第45回関東腎研究会

(2018年1月20日、大手町)

ヒト由来幹細胞から‘腎臓‘を作る戦略

Eiji Kobayashi, MD,PhD

Keio University, School of Medicine

COI: EK is a medical adviser for Sysmex Ltd. and , Screen Ltd.

自己紹介

ホームページアクセスください

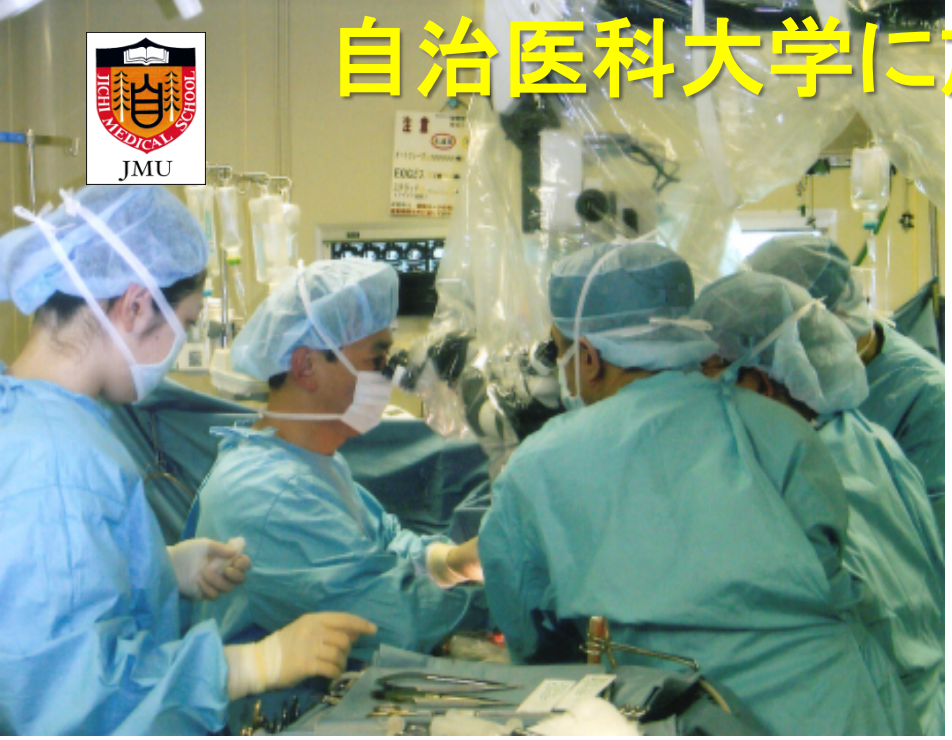
慶應義塾大学医学部臓器再生医学寄附講座
小林英司研究室

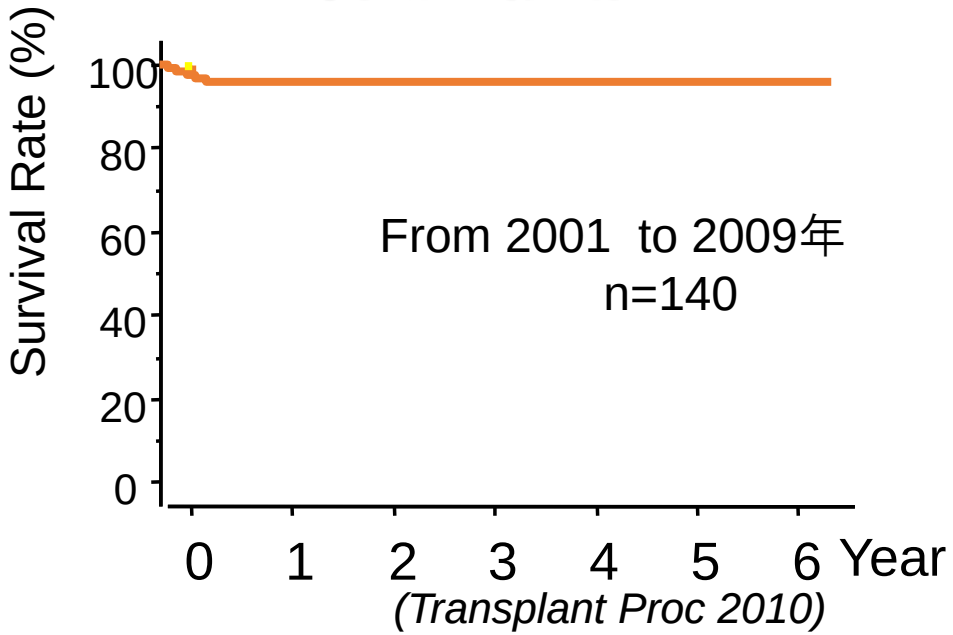
**地域病院で腎臓内科医から技術を教わり、
腎不全患者さんが教えてくれたこと**

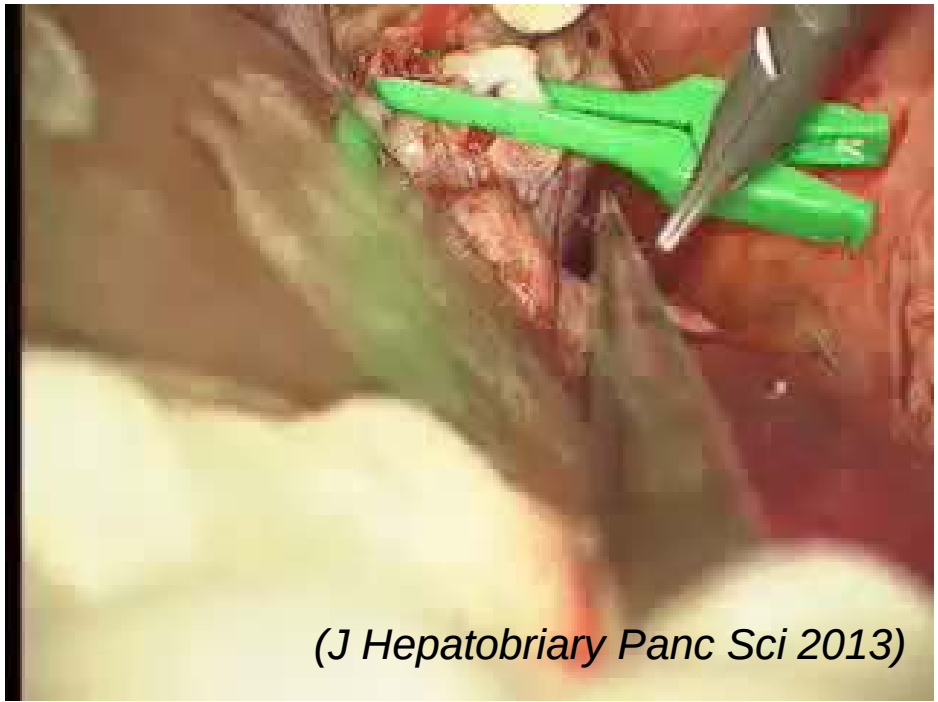
「生きた学問へ」 ―研修医時代編―

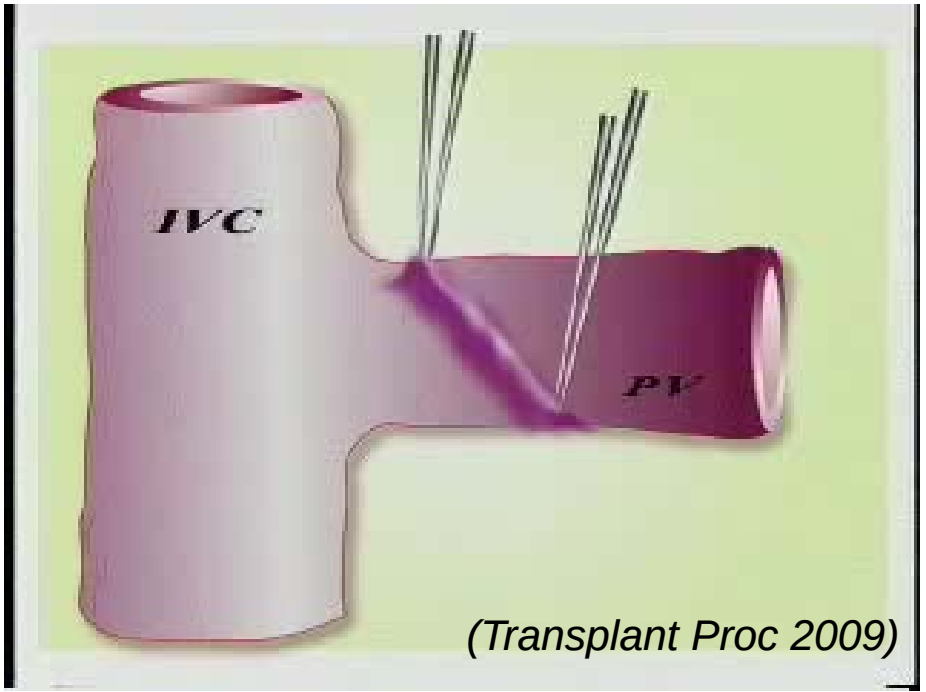
2017年6月号 「地域病院で腕を磨く(1):血液透析シャント・腹膜透析シャント」

自治医科大学に於ける生体肝移植









イスタンブール宣言

(Istanbul, 30th April – 3rd May 2008)

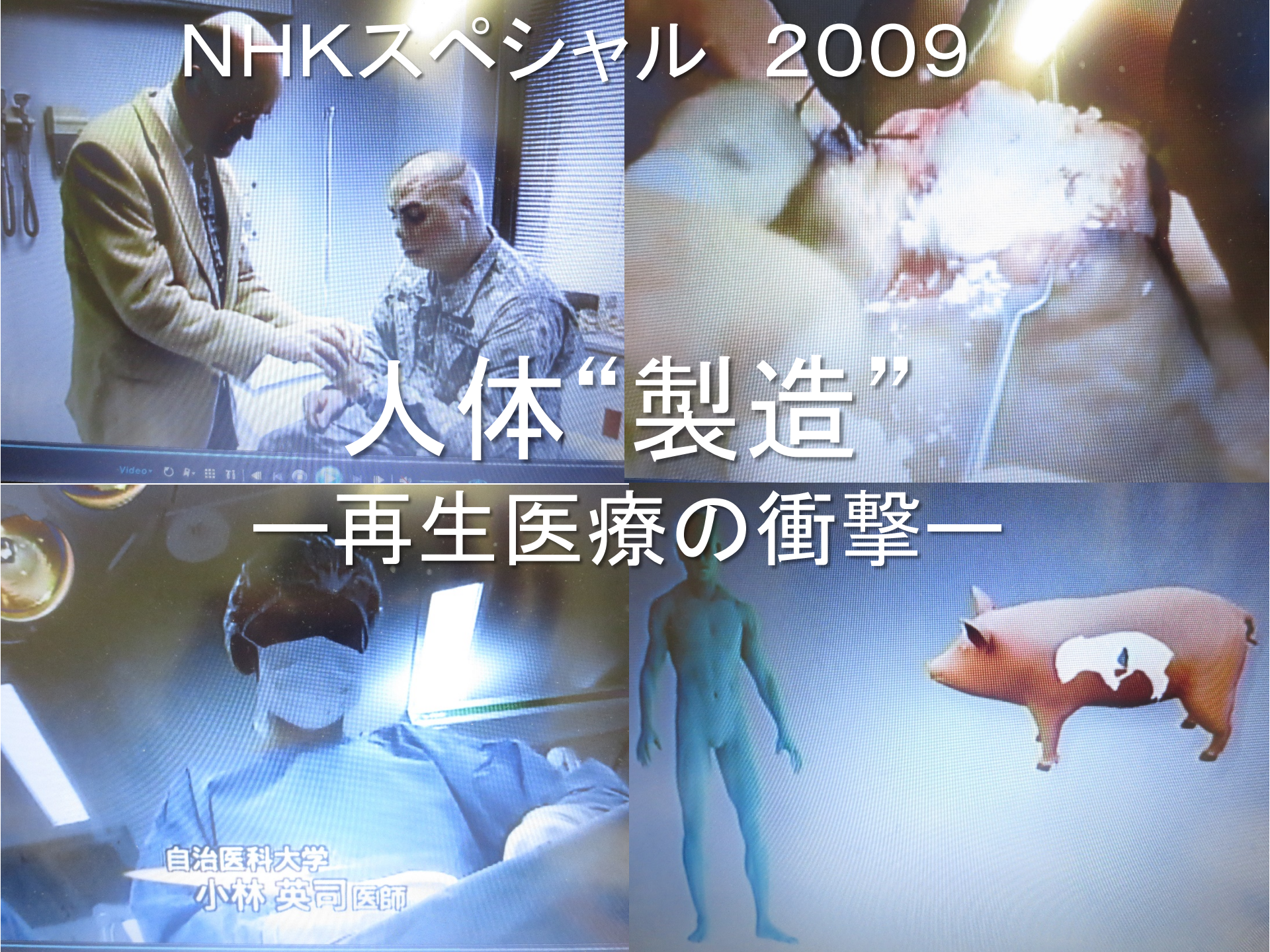
1．Organ trafficking（臓器取引）、Transplant tourism（移植ツーリズム）、Transplant commercialism（移植商業主義）等の内容を明確して、人道的、社会的、国際的に問題があるものに対し世界的に反対すること。

