New approach:

Tailor-made immunosuppressed pig/monkey models for regenerative medicine

2019.11.29
CiRA 2019 International Symposium Luncheon seminar

Axcelead Drug Discovery Partners Inc.
Integrated Biology
Toshiyuki Maki, Ph.D.

AXCELEAD
Drug Discovery Partners

Corporate Overview

- **Company name**
  Axcelead Drug Discovery Partners, Inc.
- **Established**
  July 1, 2017
- **History**
  Originated from Takeda spin-out
- **CEO**
  Yoshinori Ikeura, Ph.D.
- **Location**
  Shonan Health Innovation Park
  Fujisawa, Kanagawa, Japan
- **Number of Employees**
  >200 researchers
- **Scope of Business**
  One-stop integrated drug discovery services
- **Major shareholders and ration of shares held**
  Drug Discovery Gateway Fund 100%

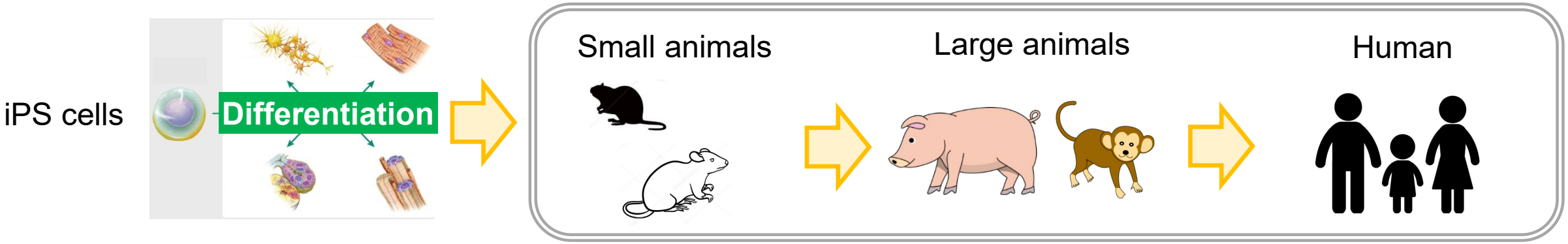
We offer a one-stop-shop service

From the initial research to preclinical development, Our IDD service offers tailored services to streamline the overall drug discovery process

Full Capabilities for drug discovery are in a facility

Function	Target ID & validation	Hit Identification	Lead generation	Lead optimization	Extensive evaluation	IND/NDA enabling study
Screening	In vitro validation	HTS	In vitro evaluation	In vitro evaluation		IND enabling in vitro evaluation
Chemistry	Druggability Tool cpd	Tool cpd Hit cpd	Design and Synthesis	Candidate selection	non-GxP bulk. API synthetic route	Synthesis of metabolites
Biology	TPP Omics, BI vitro/vivo validation	Prep. of in vitro pharmacology	**In vitro Pharmacology**	**In vivo PD**	**Efficacy in disease model**	IND enabling pharmacologi cal studies.
DMPK	Druggability	Hit profiling	HT-ADME	**HT-ADME PK/PD**	Human PK. PK/PD	IND enabling DMPK studies.
Safety	Druggability Molecular & Anatomic Pathology	Hit profiling	In vitro toxicology	In vitro toxicology. In vivo toxicodynamics.	In vivo toxicology	IND enabling safety studies

Preclinical studies in large animals are needed to ensure safety and efficacy prediction of regenerative medical products in human



- Predicting the results of clinical trials may be difficult based on the results of small animal studies alone.
- From the number of transplanted cells that are effective in large animal studies, it is possible to predict the number of transplanted cells that will be clinically effective

Today's topics !

Axcelaed establishes large animal models that accept human cells.

