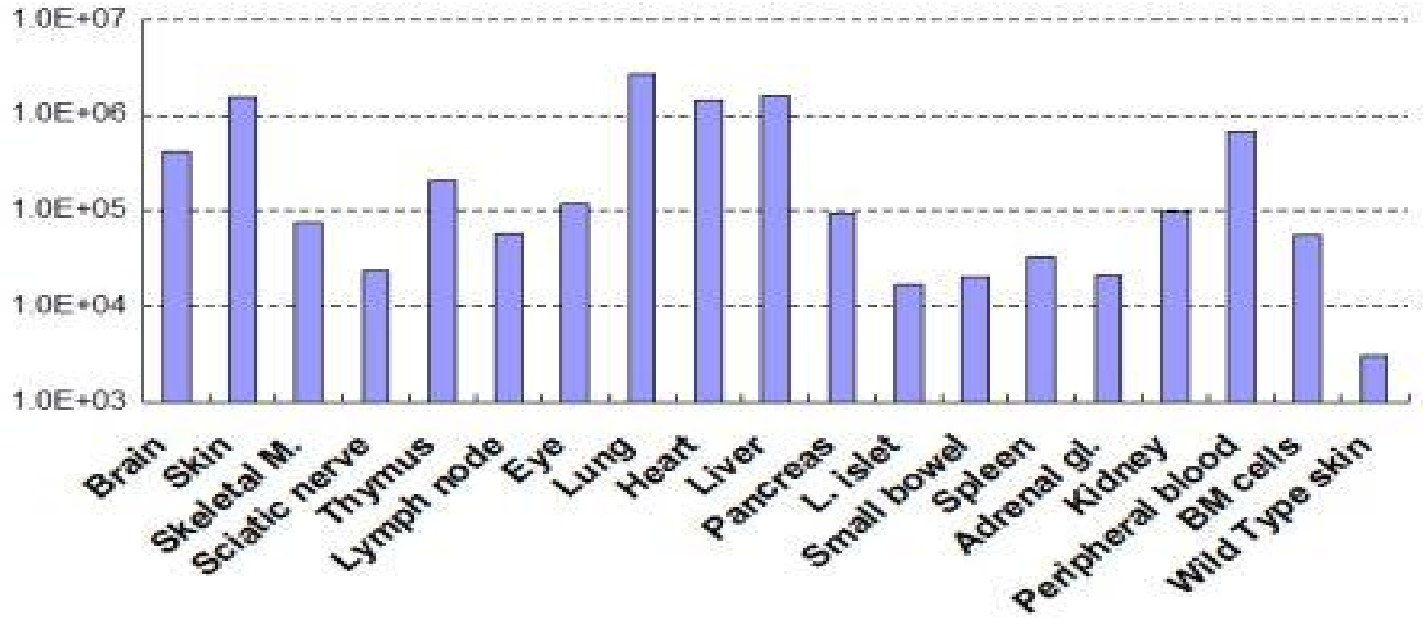


Luciferin + *Luciferase* → ***Luminescence***

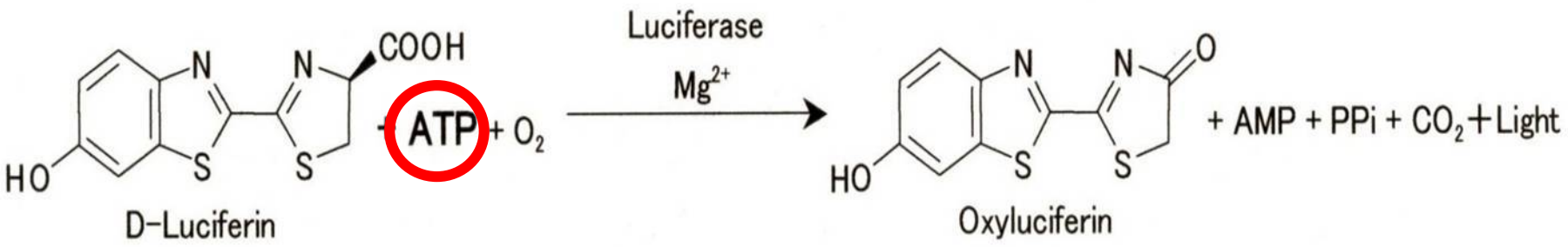


(Hakamata Y, et al. Transplantation 2006)

Light Emission of Luciferase Transgenic Rat



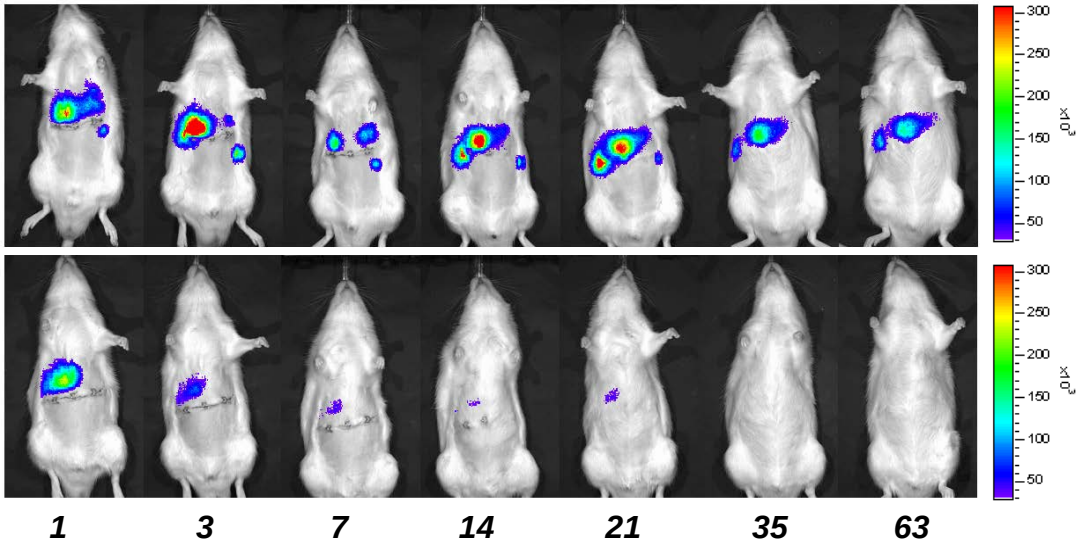
(Hakamata Y, et al. Transplantation 2006)

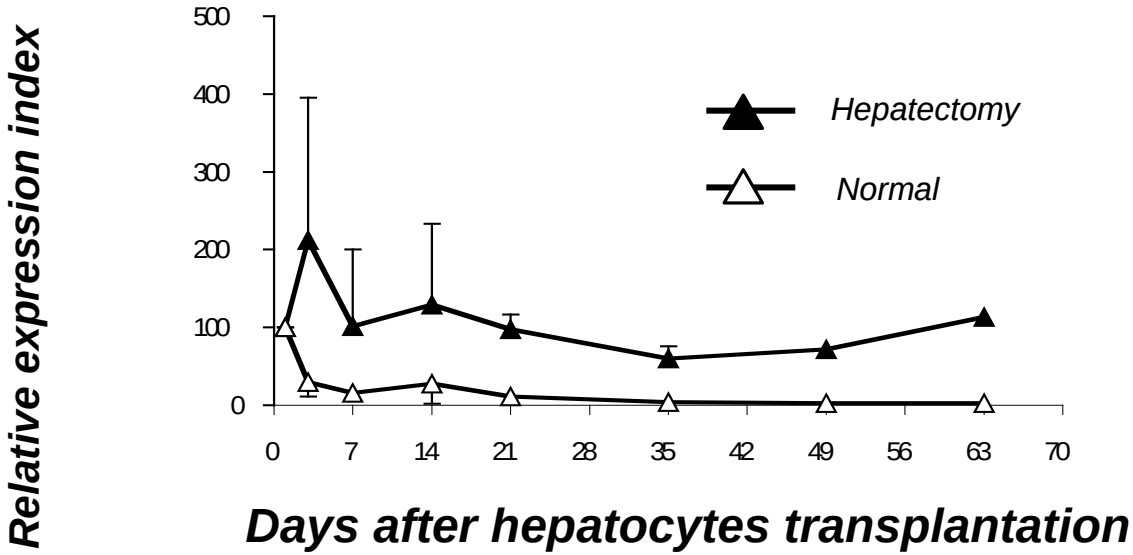


Chase the transplanted hepatocytes

Hepatectomized

Normal

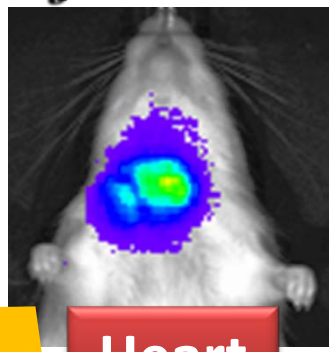




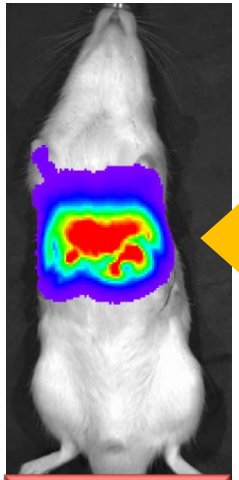
(Hakamata Y, et al Transplantation 2006)