2．死体（脳死、心停止）ドナーを自国で増やし、自国での臓器移植を増やすよう呼びかけること。そのために国際的協力をすること。

3．生体ドナーは、ドナー保護を最優先し、選定や移植に関わる総合的な保障等の制度を国家的に取り組むよう呼びかけること。

152 professionals from 78 countries

（Lancet　2008年7月6日）


NHKスペシャル 2009
人体“製造”
―再生医療の衝撃―
自治医科大学
小林 英司 医師

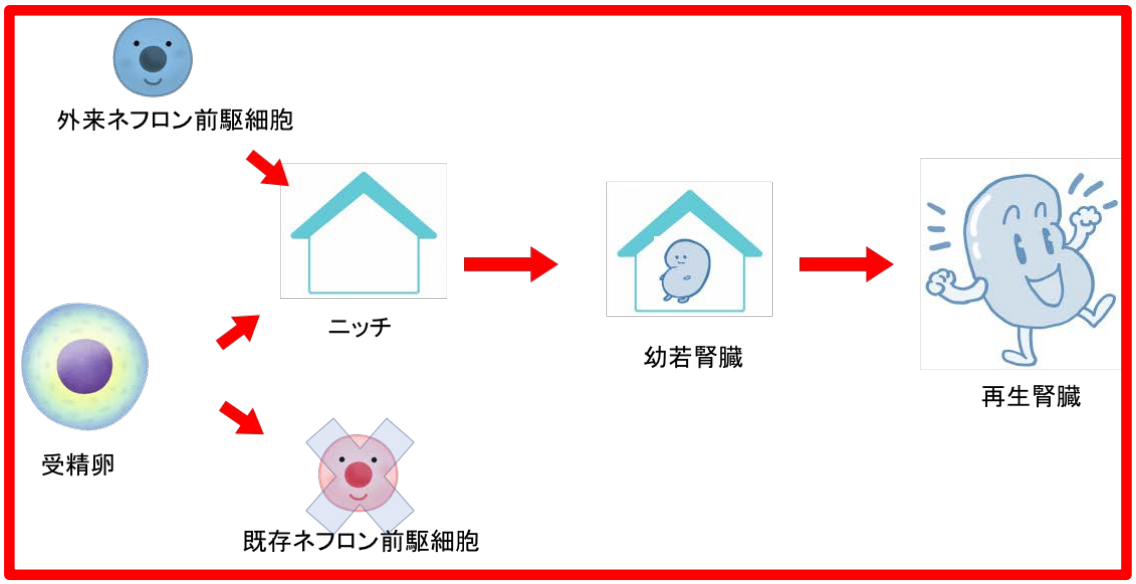
動物を利用したヒト臓器作成研究の現状

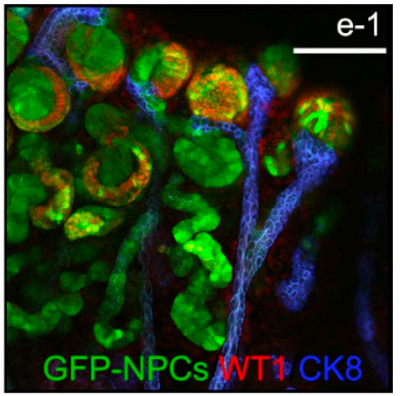
動物の利用形態			研究の現状
①動物性集合胚を用いて動物体内でヒト臓器を作製	小動物による実証研究（膵臓、腎臓）	⇨	臓器欠損ブタの作製（膵臓）
②ヒト幹細胞を動物体内に移植してヒト臓器を作製	小動物による実証研究（肝臓）	⇨	免疫不全ブタの作製
③動物の臓器原器にヒト幹細胞を注入してヒト臓器を作製	小動物による実証研究（腎臓）	⇨	ブタの臓器原器をラット、アカゲザルに移植（膵臓）
④動物臓器の骨格部分（スキャフォールド）を足場にしてヒト幹細胞からヒト臓器を作製	小動物による実証研究（心臓、腎臓、肝臓、肺）	⇨	ブタ臓器を使用してヒト臓器のサイズの足場を作製（肝臓）

小林英司：内閣府生命倫理調査会資料（2013年2月7日）

腎臓再生プロジェクト：Yamaton-K

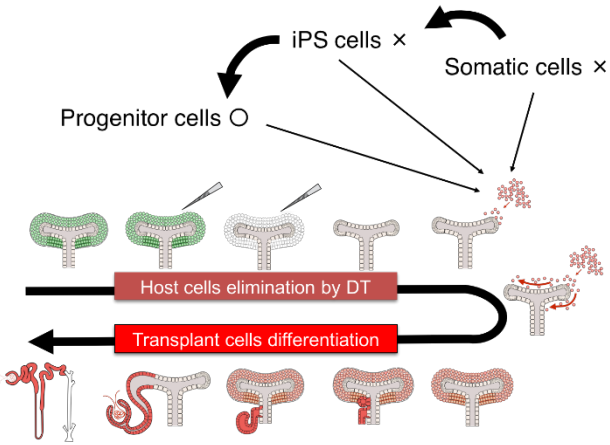
学校法人
慈 恵 大 学
See the patients,not the disease. 病気を診ずして病人を診よ

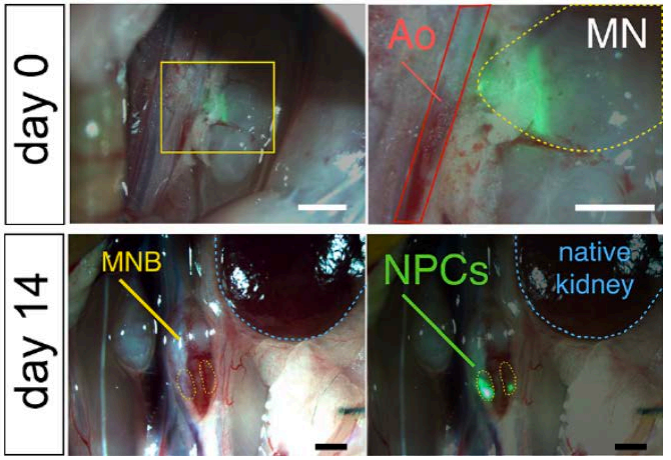




Prof. T. Yokoo

The Jikei University
Nephrology and Hypertension

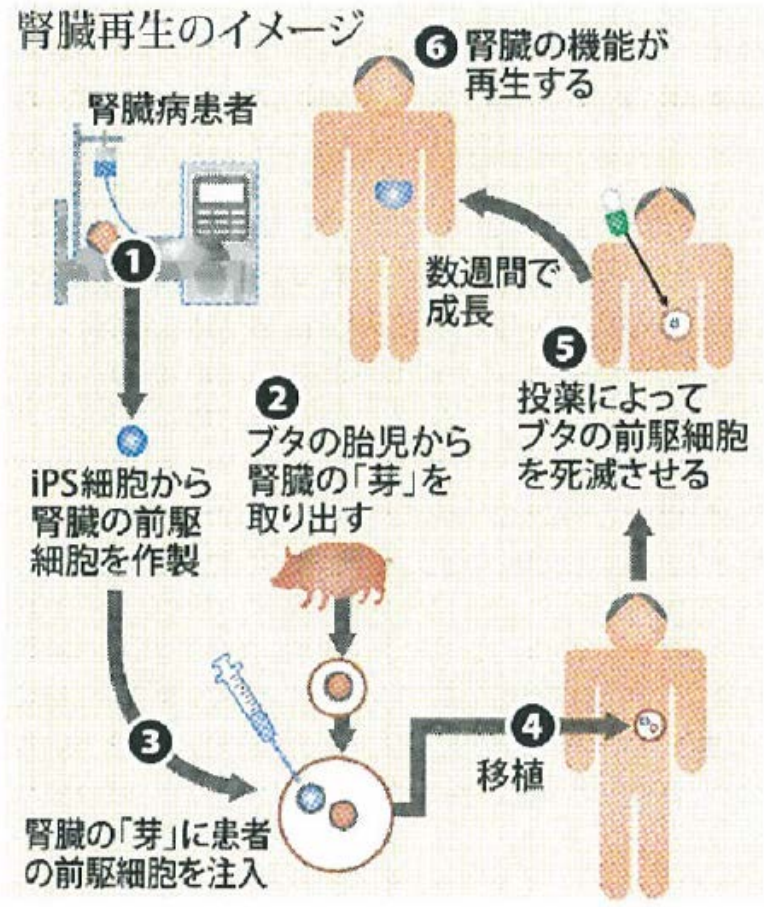




(Yanamaka S, et al. Nature Com 2017)