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Pharmacological studies using large animal models

Pig

- Microminipig（MMP）
- Göttingen minipig

➢ Cardiovascular anatomy and physiology similar to human
➢ Body and organ size similar to human

Monkey

- Cynomolgus monkey

➢ Higher brain function similar to human

Animal facility

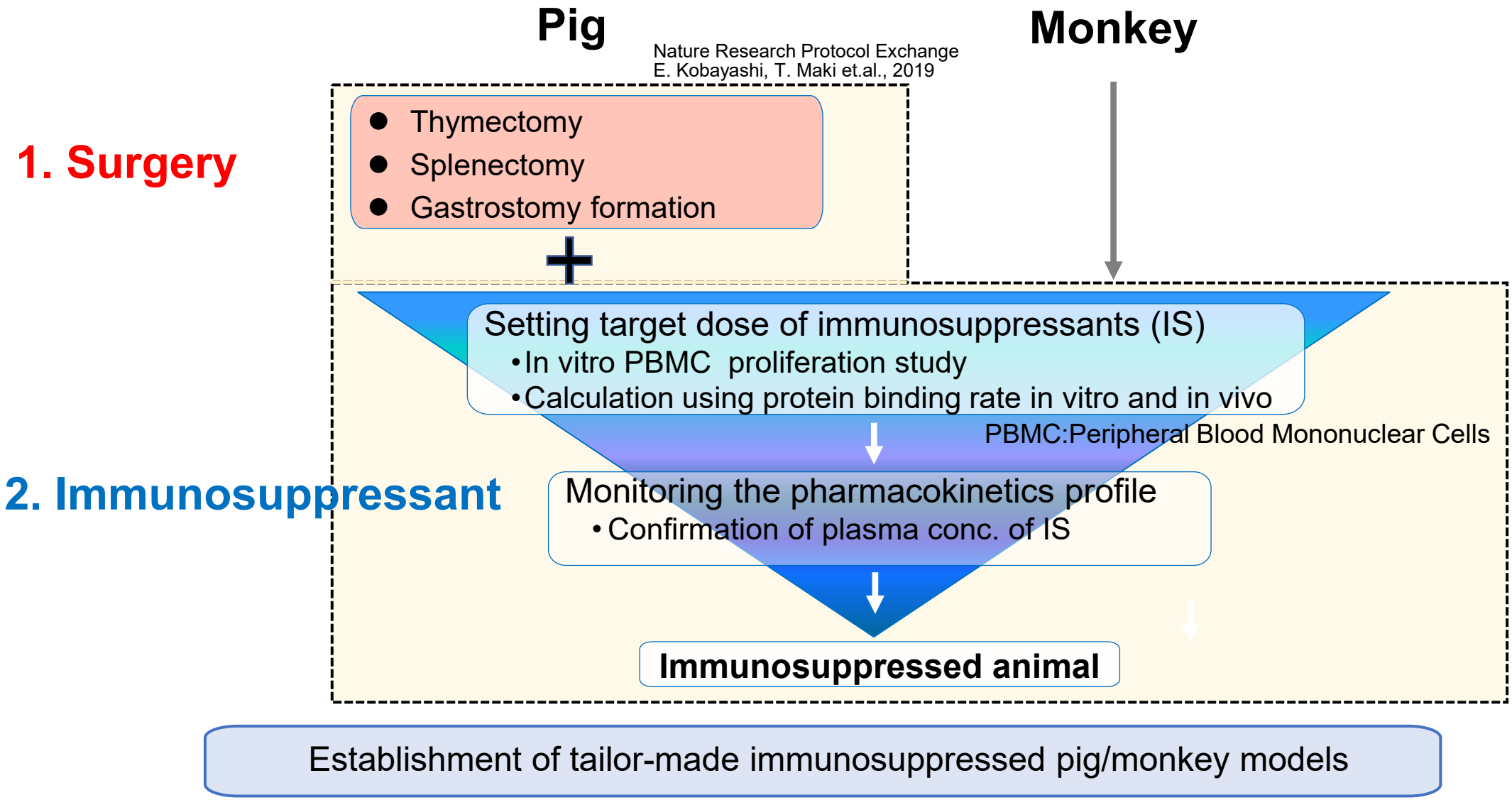
- AAALAC approved
- Cleaning everyday
- Surgical room
- Cell culture equipment, a P2/clinical sample area in the same facility


Breeding cages for pig


Surgical room


P2/Clinical sample area

Procedure for establishing immunosuppressed pig/monkey models



1. Immunosuppression by Surgery in pig

Removing immune organs

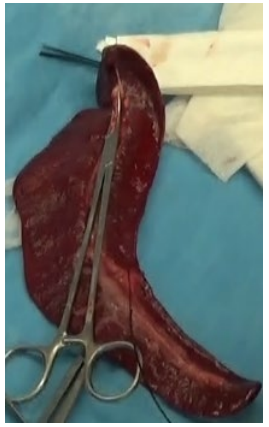
Adviser: Dr. Kobayashi (Keio Univ.).