Real-Time Detection System for Perfect Microsurgery

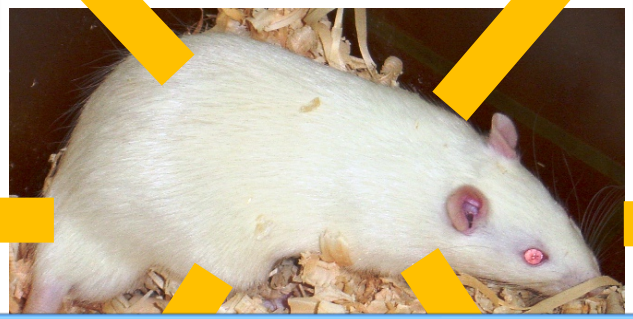
Skin



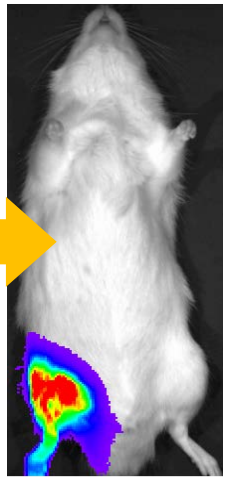
Heart



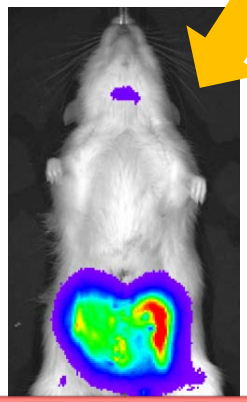
Liver



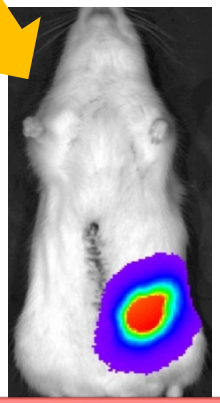
"Firefly" Transgenic LEW Rat



Extremities



Small Bowel



Kidney

Luminescence technology in preservation and transplantation for rat islet.

Negishi K, et al. Islets. 2011

Impact of normothermic preservation with extracellular type solution containing trehalose on rat kidney grafting from a cardiac death donor

Iwai S, et al. **PLoS One. 2012**

A luminance-based heart chip assay for assessing the efficacy of graft preservation solutions in heart transplantation in rats

Maeda M, et al. **Heart Asia. 2013**

Luminescence-based assay to screen preservation solutions for optimal ability to maintain viability of rat intestinal grafts

Kasahara N, et al. **Transplant Proc 2013**

Use of Mesenchymal Stem Cell-Conditioned Medium to Activate Islets in Preservation Solution

Kasahara N, et al. **Cell Med. 2013**

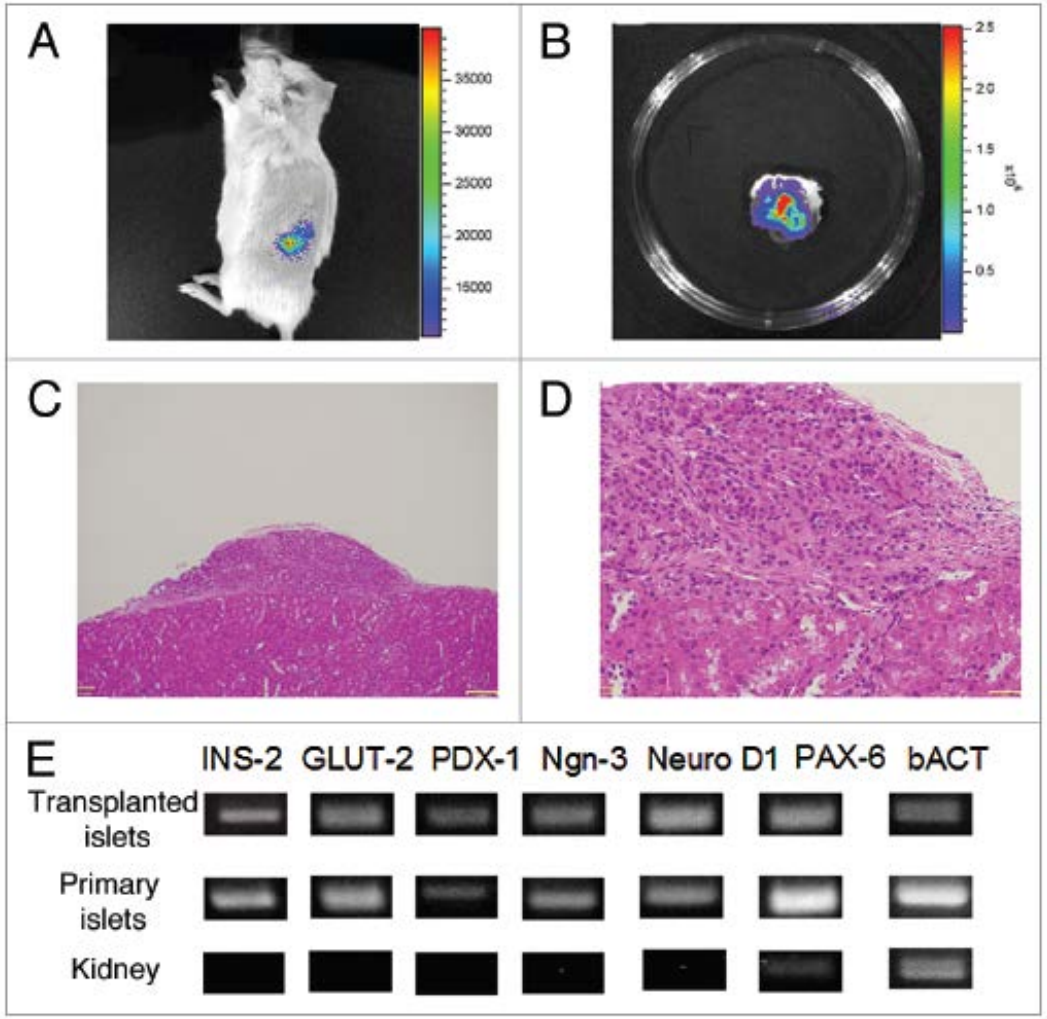
Evaluation of liver preservation solutions by using rats transgenic for luciferase

Doi J, et al. **Transplant Proc 2014**

Muscle is a target for preservation in a rat limb replantation model

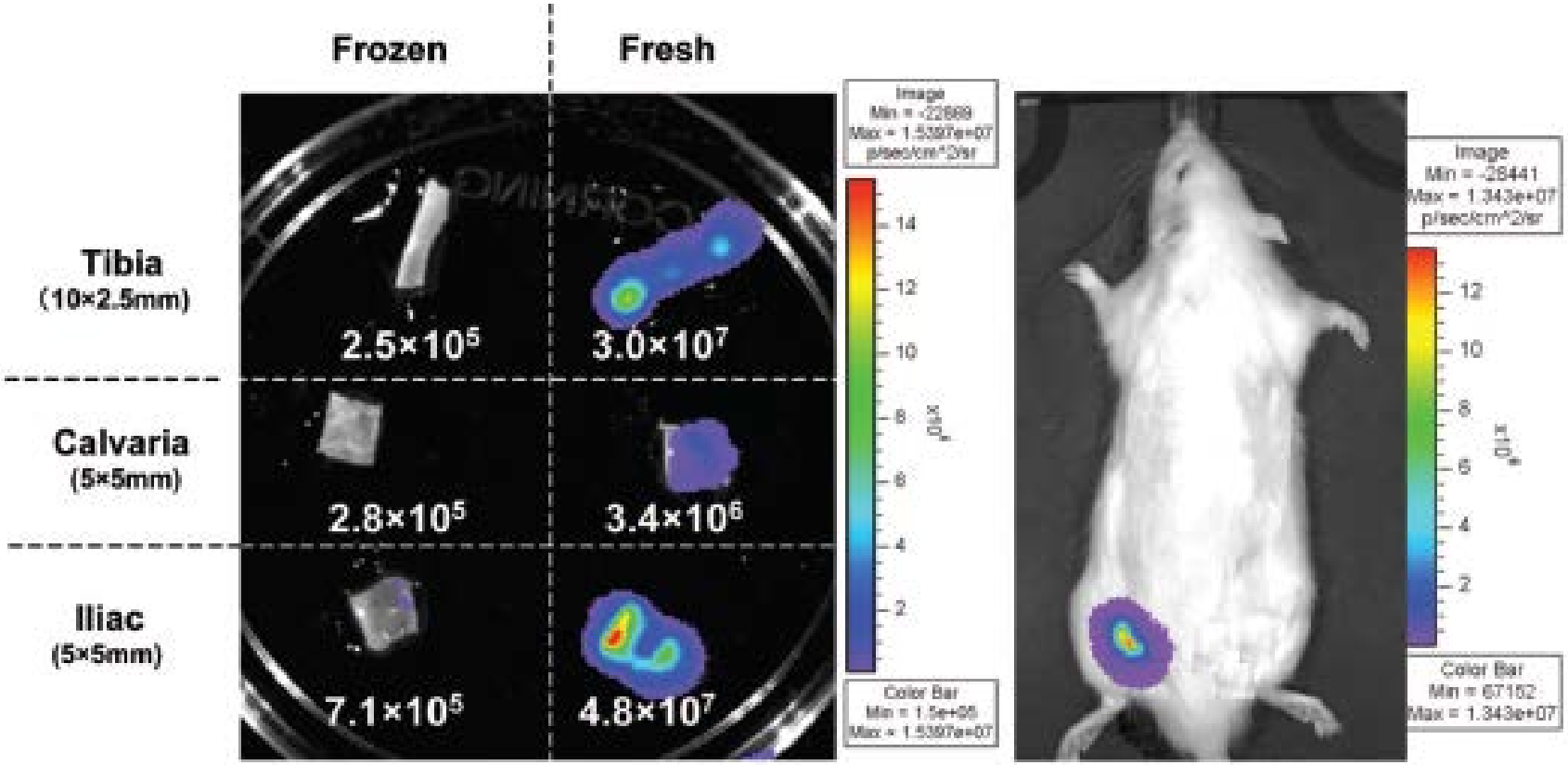
Iijima Y, et al **Plast Reconstr Surg Glob Open. 2013**