質疑のポイント

＜腎再生＞初の臨床研究　患者のｉＰＳ使用、年内開始



毎日新聞（２０１８年１月５日）

乗り越えるべき課題と戦略

①患者iPS細胞の安全性

再生医療等の安全性の確保等に関する法律（平成25年法律第85号）

知財（2012年7月6日　日本　登録番号5030039　）

②ブタ胎仔の臓器の芽の安全性

異種移植の実施に伴う公衆衛生上の感染症問題に関する指針

③臓器の芽へのヒトの細胞注入技術

知財（2012年6月5日　ニュージーランド　登録番号590122　）

④移植後の免疫抑制法

知財（未登録　免疫抑制薬のコンビネーションによるヒト組織生着法）

⑤Cell Fate Controlにおける安全性

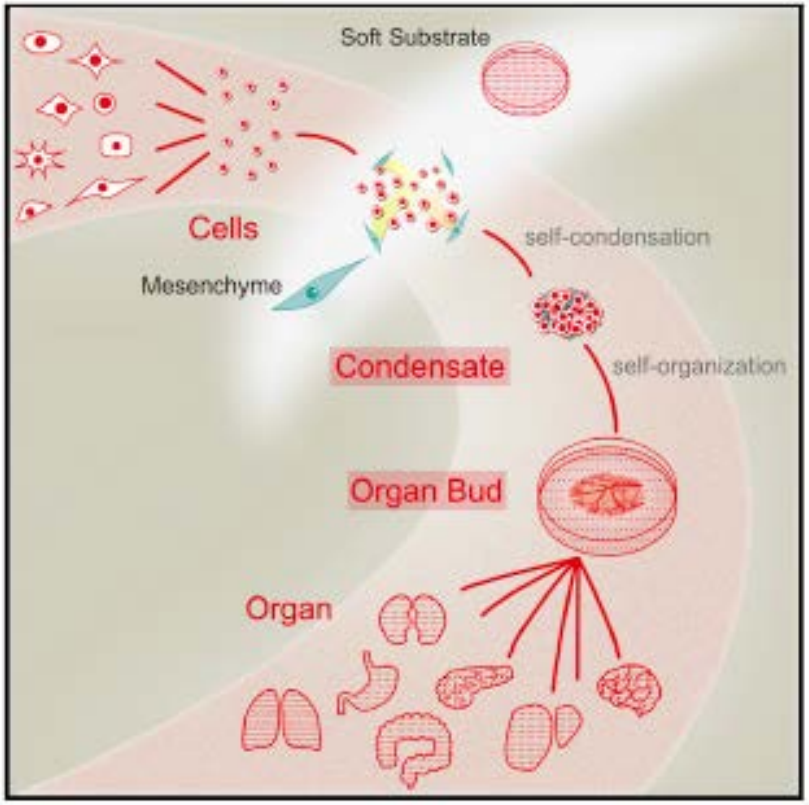
遺伝子治療医薬品の品質及び安全性の確保に関する指針（2004）

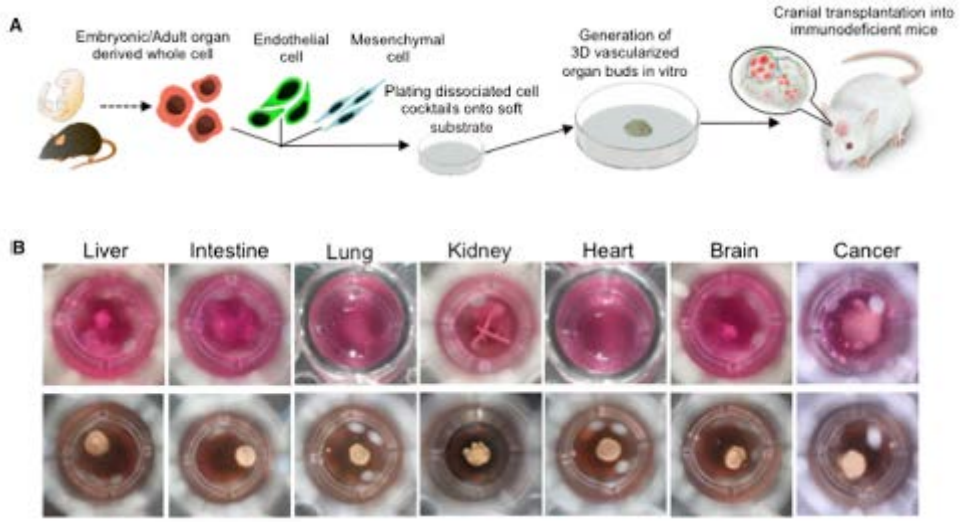
⑥尿路作成技術

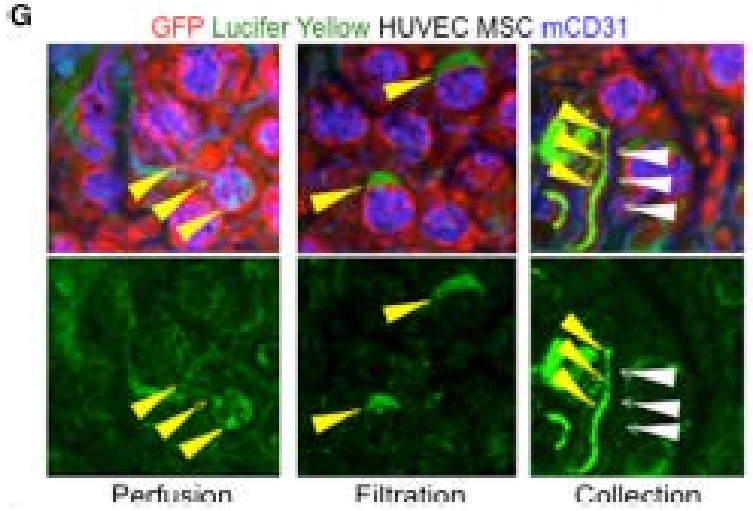
知財（移植用臓器及び臓器構造体（特願2014－257957）

（小林英司　2018年1月20日、関東腎研究会　）

Vascularized and Complex Organ Buds from Diverse Tissues via Mesenchymal Cell-Driven Condensation

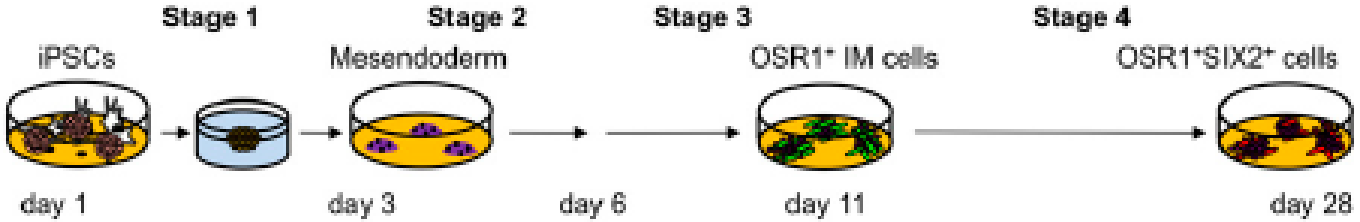




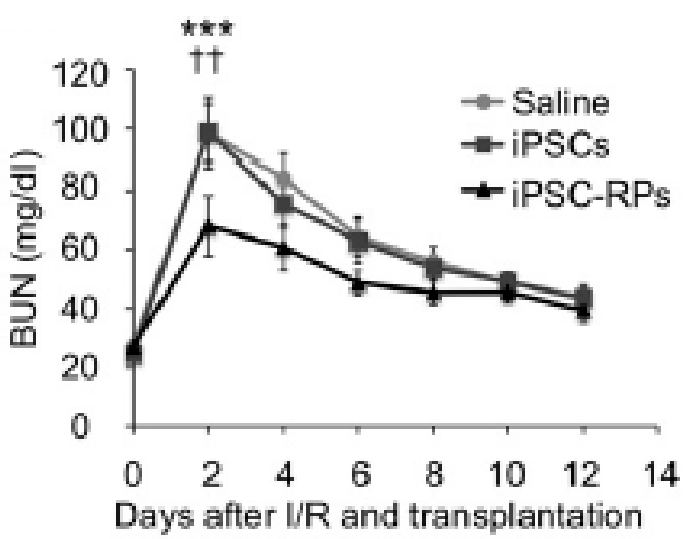


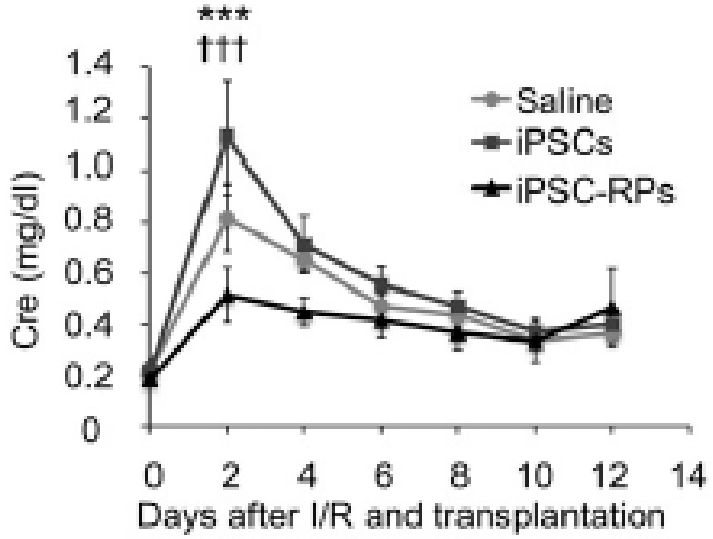
Takebe T, Enomura M, Yoshizawa E, Kimura M, Koike H, Ueno Y, Matsuzaki T, Yamazaki T, Toyohara T, Osafune K, Nakauchi H, Yoshikawa HY, Taniguchi H. Cell Stem Cell. 2015

Cell Therapy Using Human Induced Pluripotent Stem Cell-Derived Renal Progenitors Ameliorates Acute Kidney Injury in Mice



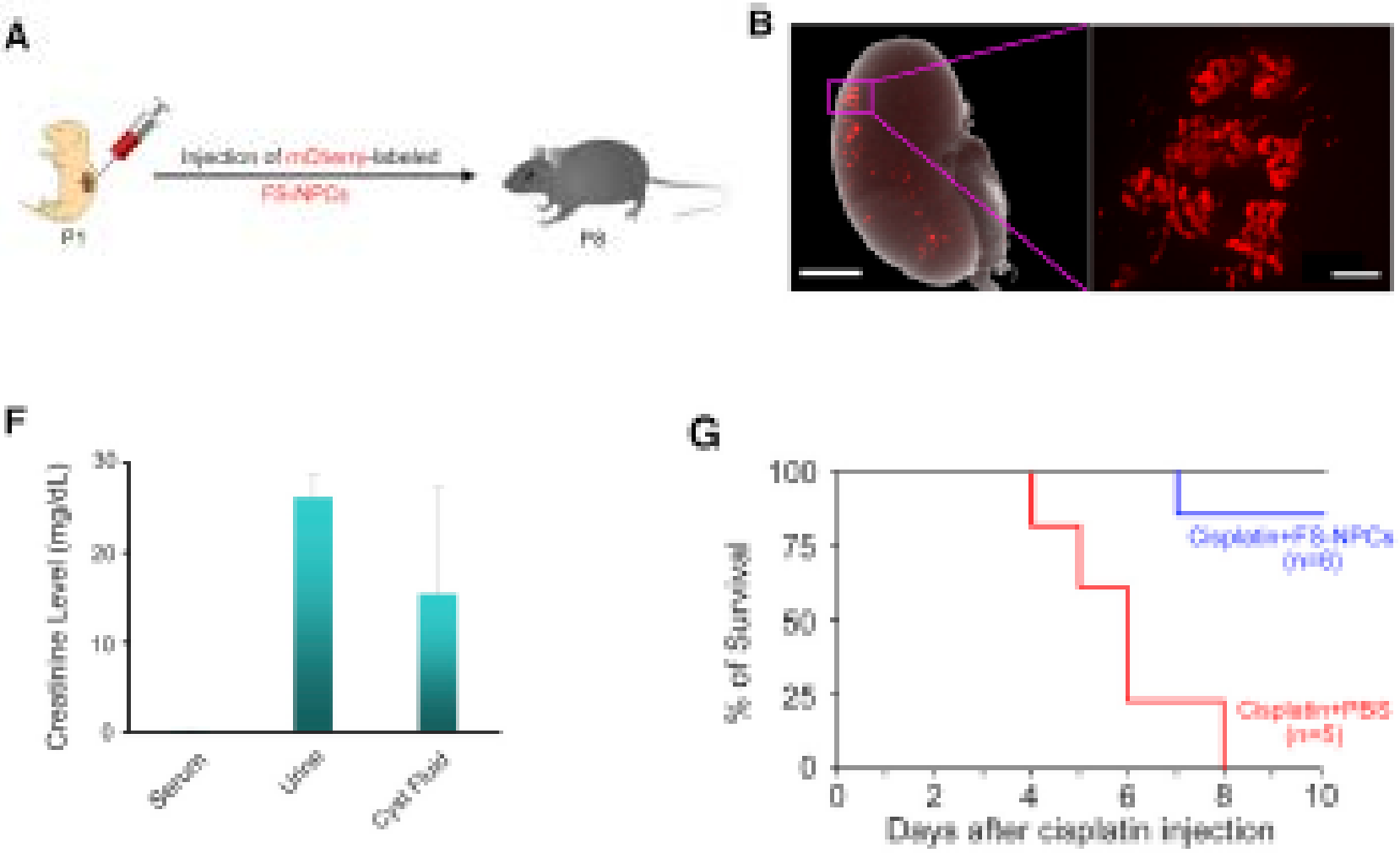
Stage 1	Stage 2	Stage 3	Stage 4
Suspension culture	Adhesion culture		
DMEM/F12 GlutaMAX			
2% FBS, 0.5% PS	10% KSR, 55 μM 2ME, 1% NEAA, 0.5% PS		
Activin A (100 ng/ml)	BMP7 (100 ng/ml)	TGFβ1 (5 ng/ml)	TGFβ1 (5 ng/ml)
CHIR99021 (1 μM)	CHIR99021 (1 μM)	TTNPB (1 μM)	DMH1 (0.5 μM)