■Thymectomy

- Commercially available thymectomized micro-mini pig (Fuji Micra Inc.)
- Thymectomy of Göttingen minipig in AXL

■Splenectomy

- Ligation of short gastric arteries, splenic artery, omental artery and removal of spleen



Other operations

■Gastrostomy formation

Objective

- Route for immunosuppressants
- Route for liquid nutrition product

■Insertion of central vein catheter

Objective

- Blood sampling for pharmacokinetics
- Route for intravenous feeding

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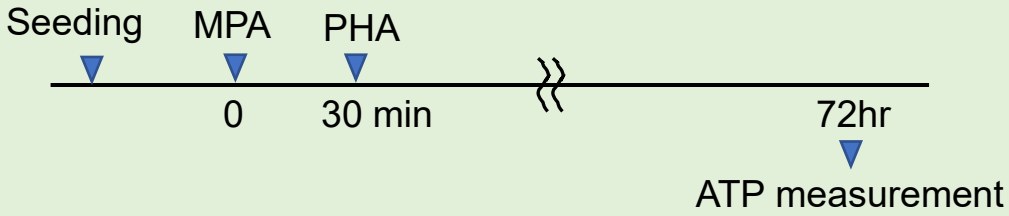
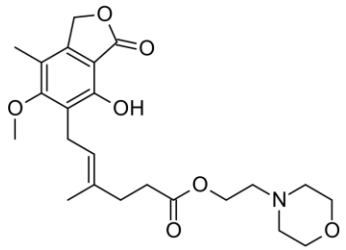
2. Immunosuppression by immunosuppressant in pig and monkey
=*in vitro* PBMC proliferation study=

- Collecting PBMC from pig plasma
- Seeding PBMC ($5x10^4$ cells/well)