Luminescence Technology in preservation and transplantation for rat islet



(K Negishi et al. Islets 3(3):111-117 2011)

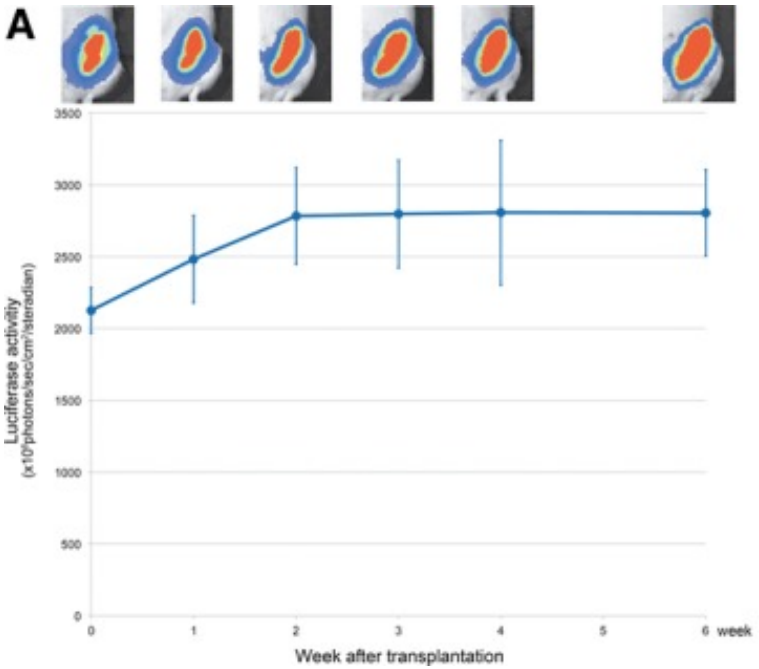
Luminescence Imaging of Regenerating Free Bone Graft in Rats



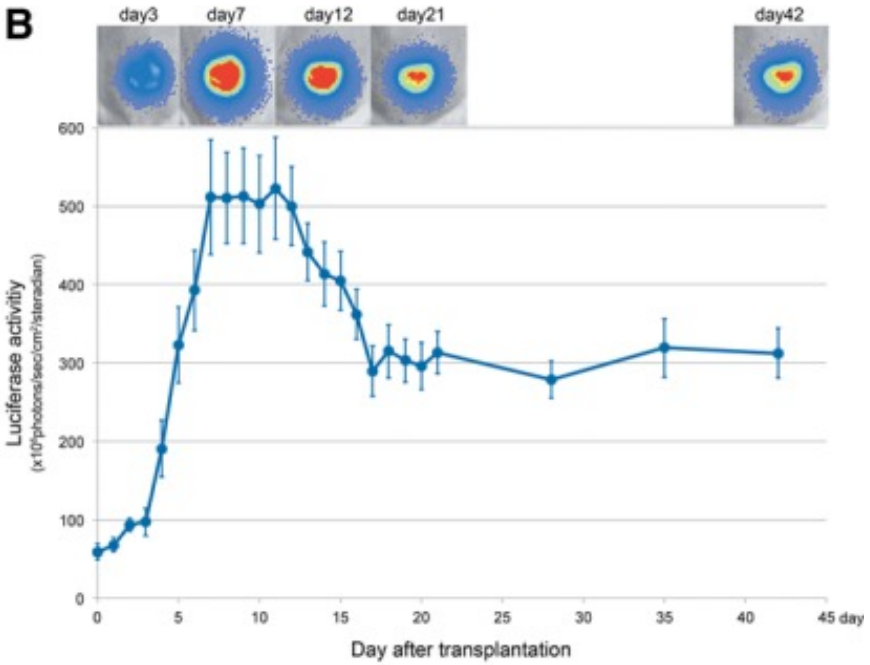
(A Yamaguchi et al. Plast Peconstr Surg 127(1):78-87 2011)

The Fate of Nonvascularized Fat Grafts: Histrogical and Bioluminesent Stusy

Vascularized Graft

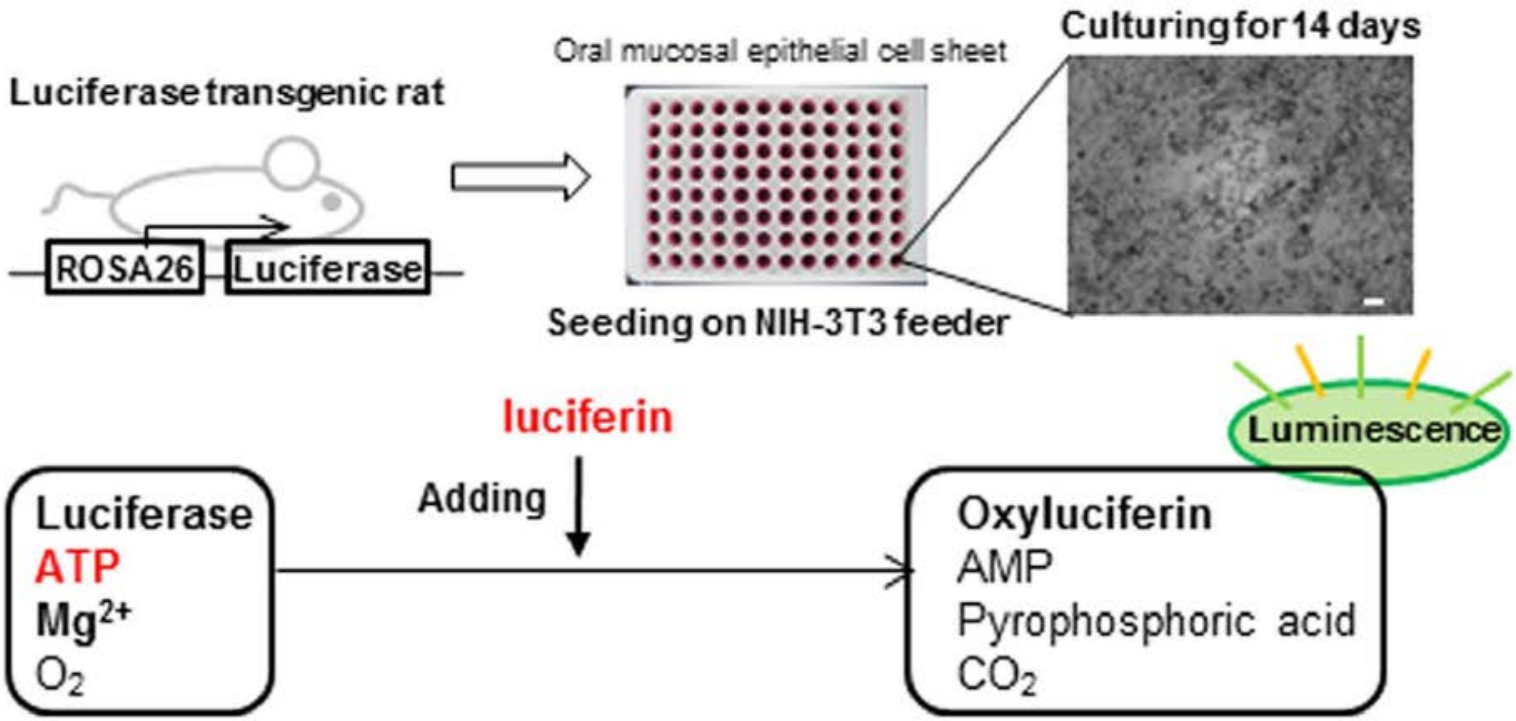


Non-Vascularized Graft



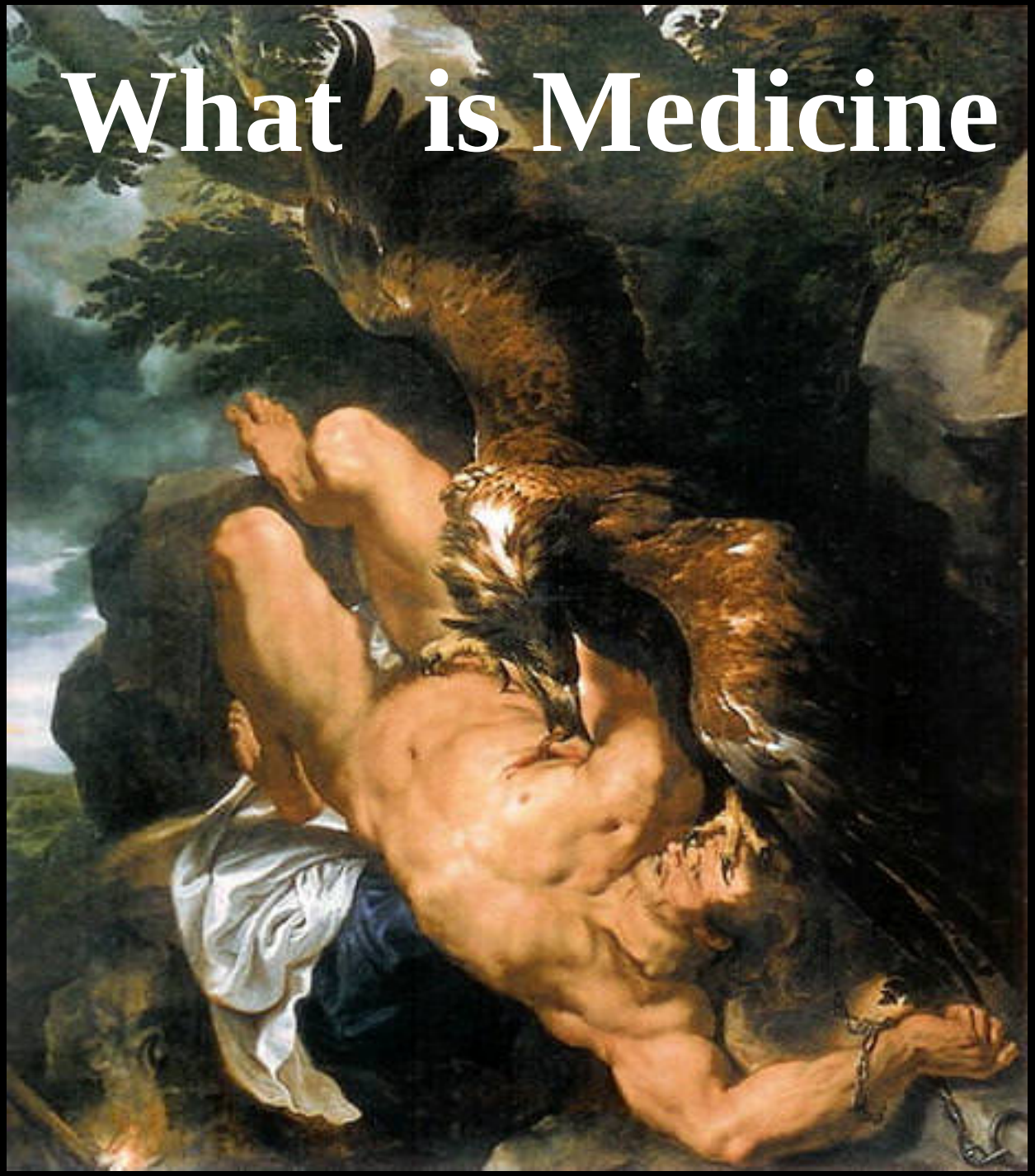
(Sunaga A, et al. Plast Reconstr Surg Glob Open 2013)