(Toyohara T, & Osafune K. Stem Cells Transl Med. 2015)

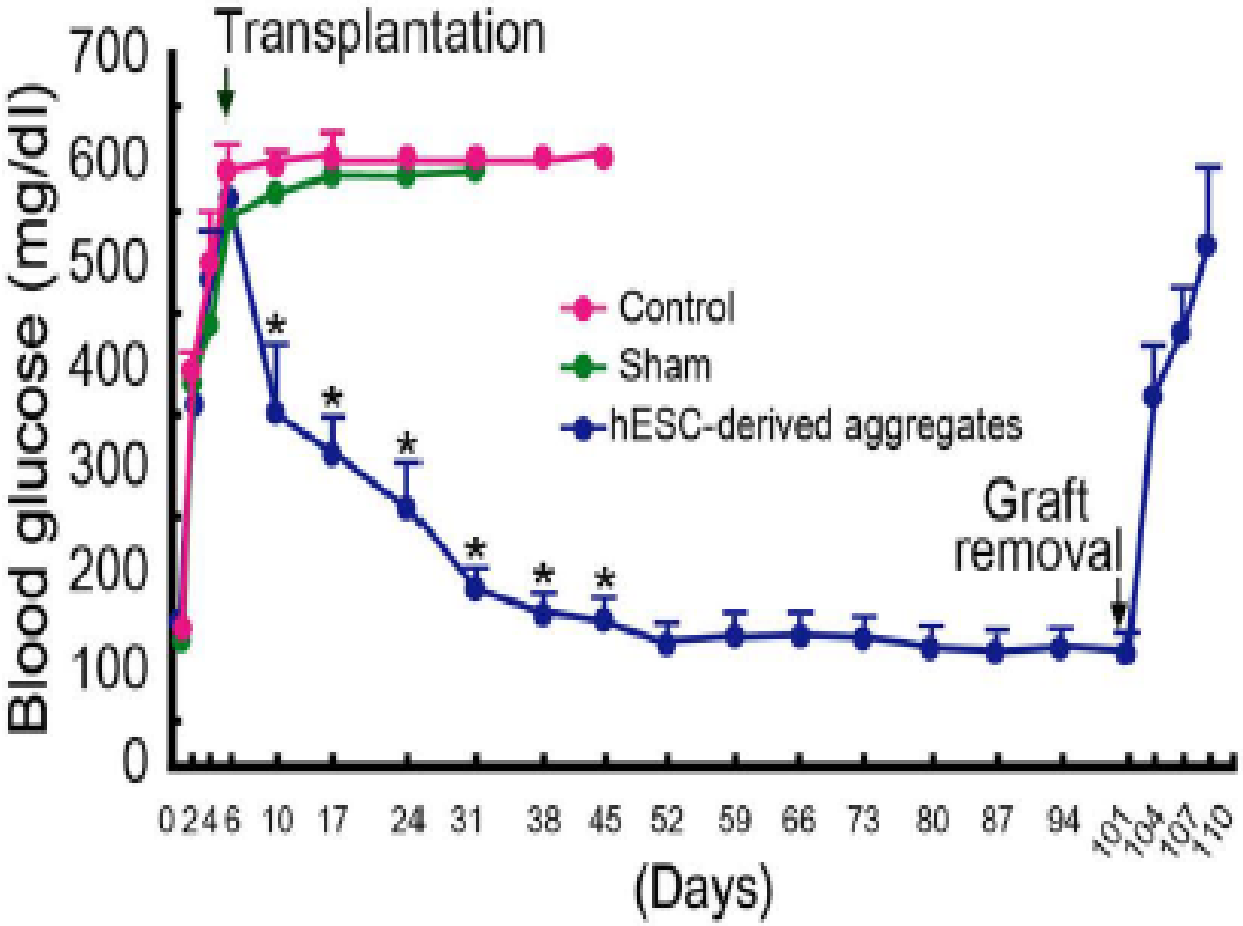
3D Culture Supports Long-Term Expansion of Mouse and Human Nephrogenic Progenitors



(Li Z, Araoka T, et al. Cell Stem Cell. 2016)

課題　1　　Organ budの機能発現には時間がかかる

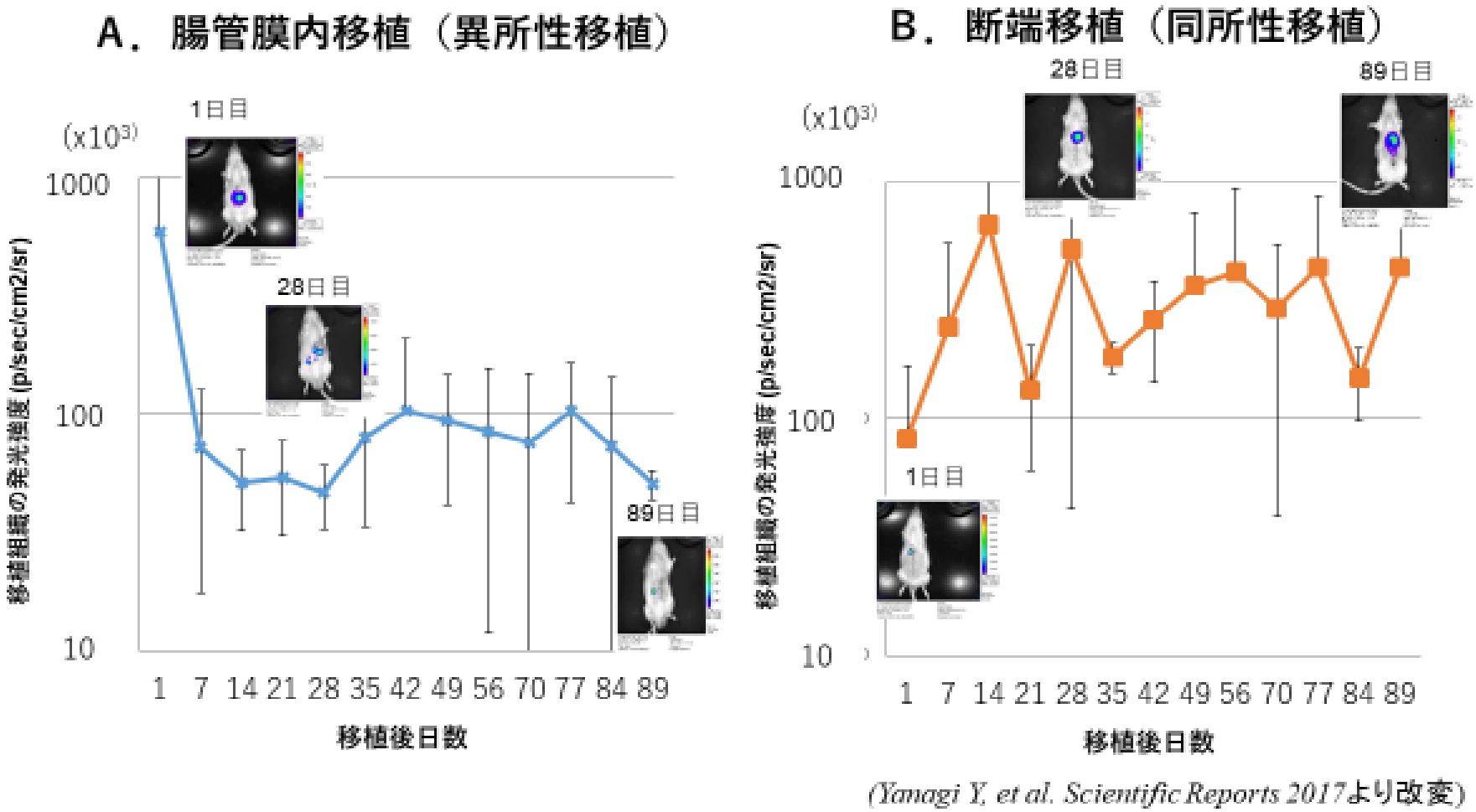
Pancreatic Islet-Like Three-Dimensional Aggregates Derived From Human Embryonic Stem Cells Ameliorate Hyperglycemia in Streptozotocin-Induced Diabetic Mice



(Shim JH, et al. Cell Transplant 2015)