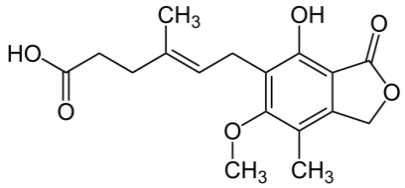
PBMC:Peripheral blood mononuclear cells

PHA:Phytohaemagglutininin



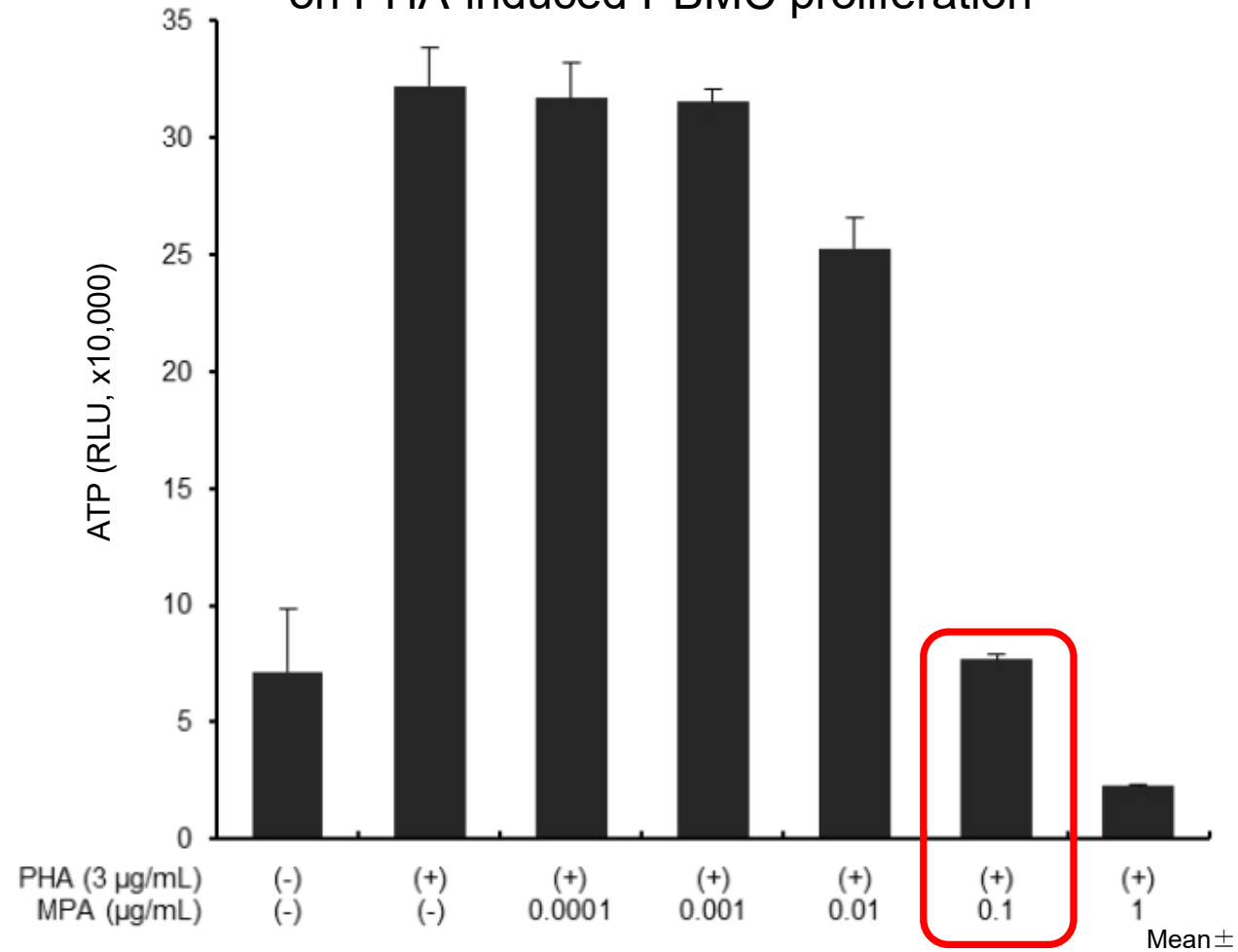


mycophenolate mofetil (MMF)
(Pro-drug)



Mycophenolic Acid (MPA)
(active form)

Effect of mycophenolic acid (MPA)
on PHA-induced PBMC proliferation



Mean±S.D., n=3

Nature Research Protocol Exchange
E. Kobayashi, T. Maki et.al., 2019

PHA-induced PBMC proliferation was inhibited by MPA at 0.1 μg/mL

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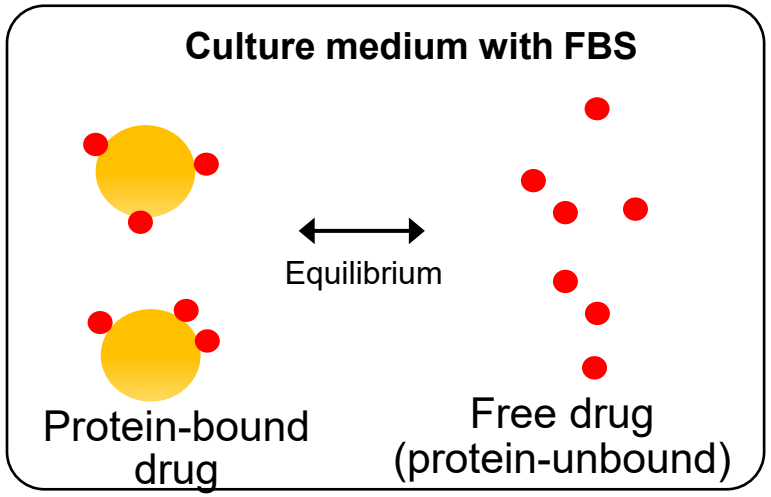
2. Immunosuppression by immunosuppressant in pig and monkey
= *targeted plasma conc. of drug* =

Calculation using unbound fraction (fu) in culture medium and in vivo plasma

Protein
(mainly albumin)