Ebselen Preserves TissueEngineered Cell Sheets and their Stem Cells in Hypothermic Conditions

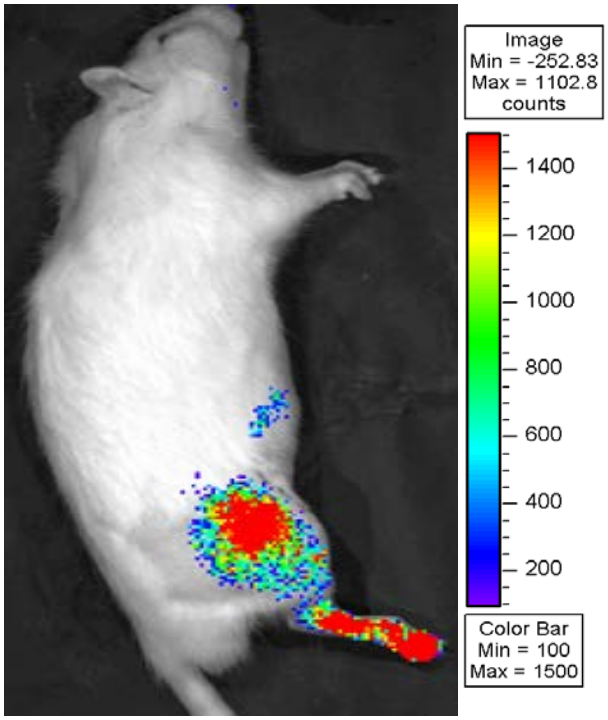


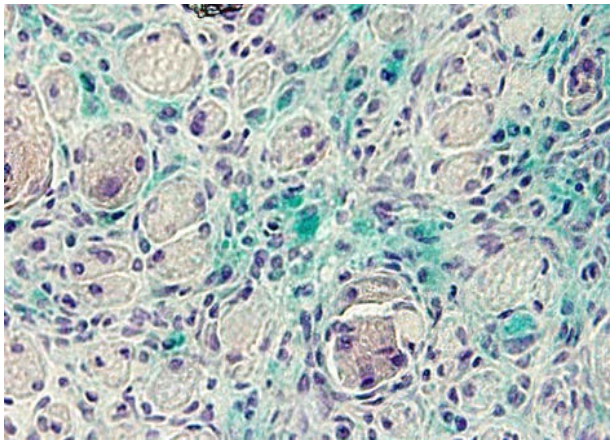
(Katori R et al. Scientific Reports 2016)

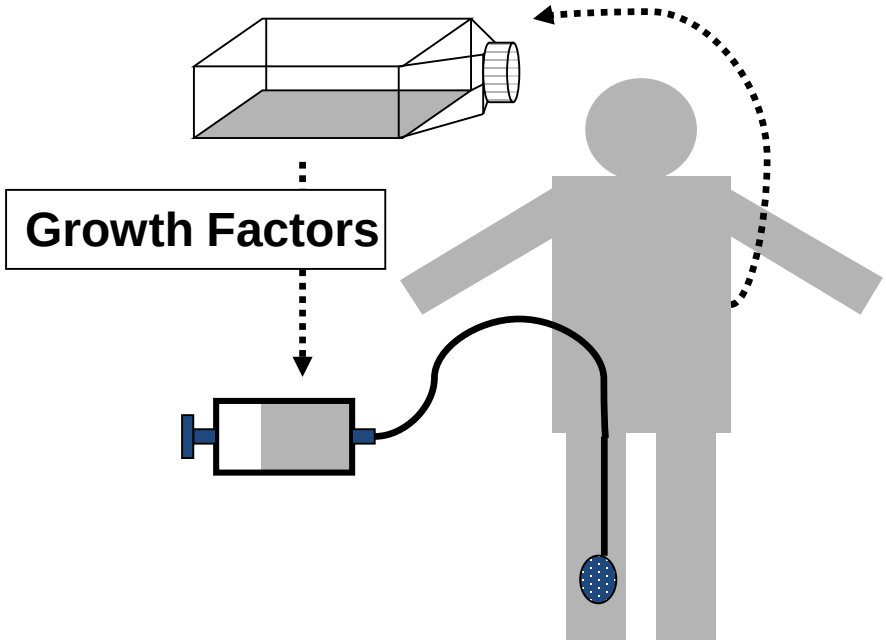
What is Medicine ?