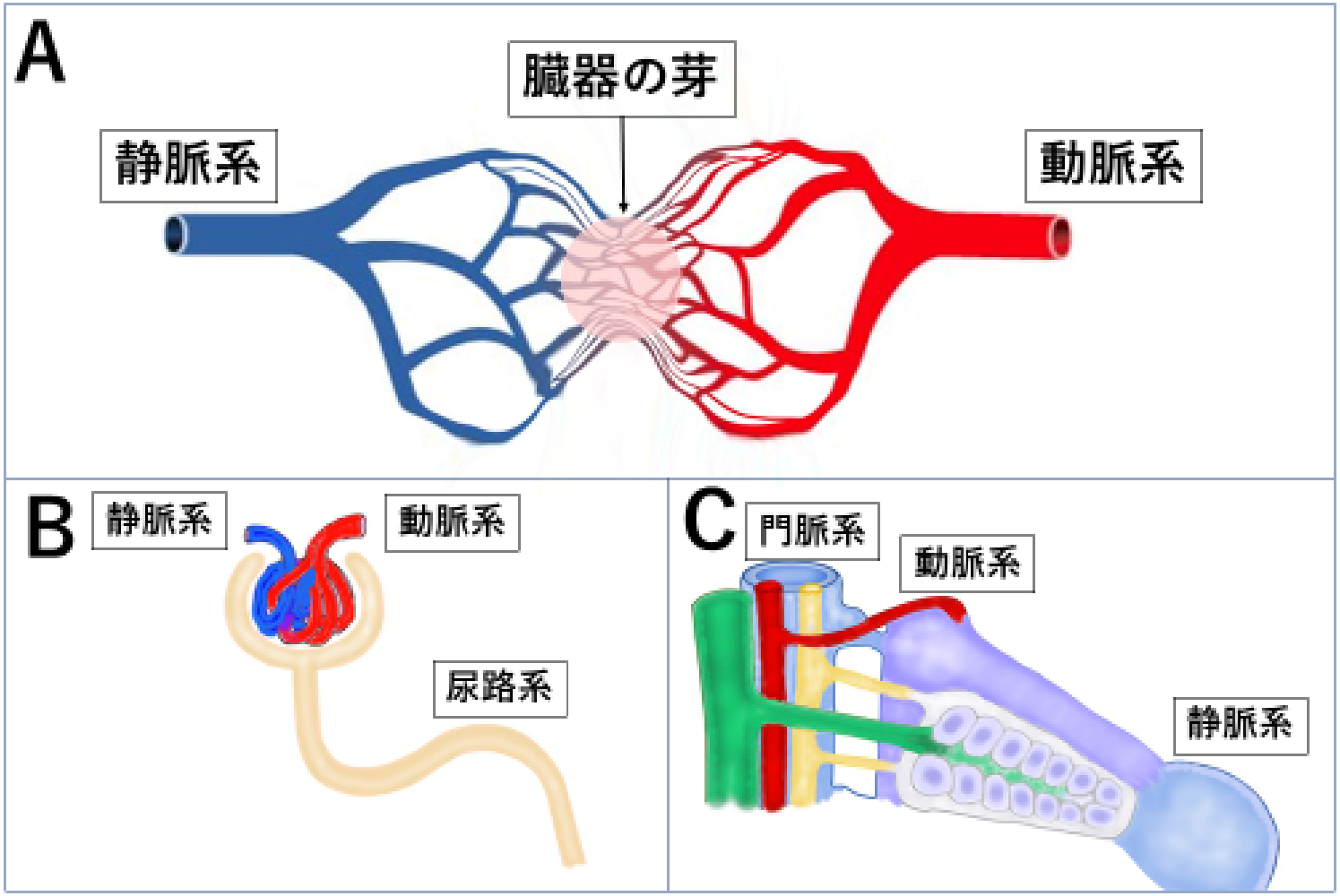
課題　2　Organ budの移植場所が機能に影響する

肝芽は、異所性では育たない



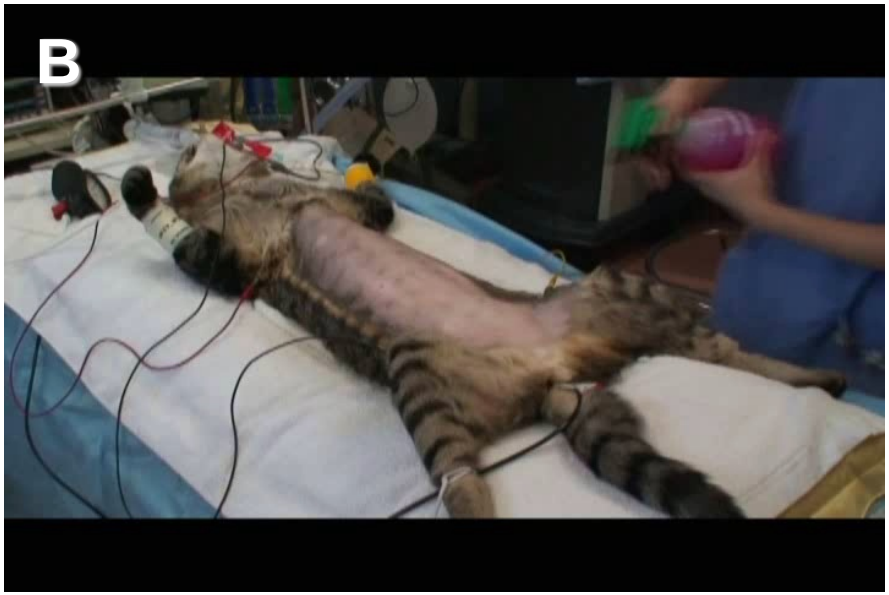
(Yanagi Y, et al. Scientific Reports 2017より改変)