● Mycophenolic acid

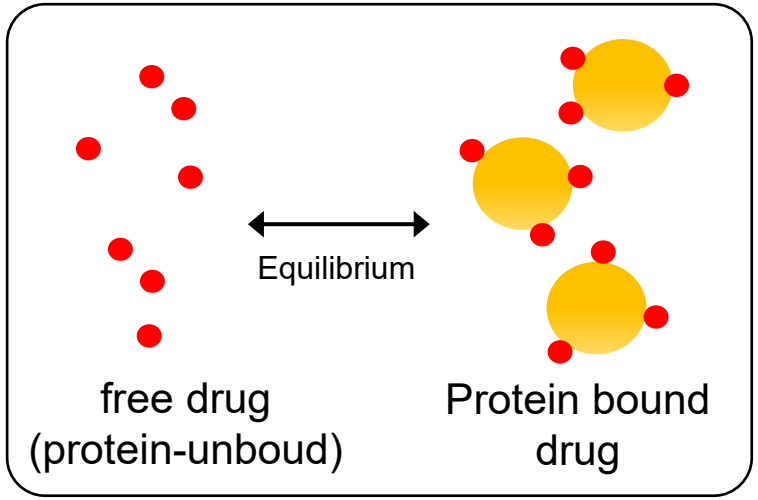
PBMC proliferation study



Concentration in culture medium (μg/mL)	
Total conc.	Free drug
0.1 →	0.059

fu:0.59

Targeted plasma drug conc. in pig



Concentration of plasma in animal (μg / mL)	
Free drug	Total conc.
0.059 →	**1.18**