Application of Somatic Stem Cells for Regenerative Medicine

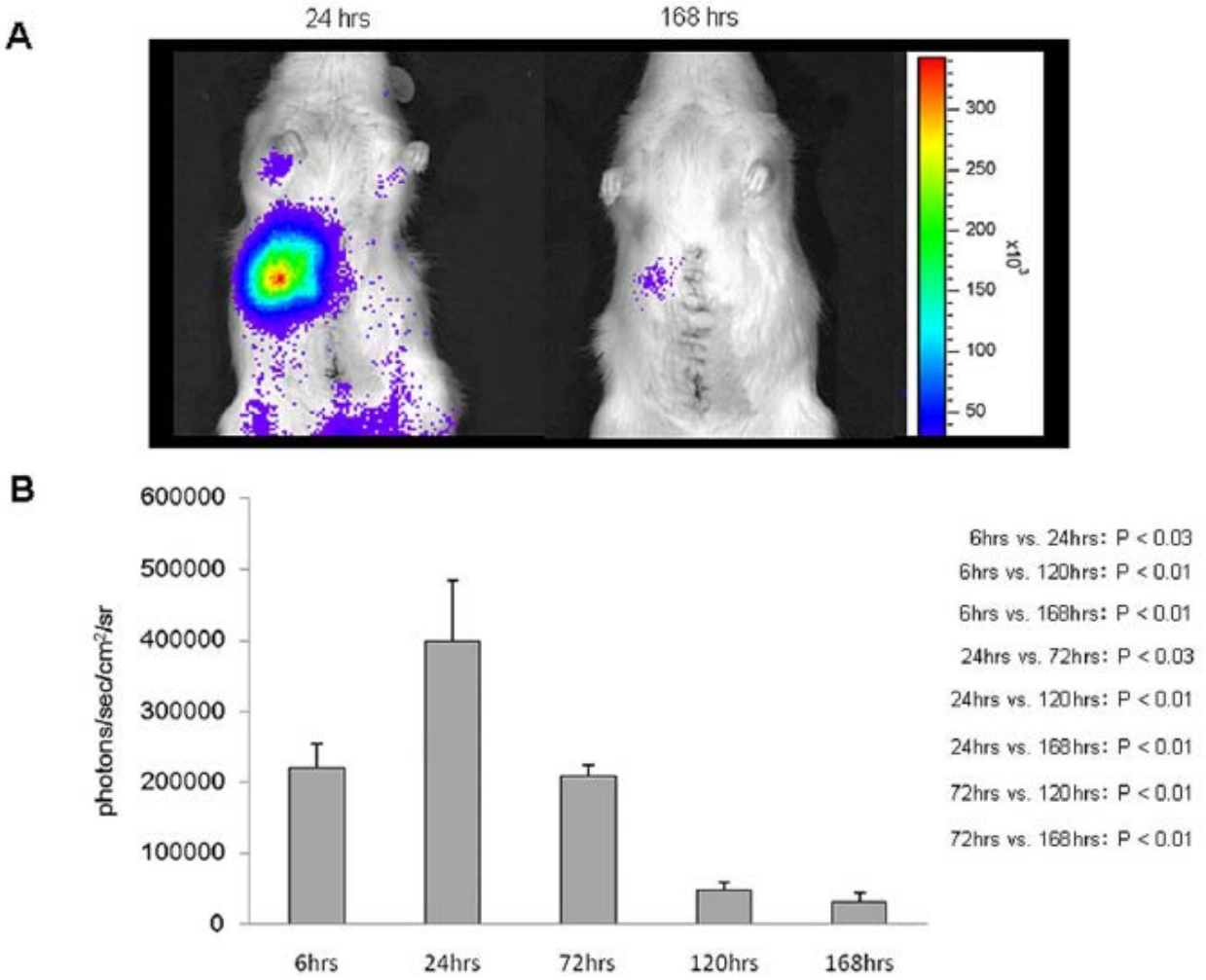






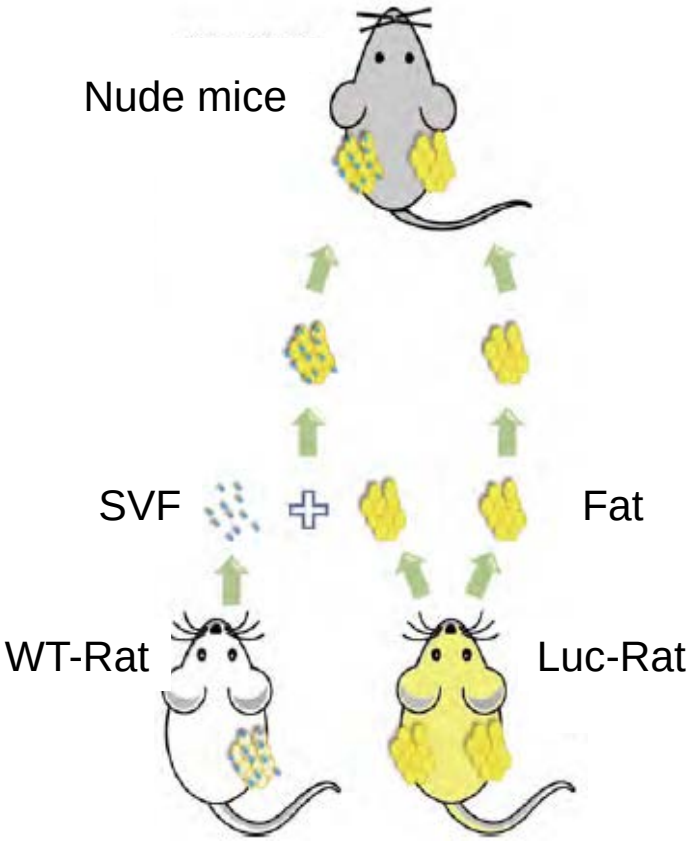
(Hara M, et al. J Autoimmun 2008)

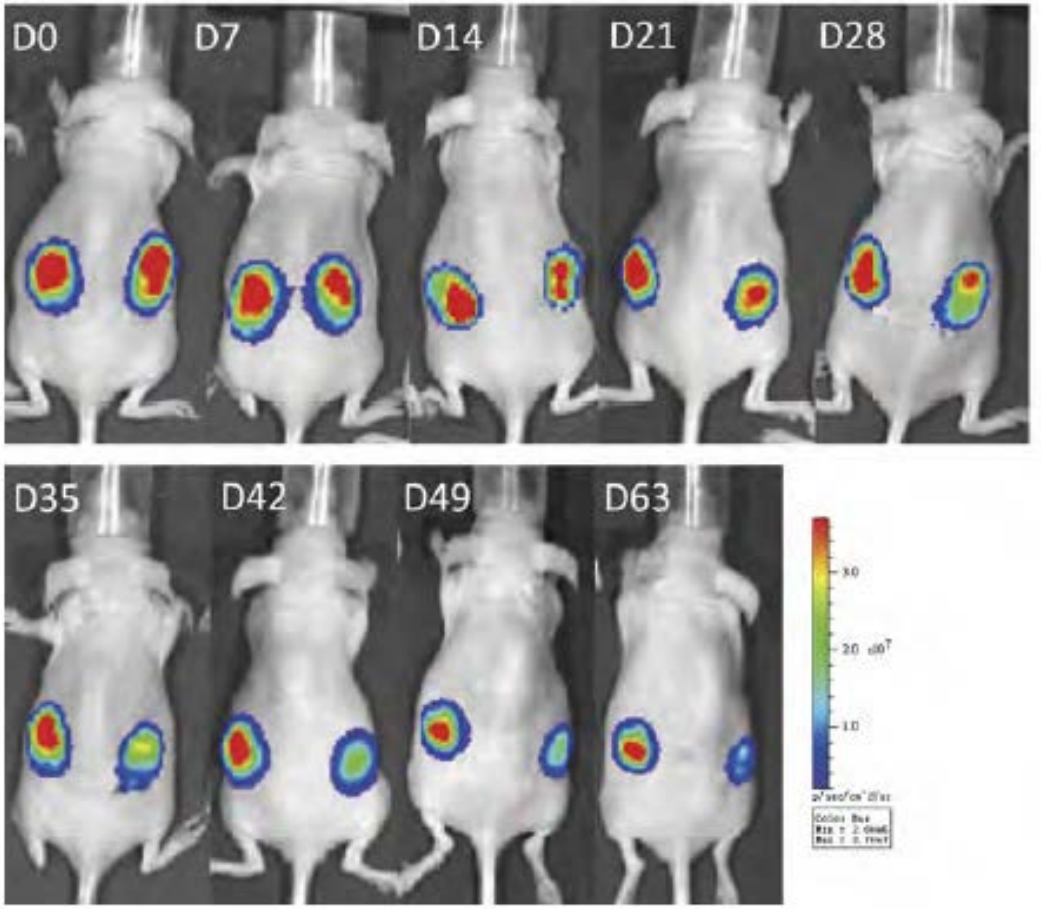
Bone Marrow-Derived Mesenchymal Stem Cells Ameliorate Hepatic Ischemia Reperfusion Injury in a Rat Model



(H Kanazawa et al. PLoS One 6(4):e19195 2011)

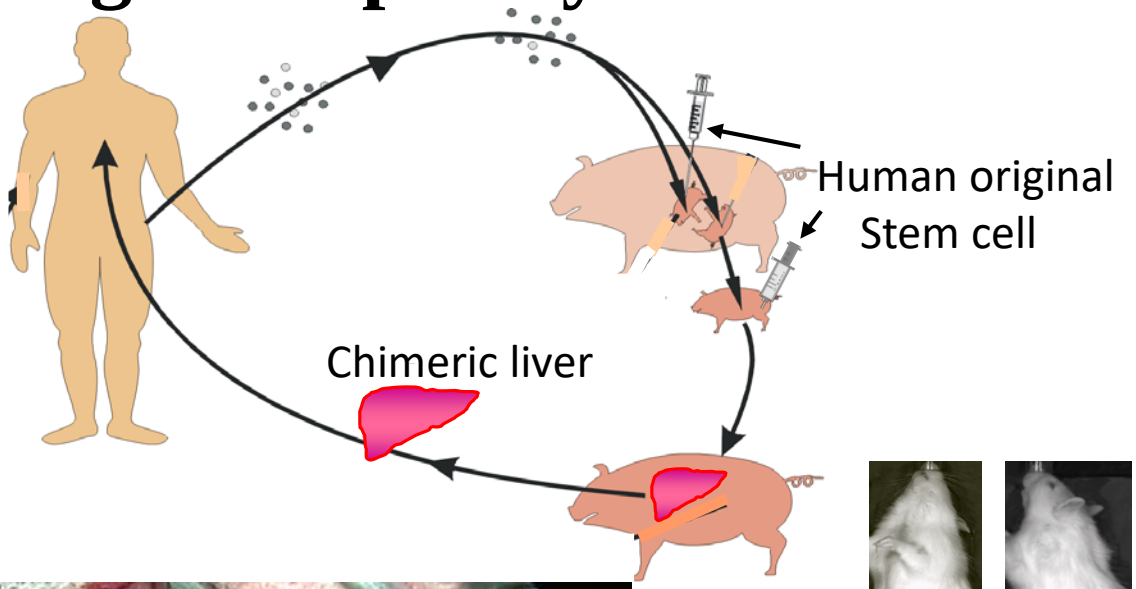
In Vivo Bioimaging Analysis of Stromal Vascular Fraction-Assisted Fat Grafting: The Interaction and Mutualism and Cells and Grafted Fat