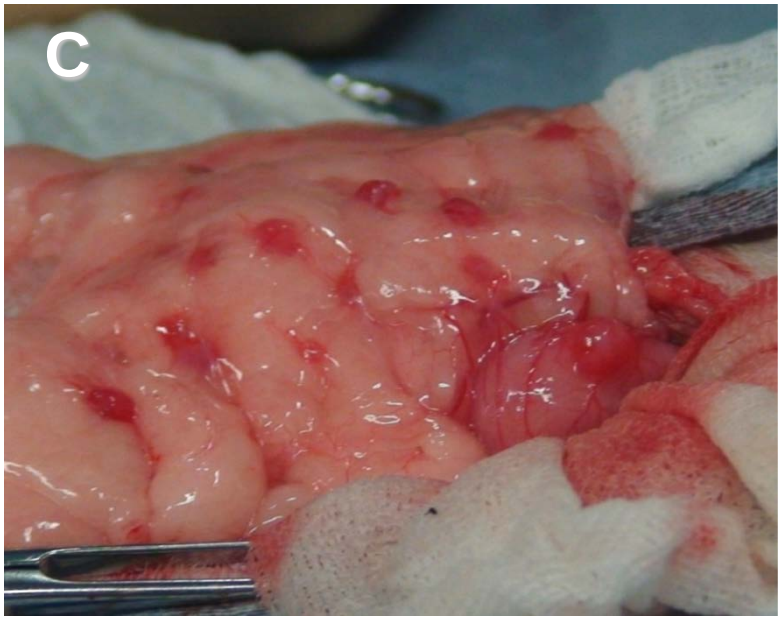
外科技術を応用した「臓器の芽」の移植法（腎、肝）

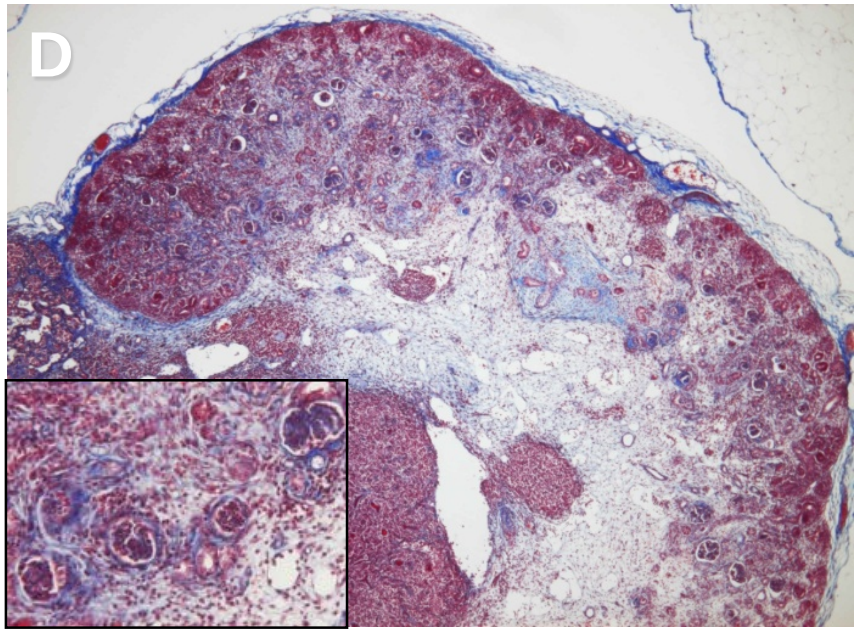


（小林英司、日本外科学会誌（総説）２０１８）

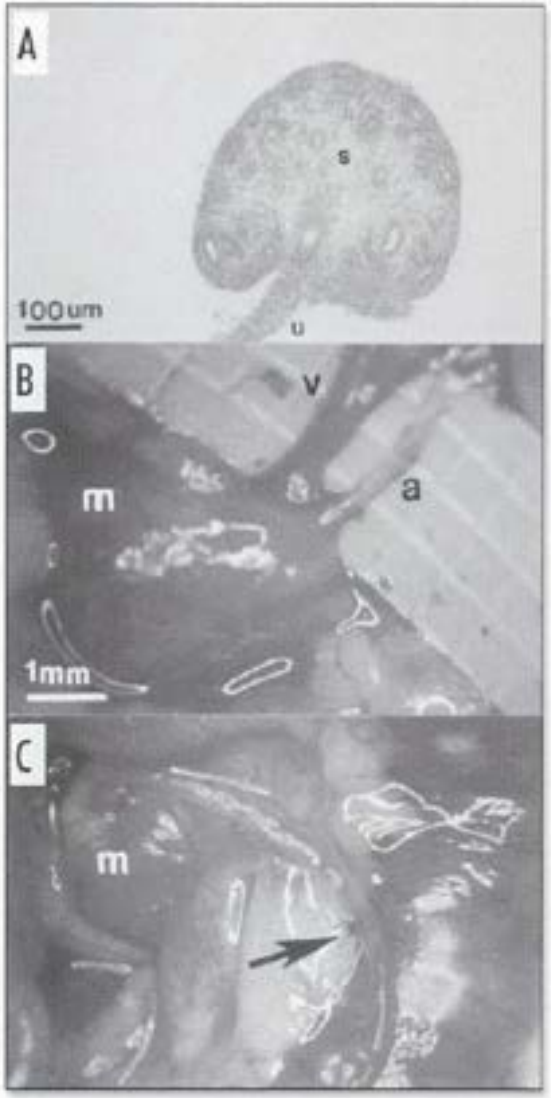
Yamaton K計画スタート（2008年）

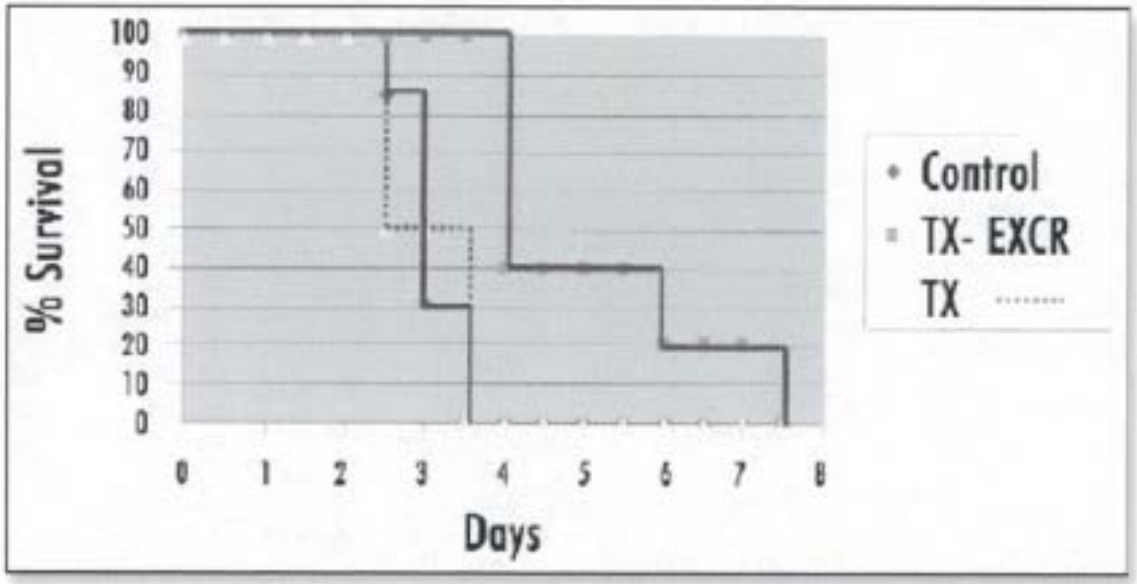






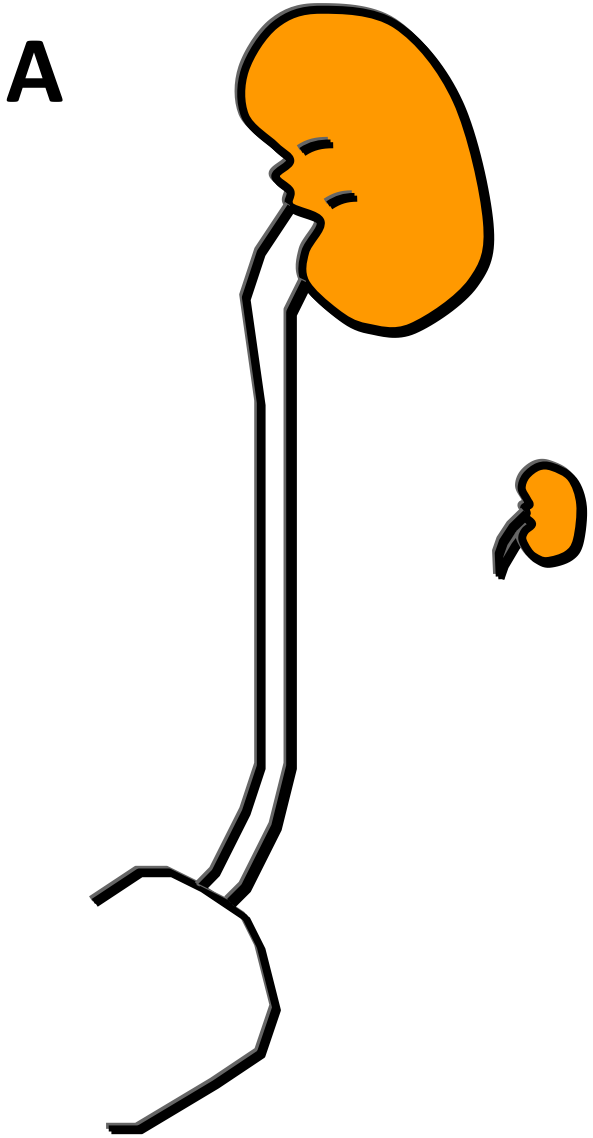
Prolongation of life in anephric rats following de novo renal organogenesis.

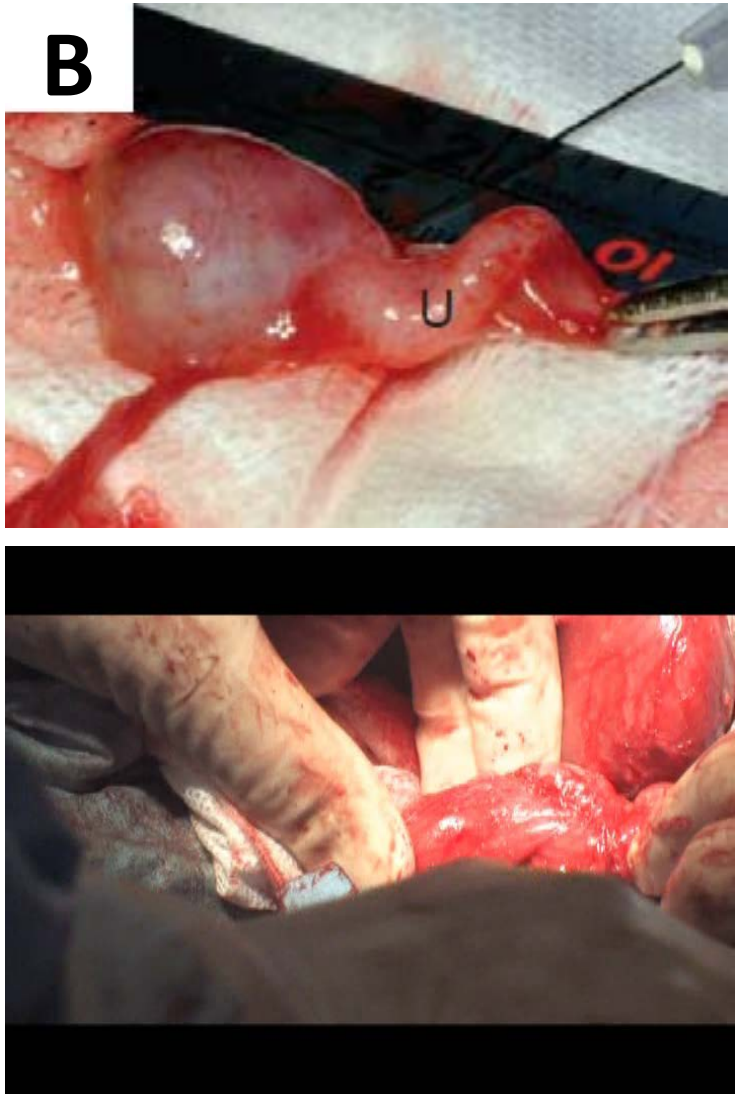




(Rogers SA, Hammerman MR. Organogenesis. 2004)

臨床に使える尿路作成への挑戦





クローン豚由来メタネフロスの尿管吻合

(A :Yokote T, et al. PNAS 2015)

「クロアカ・グラフト」開発（2012年）

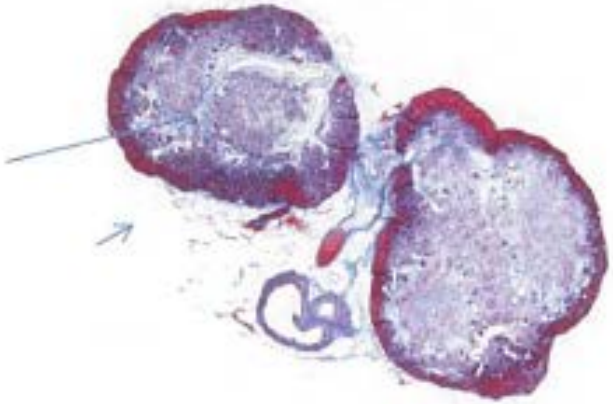
「クロアカ」とは

In zoological anatomy, a **cloaca** (/kloʊˈeɪkə/) is the posterior opening that serves as the only such opening for the intestinal, reproductive, and urinary tracts of certain animal species.