fu:0.05

Total = protein-bound drug + free drug

The targeted plasma concentration of drug was calculated to be **1.18 μg/mL**

2. Immunosuppression by immunosuppressant in pig and monkey

= *Pharmacokinetics of mycophenolate mofetil (MMF)* =

Administration of mycophenolate mofetil(MMF) in pig

Day 0 — Day 7

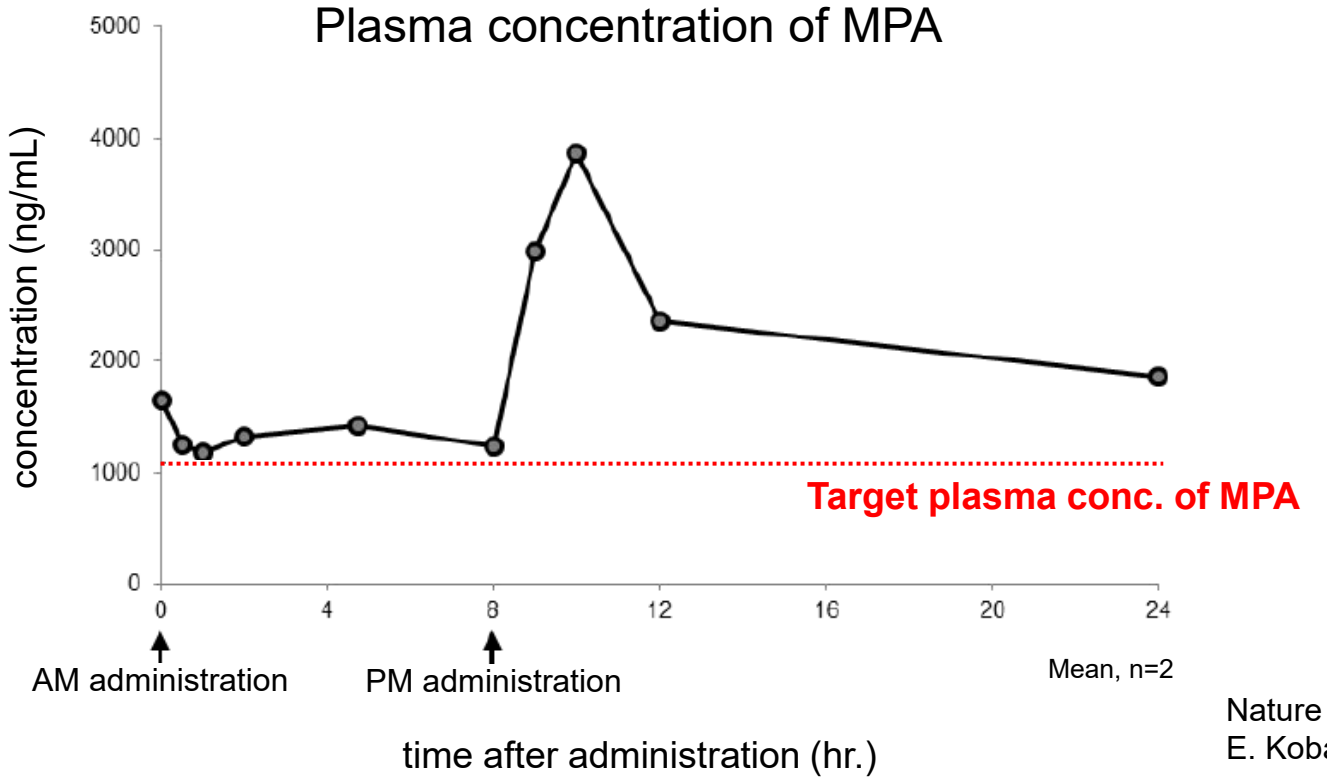
Collecting plasma for pharmacokinetics

MMF (**20 mg/kg**) MMF (**30 mg/kg**)

9:00 17:00 21:00 9:00

0 0.25 0.5 1 2 4 8 9 10 12 24

Time after AM administration (hr.)

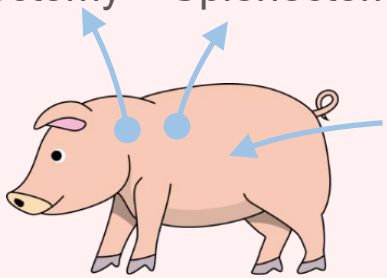


Nature Research Protocol Exchange
E. Kobayashi, T. Maki et.al., 2019

Plasma concentrations of MPA were kept higher than the targeted concentration throughout the day

Efficacy and engraftment study can be conducted using immunosuppressed animals

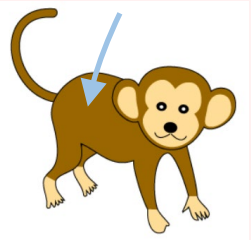
Thymectomy Splenectomy



Gastrostomy formation
Immunosuppressant

Immunosuppressed pig
[Surgery + Immunosuppressant]

Immunosuppressant



Immunosuppressed monkey
[Immunosuppressant]

Type1 diabetes
[streptozotocin-induced]

Immunosuppressed-diabetes pig models

Cell transplantation

Heart failure
[left coronary artery ligation]