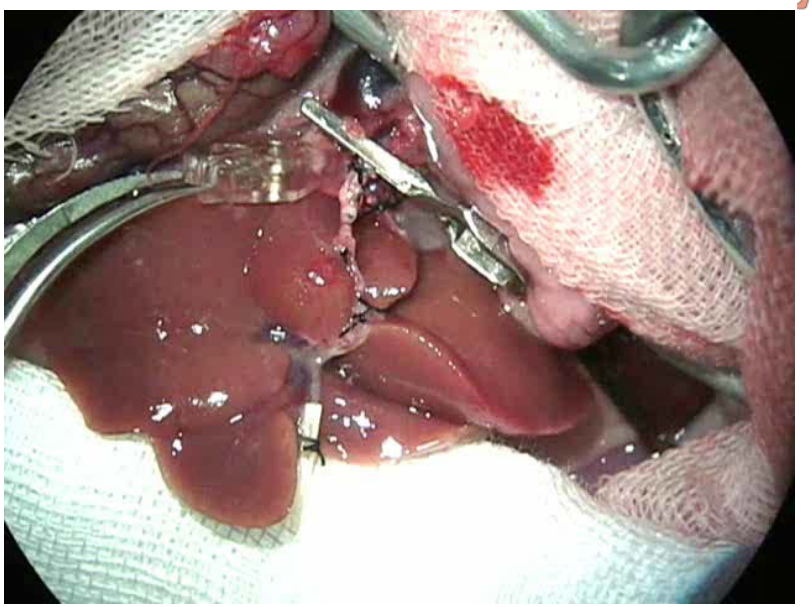


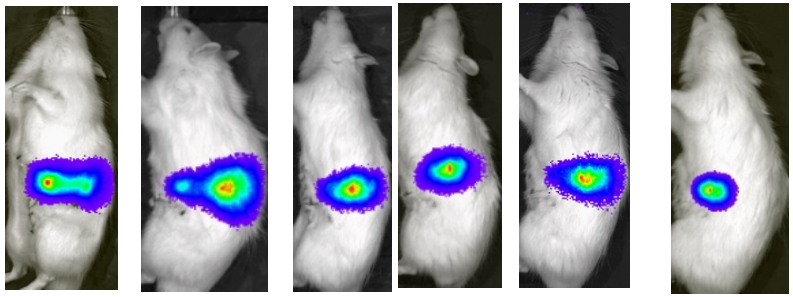


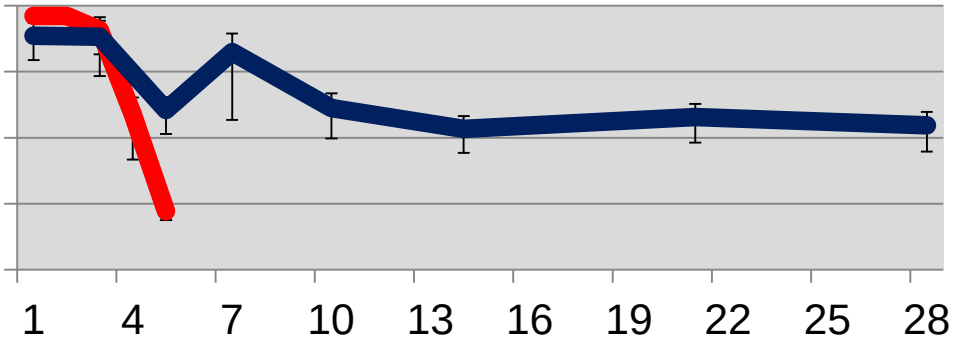
(Zhou SB, et al. Transplantation 2014)

Transplantation of engineered chimeric liver with autologous hepatocytes and xenobiotic scaffold



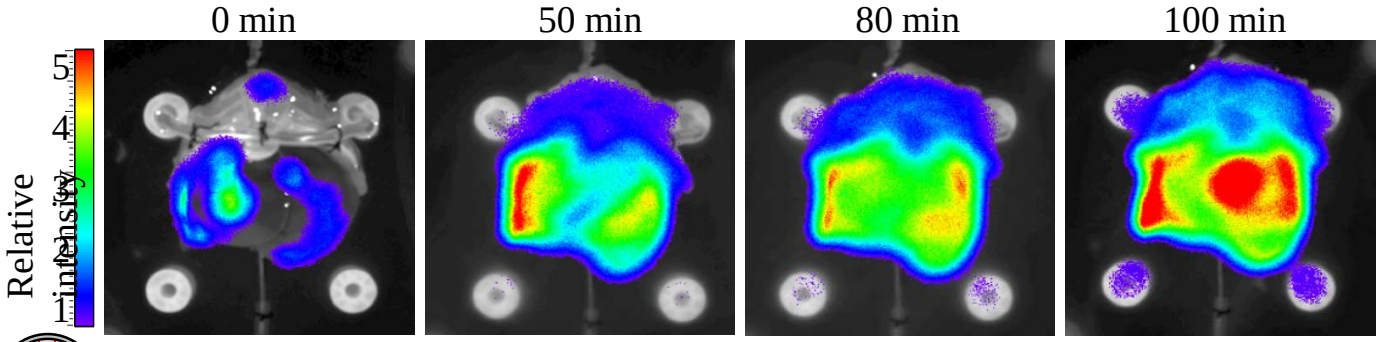


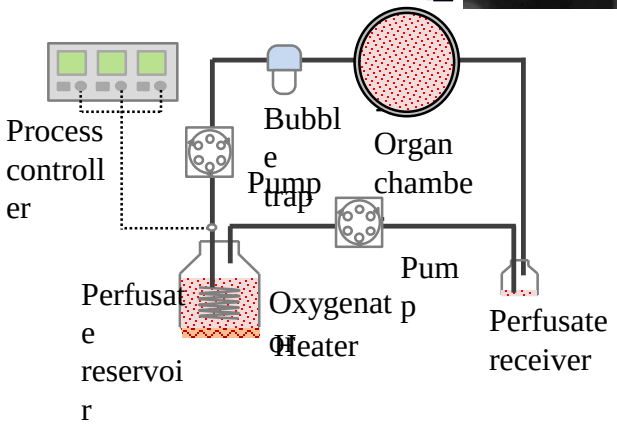


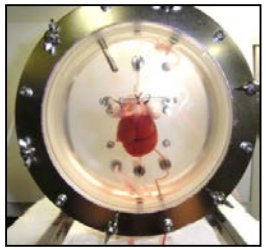


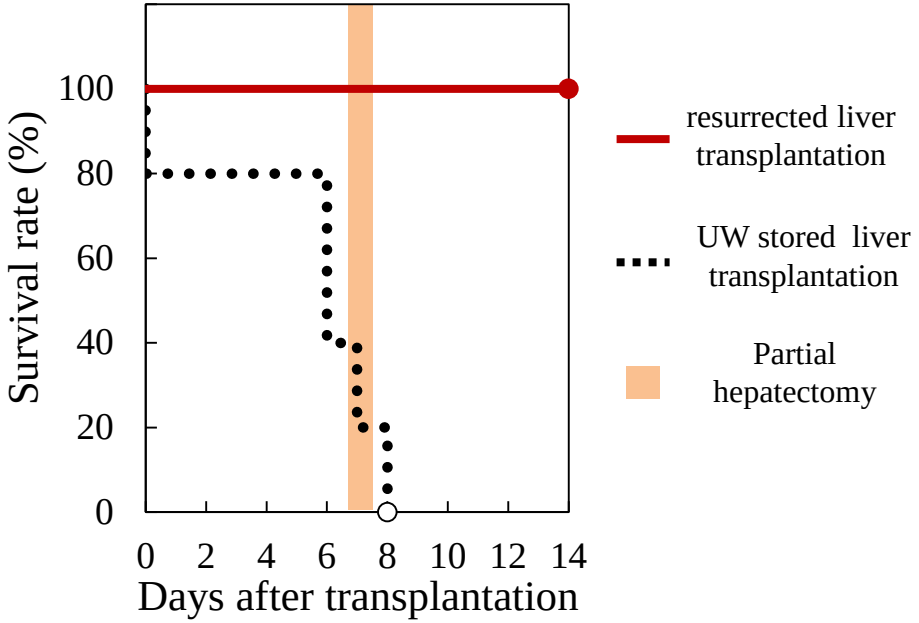
(Hata T, et al. Ann Surg 2013)