クロアカ・マキシマ（Cloaca Maxima）は、古代ローマの下水システム。

「クロアカ・グラフト」の移植とその発育

尿路と膀胱も育つ

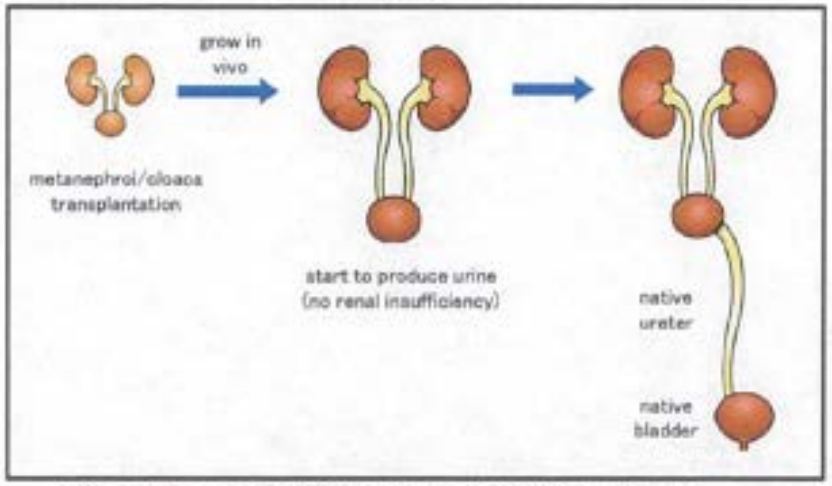


ブタクロアカの猫への移植（2012年）

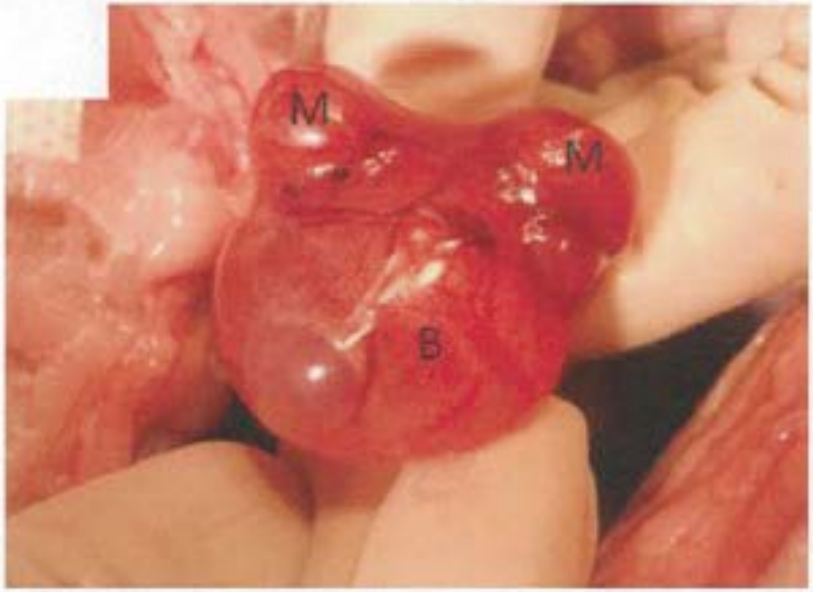
北里大学獣医学部（青森）

ブタクロアカの臨床応用に向けて

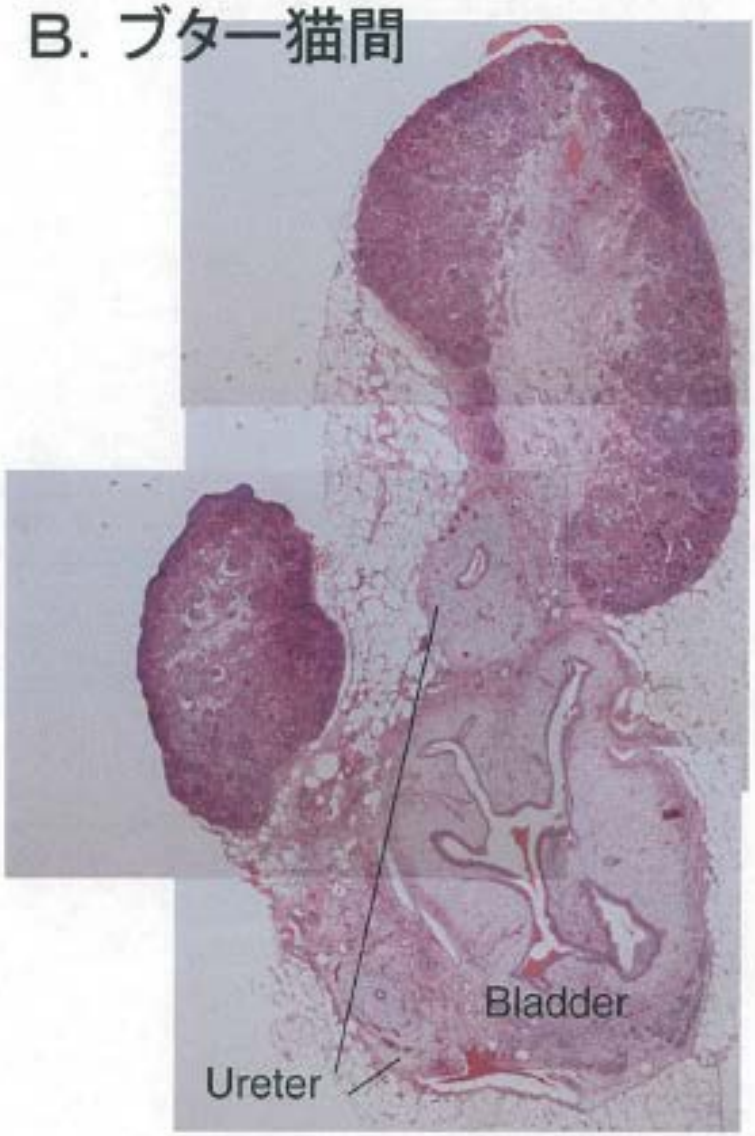
A. クローン豚間



Step-wise peristaltic ureter (SWPU) system



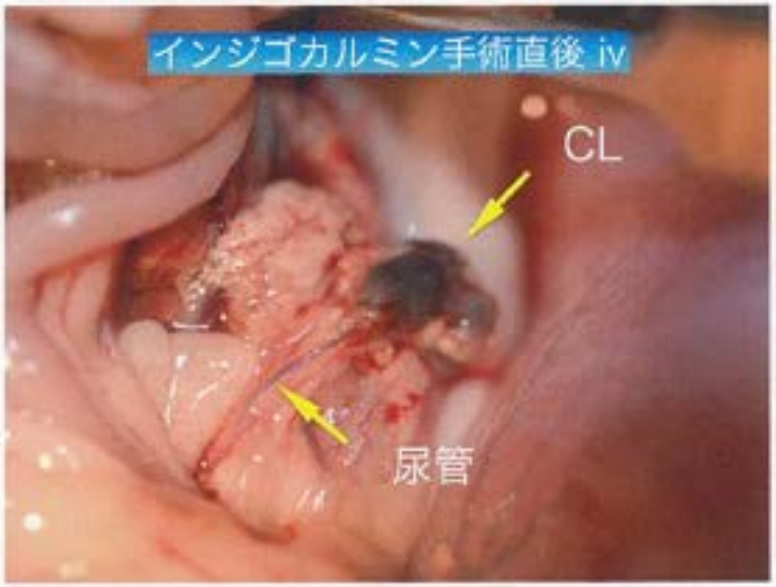
B. ブター猫間

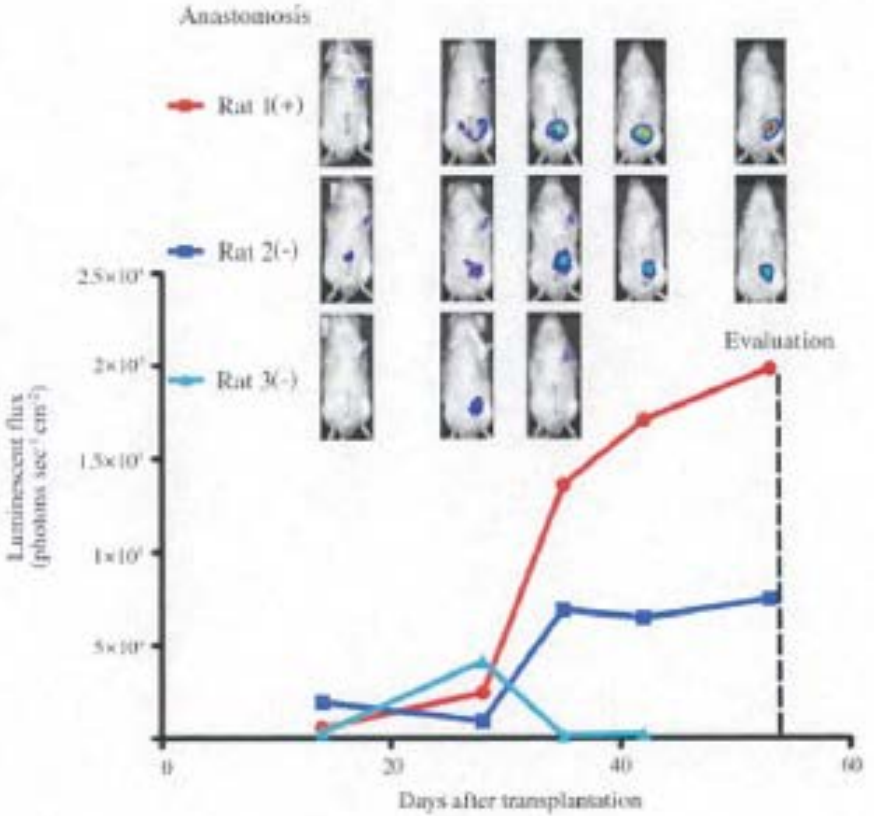


(Yokote T, et al. PNAS 2015)

尿路再建には確実なマイクロサージャリー技術が必要

Embryonic kidney function in a chronic renal failure model in rodents

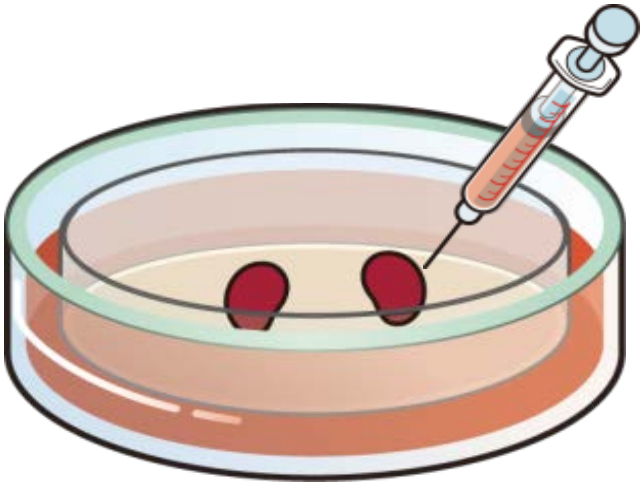


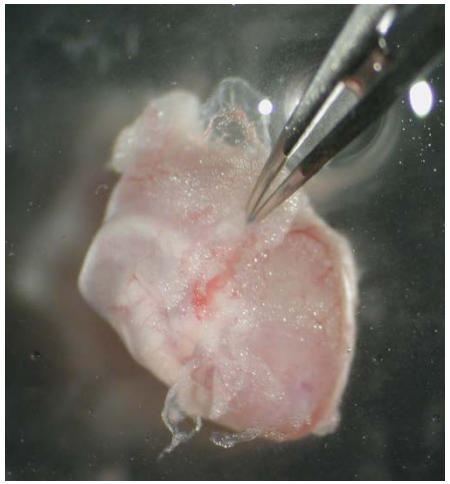


(Fujimoto E, et al. Clin Exp Nephrol. 2016)

ブタメタネフロン（腎原基）への
ヒトMSC注入によるヒトキメラ臓器作出

特許技術：人工腎臓前駆体及びその製造方法





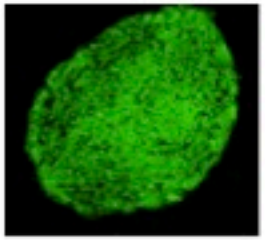
ヒトMSCを体外
で簡便に注入

（2012年6月5日　ニュージーランド　登録番号590122　）

特許技術の利用法(1):幹細胞の分化能の評価法

従来法

免疫不全マウス
へ移植

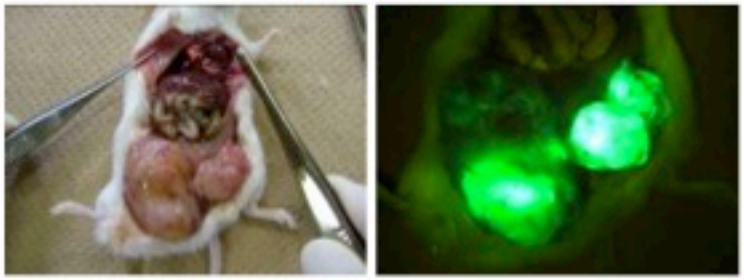


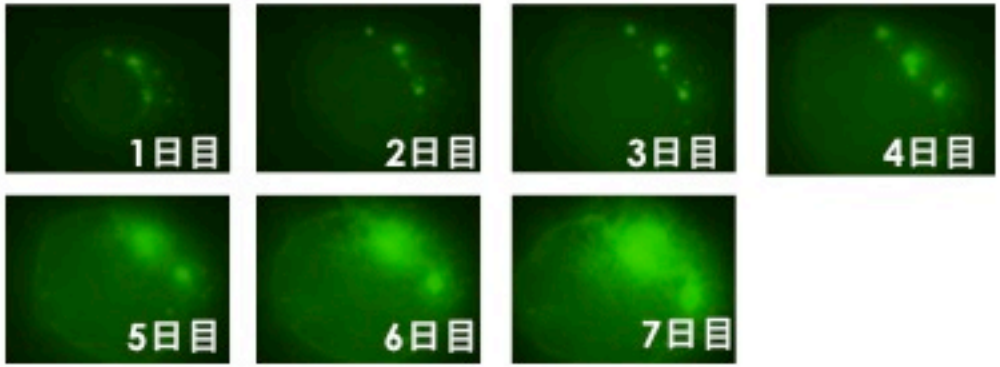
ヒトiPS細胞

新手法

器官培養

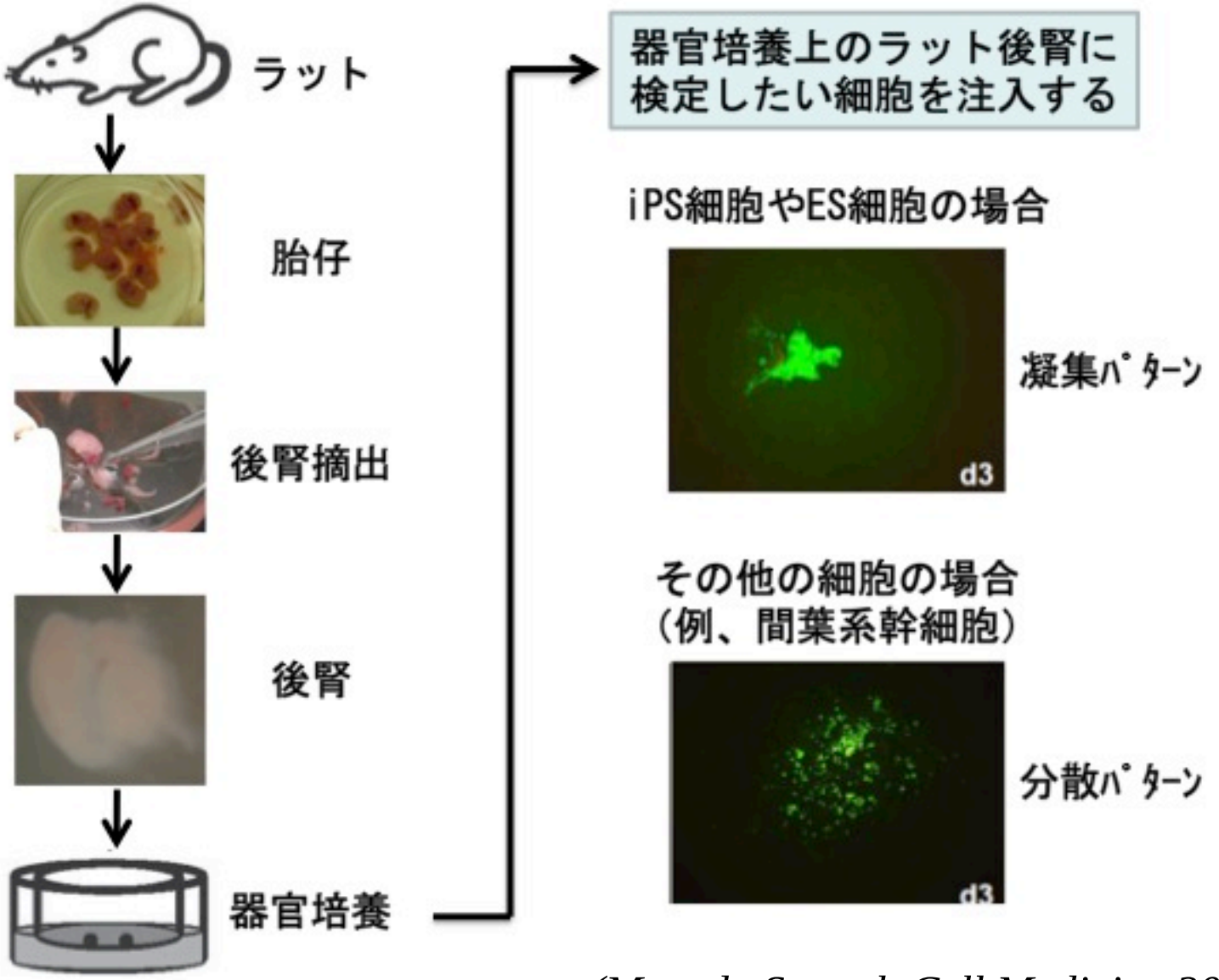
	所要期間	開始細胞数
➢ 従来法：腫瘍(+)	(2ヶ月)	(10^5個)
➢ 本法：　凝集(+)	(1-2週間)	(10^3個)