Immunosuppressed-heart failure pig/monkey models

Cell transplantation

Evaluate engraftment and efficacy

Establishment of STZ-induced type1 diabetes pig model

STZ treatment in pig

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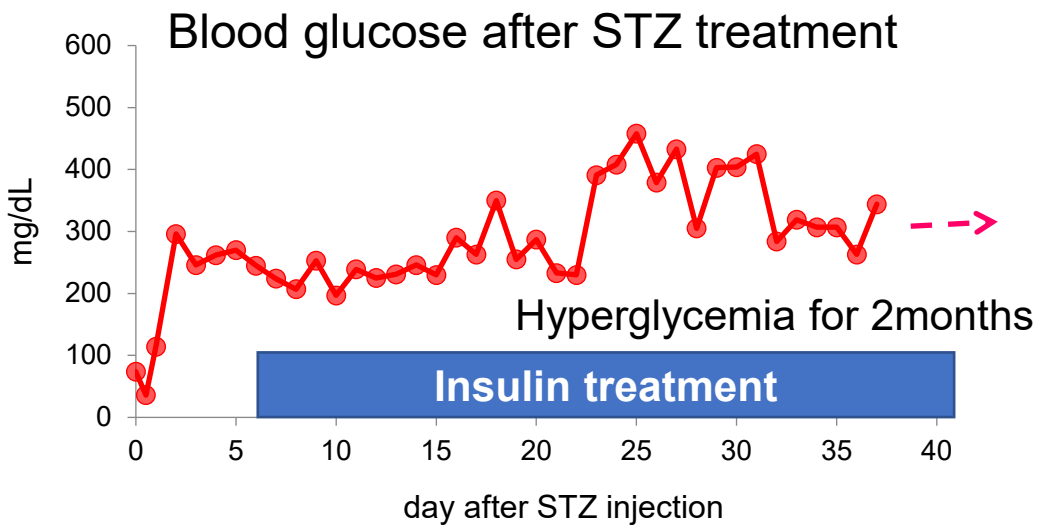
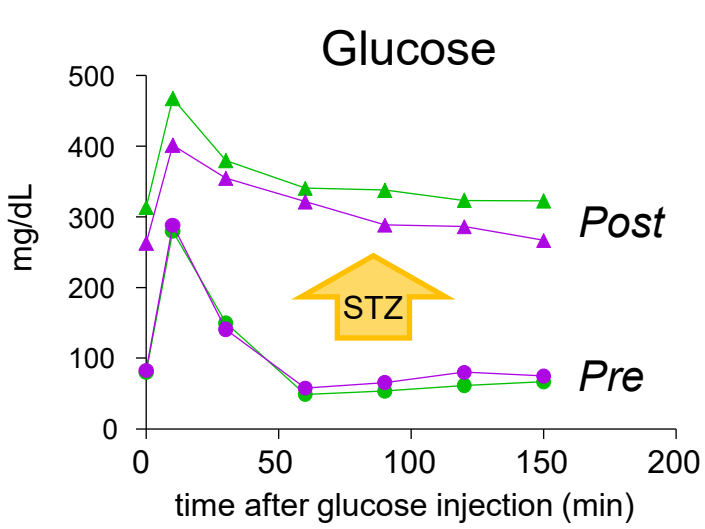
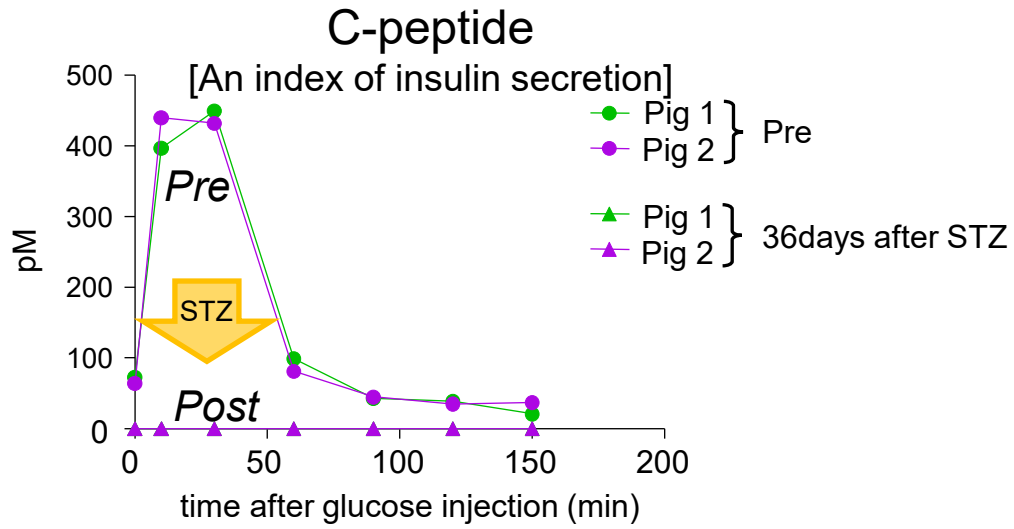
- ✓ Hyperglycemia for 2 months
- ✓ Glucose tolerance and decrease in C-peptide secretion (ivGTT)
- ✓ Stable physical condition

ivGTT : IntraVenous Glucose Tolerance Test



ivGTT (Pre and post STZ treatment)





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Establishment of heart failure pig model