Hypothermic temperature effects on organ survival and restoration



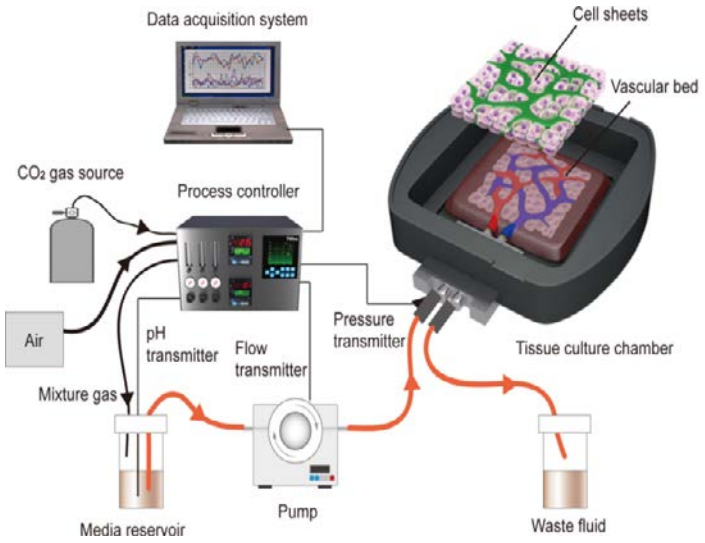


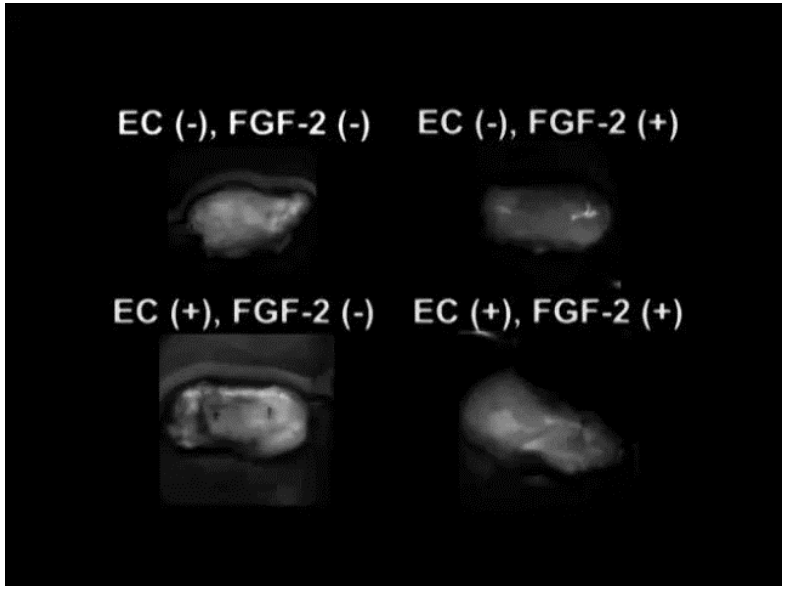


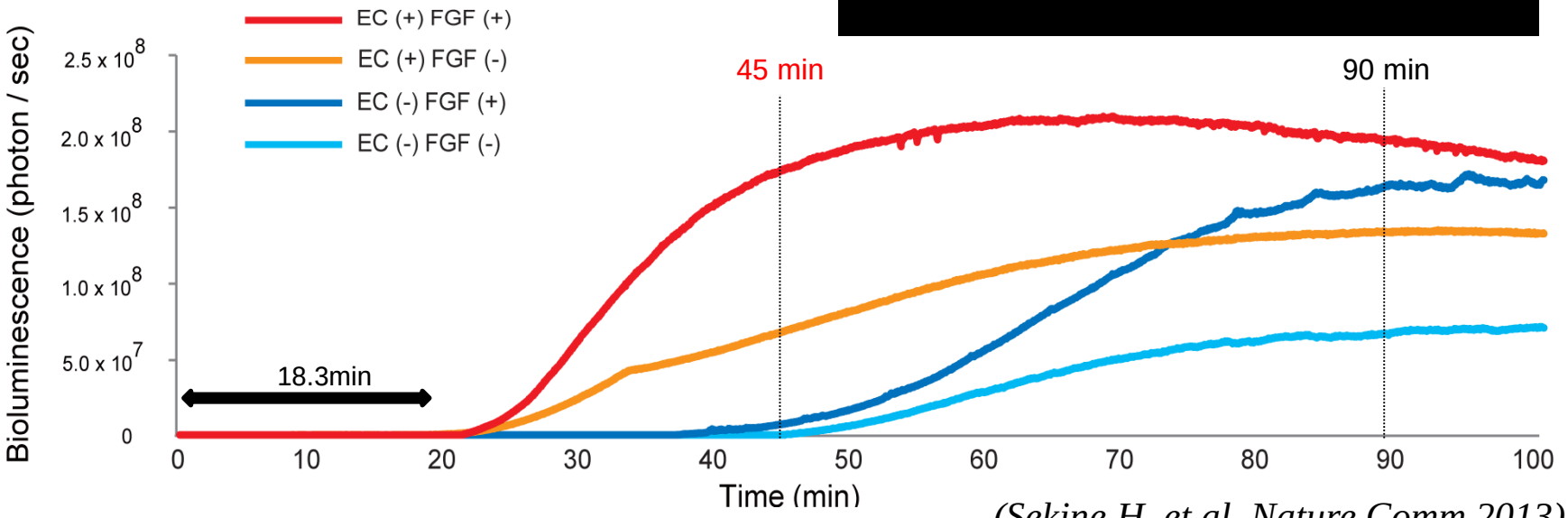


(Ishikawa J, et al. Scientific Reports 2015)

In vitro fabrication of functional three-dimensional tissues with perfusable blood vessels

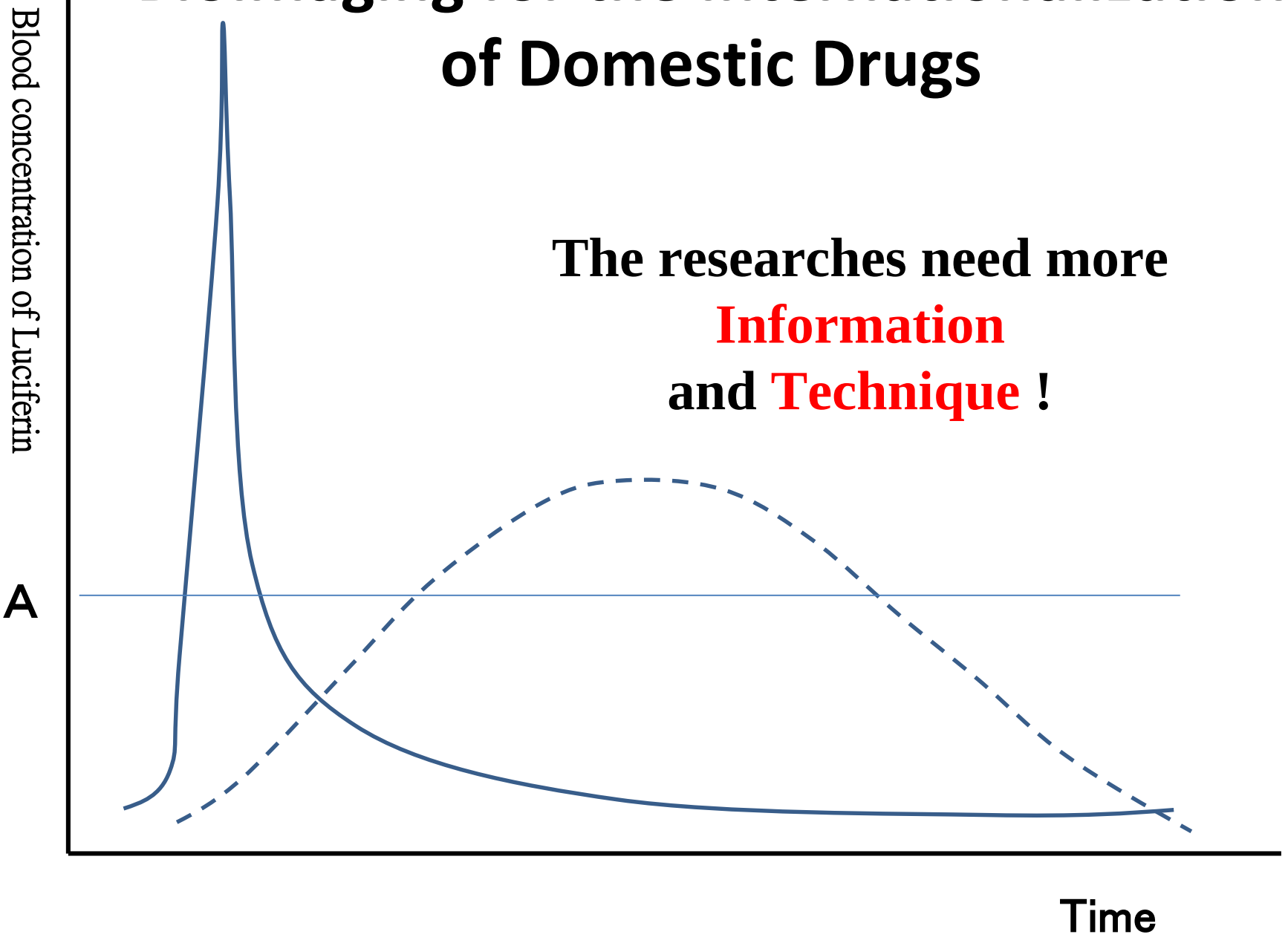






(Sekine H, et al. Nature Comm 2013)

Bioimaging for the Internationalization of Domestic Drugs

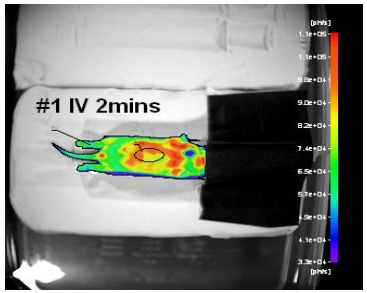


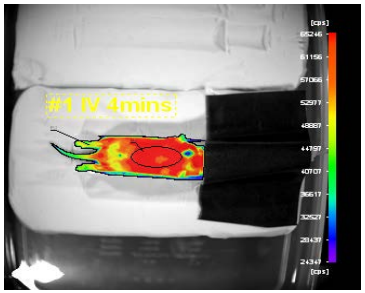
The researches need more
Information
and Technique !

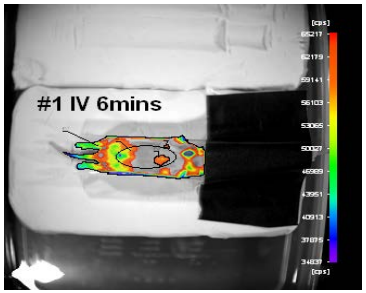
Comparison of Luminescence intensity between *iv* and *ip* in Luc Tg newborns