（2012年7月6日　日本　登録番号5030039　）

特許技術の利用法(2):ES/iPS細胞とMSC細胞の区別



(Masuda S, et al. Cell Medicine 2012)

最大なる課題

‘日本人はマウスの治療が上手‘の皮肉

患者中心であることを
忘れてはならない

THIS WEEK

EDITORIALS

FUTURE-PROOF Short-term solutions to long-term issues p.164

WORLD VIEW Business must pull up its sustainable socks p.165

SEA ME? Cuttlefish switch off to evade marine predators p.166

Stem the tide

Japan has introduced an unproven system to make patients pay for clinical trials.

Japan has been working feverishly to stay at the cutting edge of research and clinical applications in regenerative medicine. It has invested billions of yen in induced pluripotent stem (iPS) cells — made by r
develop in
tions to cr
The stra
ments wer
erative-m
patients' n
in phased

“Japan could find itself flooded with unsuccessful treatments.”

of millions of dollars. But it is not clear whether the acceleration will benefit patients or help Japan's overburdened national health system.

One of the approved treatments, HeartSheet, is made of skeletal-muscle stem cells that are taken from a patient's thigh and grown in the lab. The sheet, made by the company Terumo, is then applied to the hearts of people who have severe cardiac failure. Japan's health ministry gave “conditional approval” for clinical use of the treatment after the company carried out a phase II trial, which hinted at its safety and efficacy in seven patients (Y. Sawa *et al. Circ. J.* **79**, 991–999; 2015).

The company can market and sell the treatment. The approval comes with the condition that, within 5 years, Terumo must provide
ts treated with HeartSheet and 120 controls
is effective. Officials at the Pharmaceuticals
cy, which approves new treatments, say that
ata will be just as strict as it would be fo
ical trial.
Japanese obsessions. First, th
generative medicine
S cells — which
Nob
national project.
d t
mies of economic growth,
s
biotechnology so far.
e world
excited about the approval, too.
on are a welcome counterpoint to the nar-
biotech firm Geron, once a trailblazer in
given up on embryonic stem-cell therapies
Takahashi of the RIKEN Center for Devel-
decided to halt her trial of iPS-cell-derived
elated macular degeneration.
ay, and pay dearly: the HeartSheet treat-
ion (US$122,000). Last month, the health
ocedures covered by national health insur-
patients still pay 10–30% of the cost for a drug that is not known to be effective. As they do so, they basically subsidize the company's clinical trial.

Japan has turned the drug-discovery model on its head. Usually, the investment — and thus the risk — is borne by drug companies, because they stand to gain in the long run. Now the risk is being outsourced. By the time it is clear whether a treatment works or not, the companies will have already made revenue from it.

The government argues that its system will encourage firms to bring to market regenerative-medicine treatments that might work. They will, at least, work well enough to make it past small initial trials. Many drugs do that, and then most of them fail at phase III.

“Japan could find itself flooded with unsuccessful treatments.”

Biotech companies in other countries are keen on the idea and have pushed their own regulatory bodies to follow Japan's lead. This is a bad move. Regulatory agencies around the world should resist pressure to create such fast-track systems, at least until Japan has proved that its system works. That will take time. The country will have to demonstrate that its health-care system can withstand the costs of the new regenerative-medicine treatments, and that patients do not feel cheated. What happens when, inevitably, one of the fast-track drugs turns out to be ineffective? Company officials and government representatives say that patients will not be reimbursed, even though some might have paid up to ¥4.5 million (the rest covered by health insurance) for an ineffective treatment.

Japan's drug authority must guarantee that the post-commercialization evaluation of the drugs will be as rigorous as it says. It will not be easy to rein in a drug that has already been approved, whether that approval is conditional or not. If lax evaluation means that ineffective drugs are not revealed, or are not taken out of circulation, Japan could find itself flooded with unsuccessful treatments. And that would not be good for patients, the government or the biotech companies that want to see their truly effective medicines noted as such. ■

(Nature Dec 2015)

戦略：ヒト由来細胞を受け入れるブタを作る

1. ヒト由来細胞の安全性・有効性の検証

2. ヒト由来幹細胞注入でヒト組織・臓器を作る

免疫不全ブタの利用と課題

Ito T, et al. Generation of recombination activating gene-1-deficient neonatal piglets: a model of T and B cell deficient severe combined immune deficiency PLoS One. 2014

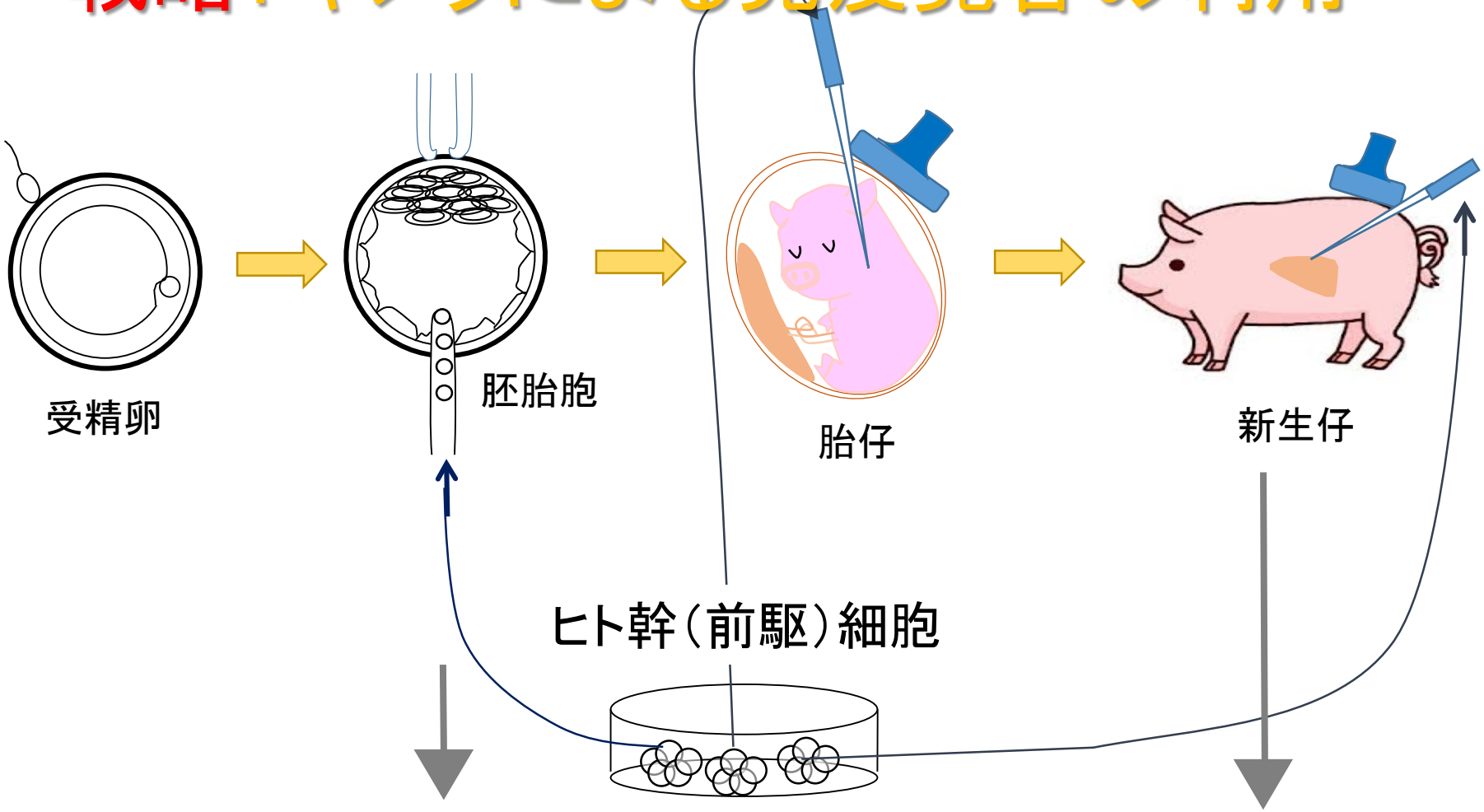
Huang J, et al. RAG1/2 knockout pigs with severe combined immunodeficiency J Immunol. 2014

Lee K, et al. Engraftment of human iPS cells and allogeneic porcine cells into pigs with inactivated RAG2 and accompanying severe combined immunodeficiency Proc Natl Acad Sci U S A. 2014

Watanabe M, et al. Generation of interleukin-2 receptor gamma gene knockout pigs from somatic cells genetically modified by zinc finger nuclease-encoding mRNA PLoS One. 2013.

Suzuki S, et al. Il2rg gene-targeted severe combined immunodeficiency pigs Cell Stem Cell. 2012.

戦略：キメラによる免疫寛容の利用



小動物	*(Yamaguchi T, et al. Nature2017)*	*(Hata T, et al. Ann Surg 2013)*
ブタ	*(Wu J, et al. Cell 2017)*	*(unpublished)*

Acknowledgement /共同研究者

慈恵医科大学
高血圧内科　横尾隆

明治大学　農学部
発生工学研究室　松成ひとみ、長嶋比呂志

北里大学
獣医学部　岩井聡美

慶應義塾大学　医学部
循環器内科　遠山周吾、藤田淳、福田恵一
心血管外科　川口新治、平野暁教、志水秀行
消化器外科　八木洋、北川雄光
消化器内科　杉本真也、佐藤俊朗

自治医科大学
先端医療技術開発センター　花園　豊

東京医科歯科大学
再生医学　関矢一郎

日本獣医生命大学
獣医看護　袴田陽二

京都大学　医学部
胆管膵・移植外科　八木真太郎、上本伸二

九州大学　医学部
小児外科　柳佑典、田口智章

佐賀大学　医学部
臓器再生医工学講座　中山功一

東京女子医科大学
先端生命医科学研究所　関根秀一、清水達也

国立成育医療センター
先端医療開発室　許懐哲、絵野沢伸

理化学研究所
多細胞システム形成研究センター
石川潤、辻孝