Left coronary artery ligation in pig

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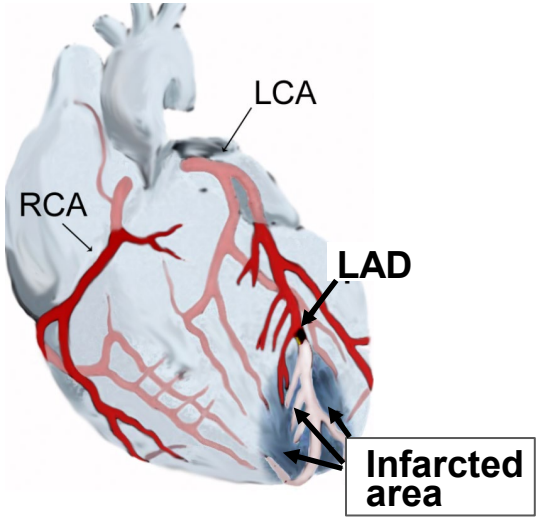
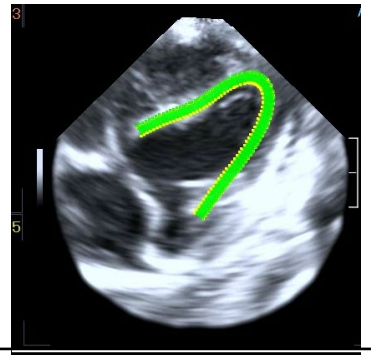
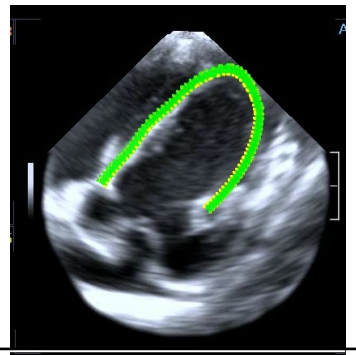
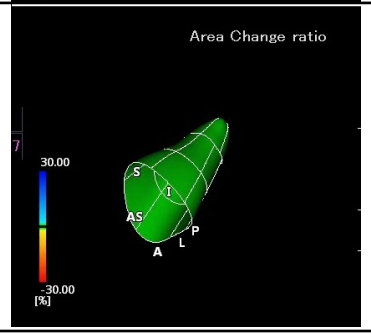
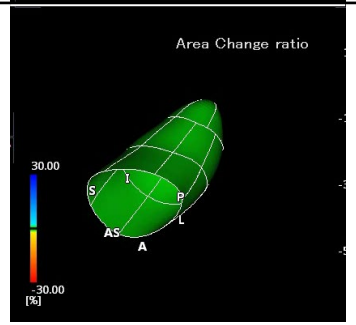
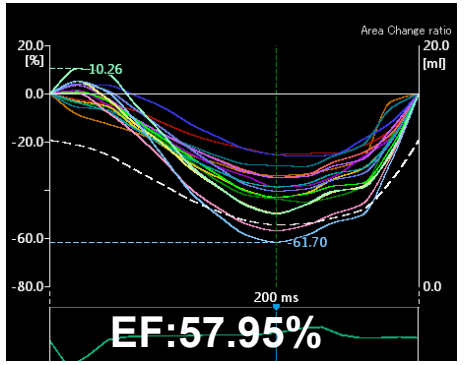
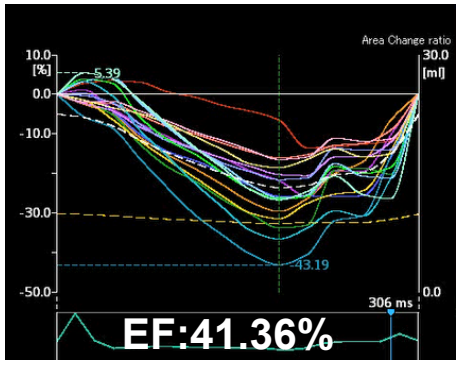
- ✓ Myocardial infarction
- ✓ Decrease in ejection fraction 2weeks after the surgery



LAD : left coronary artery

3D Echo (Aplio i900)

	Normal [before myocardial infarction]	Heart failure [35days after myocardial infarction]
Apical 4-Chamber		
3D Plastic BAG		
Area strain curve		

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Problems and solutions in pre-clinical studies in regenerative medicine

Problems

- Gaps between clinical trails and small animal studies
- No established immunosuppressed large animal models

Solutions

Tailor-made large animal immunosuppression models by removing immune organ and treating with immunosuppressants

High clinical extrapolation is expected to improve the probability of success in clinical trials.

Evaluation platforms for development of human iPS-derived cells in Axcelead

Menu	Contents
In vitro tumorigenicity study	Detection of undifferentiated iPS cells
In vivo tumorigenicity study	Detection of tumorigenic cells (non-GLP)
Safety study	General toxicity study Safety pharmacology study
Pharmacological study	**Evaluation of engraftment and efficacy of iPSC-derived cells using model animals (pigs and monkeys)** Today's presentation Evaluation of engraftment and efficacy of iPSC-derived cells using model animals (rats and mice)
Others	Evaluation of cell delivery device

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