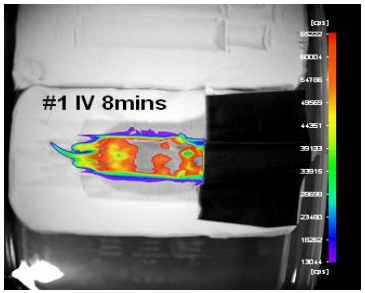
IV

30mg/kg



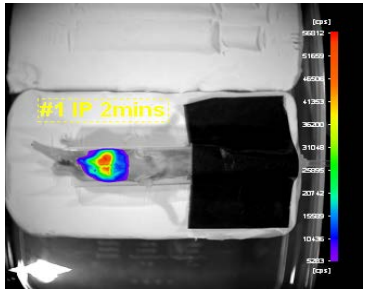


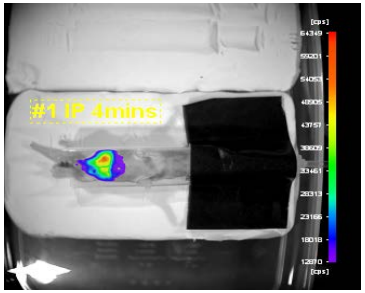


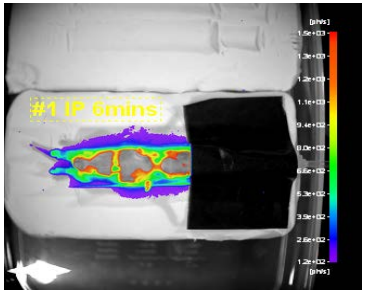


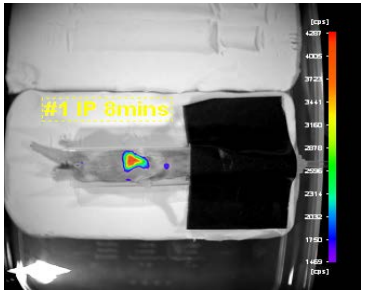
IP

150mg/kg







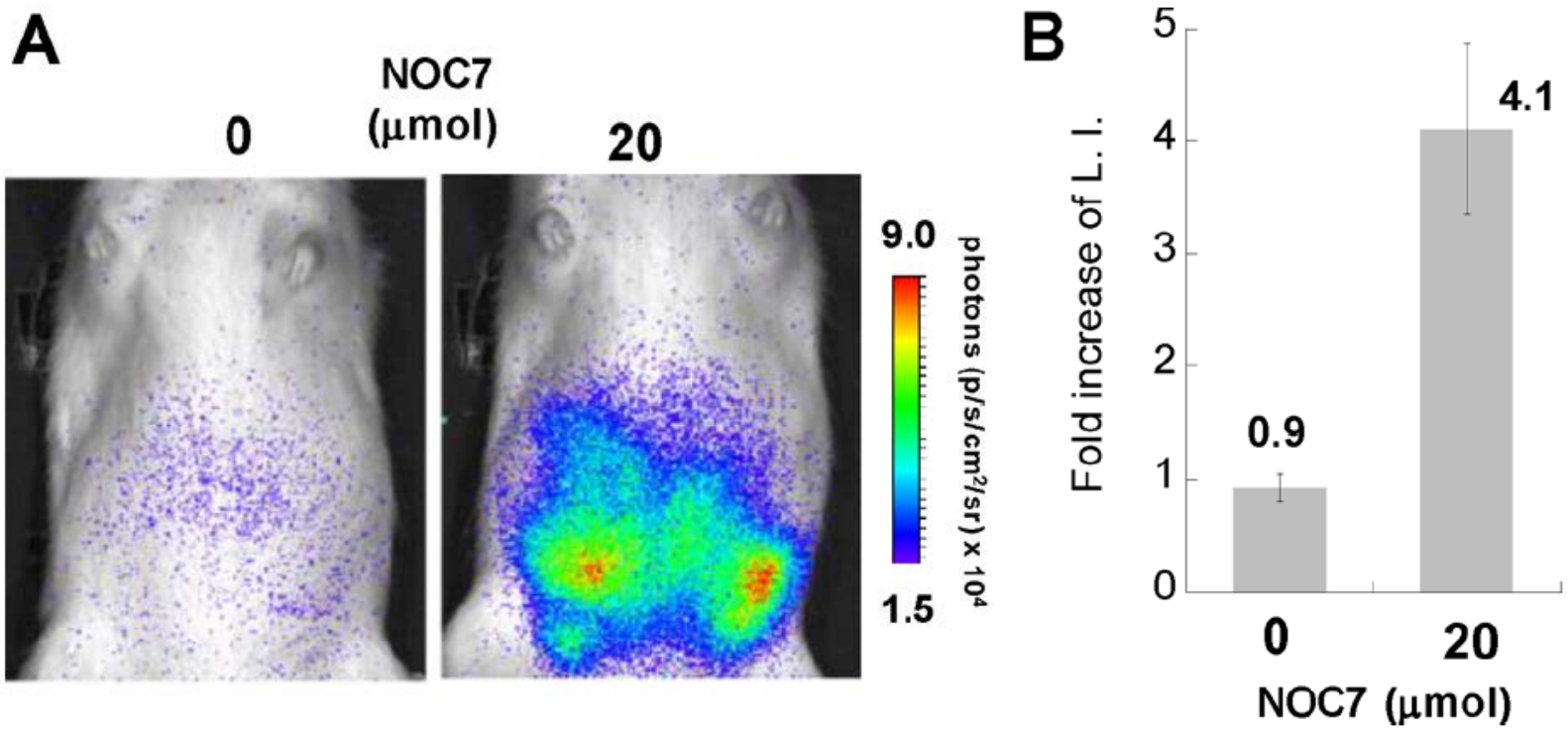


2 4 6 8

(Minutes)

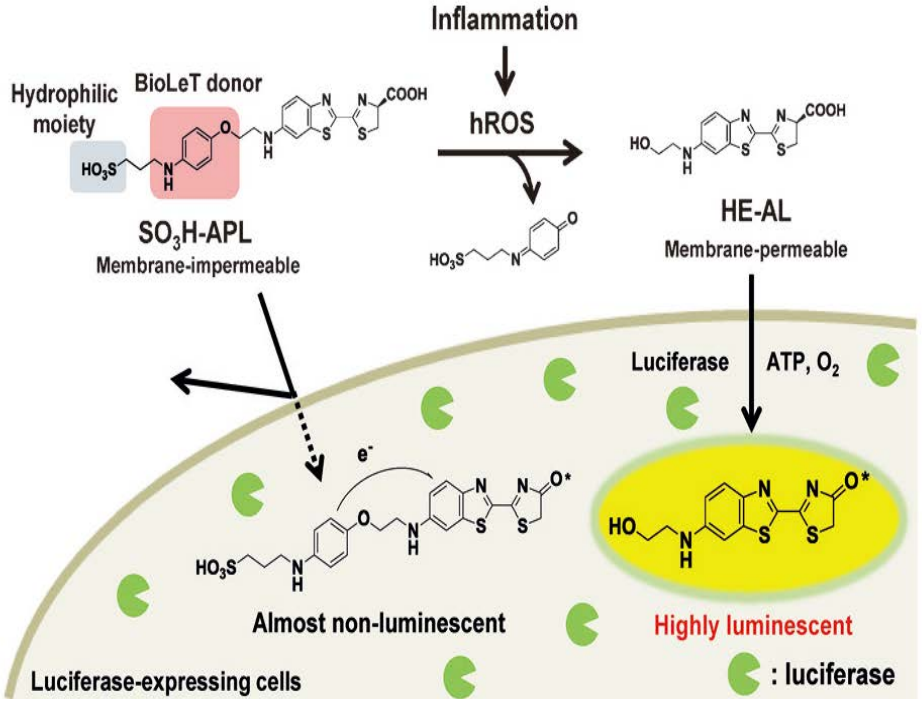
(Hakamada Y. Unpublished)

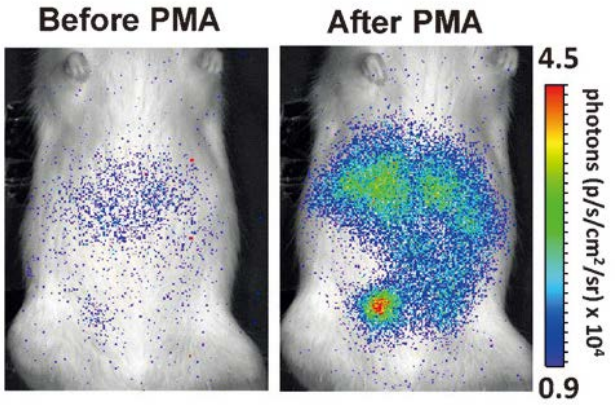
New class of bioluminogenic probe based on bioluminescent enzyme-induced electron transfer: BioLeT

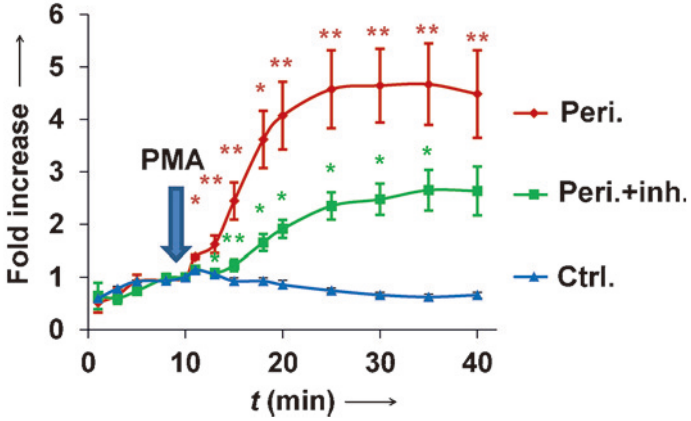


(Takakura H, et al. J Am Chem Soc. 2015)

Development of a Sensitive Bioluminogenic Probe for Imaging Highly Reactive Oxygen Species in Living Rats





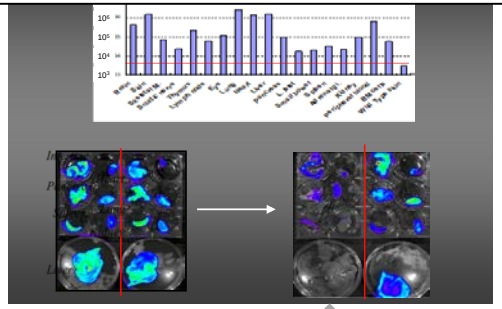


(Kojima R, et al. Angew Chem Int Ed Engl. 2015)

How to establish a new stage of International In Vivo Bioimaging Hub for new drug development ?

Domestic Drugs

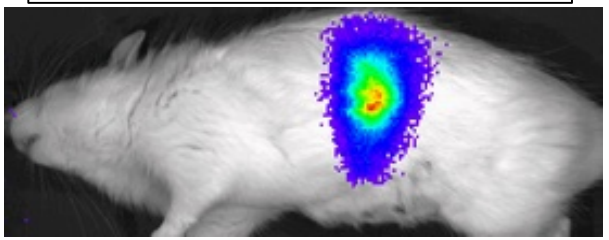
In vitro Screening System



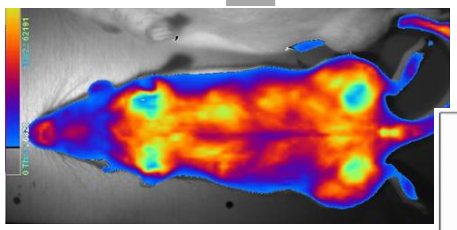
New Prove

New Imaging Machine

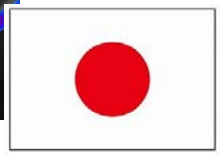
In Vivo Testing System



Surgical Technique



Firefly Tg rat



We can cooperate for the suffering patients