Axcelead

Innovation Accelerator as the Best Partner

Integrated & Translational Science

Axcelead Drug Discovery Partners Inc.

AXCELEAD
Drug Discovery Partners

Therapeutic areas

CNS、CV/MD、Oncology、Immunology、Motility

Selected Axcelead Service

Target ID、HT-ADME、DDA&TR、PP-G、
Integrated safety assessment

Platform

NGS/BI、Proteomics/Metabolomics、
Genetically modified mouse/rat、
Integrated pathology

Discovery Services

Pharmacology study using large animal、
In vivo platform、Ophthalmologic evaluation

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CNS

1. Brain Function

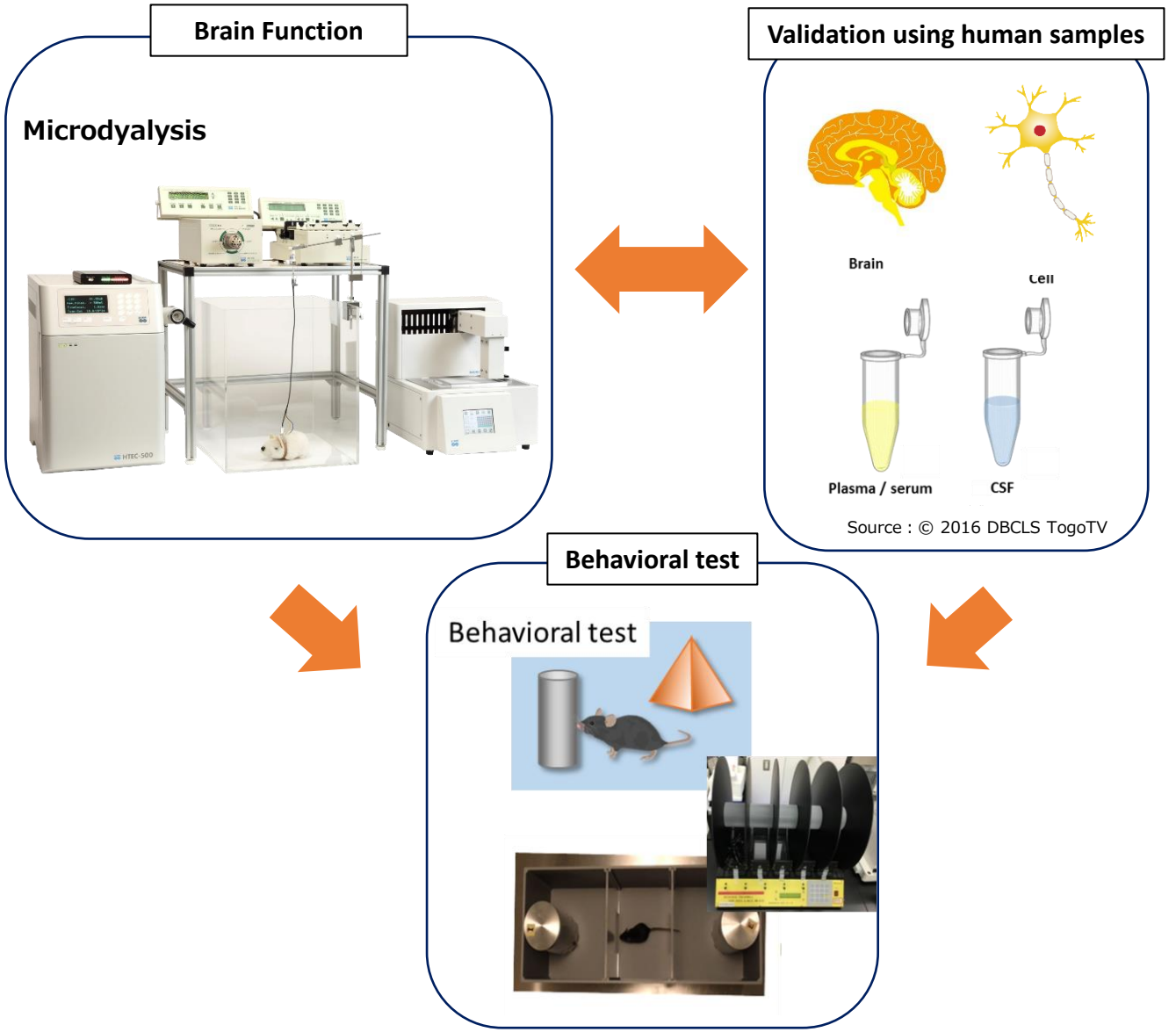
- ◆ Evaluation the potential of compound by neurotransmitter release linked to the brain function using Microdialysis.
- ◆ Improve predictability in clinical trials by biomarker search etc.
- ◆ Experience that synthesize the PET ligand in collaboration with other function

2. Behavioral test

- ◆ Various behavioral tests that are essential to evaluate the efficacy of compound in CNS area
- ◆ Experience and knowledge that have been involved in the establishing various assay systems

3. Validation using human samples

- ◆ Validation of hypothesis based on the change of gene and protein expression using patient-derived CSF, plasma, brain and cells etc.
- ◆ Sampling of CSF, plasma, brain and cells from animal model and comparison the change of patients with that of animal model
  (Back translation)
- ◆ Biomarker search by comprehensive analysis using multiomics analysis and bioinformatics



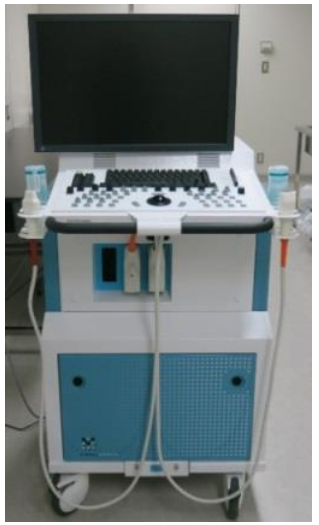
Cardiovascular disease (CV) / Metabolic disease (MD)

1. High expertise in CV / MD area

- **Experience and knowledge of various animal models**
  - Design the most appropriate *in vivo* study plan depending on target or mechanism
- **Knowledge of heart failure and diabetes mellitus involving multiple organs**
  - Comprehensively consider pathophysiology and drug effects in the heart, blood vessel, and kidney regarding heart failure or in the liver, pancreas, adipose tissue, skeletal muscle, and kidney regarding diabetes mellitus
- **MOA analysis**
  - Perform MOA analysis through *in vivo* and *in vitro* study for elevating or lowering blood pressure, cardiac function, and blood glucose
- **Unique evaluations using special equipment**
  - Non-invasive and real-time cardiac function/structure using ultra high frequency cardiac-echo apparatus
  - Direct cardiac function in isolated perfused rat heart, direct vasoconstriction/relaxation in rat aorta ring assay
  - Non-invasive and real-time fat weight using EchoMRI and X-ray CT system
  - Energy consumption analysis using Oxymax system

2. Superiority in NASH / kidney disease area

- **Various NASH models**
  - Select the appropriate model depending on target from multiple models that cause fibrosis due to chemicals or metabolic disorders
- **Superiority in the evaluation of siRNA and antibodies**
  - Stably perform long-term twice-weekly administration by retro-orbital injection
  - Evaluate KD efficacy of siRNA in hepatic cells / hepatic stellate cells
  - Measure plasma / liver siRNA concentration
- **Original kidney disease model**
  - Utilize Alport syndrome mice as a chronic kidney disease model (doi:10.1016/j.bbrep.2018.12.003)



Vevo2100 (ultra high frequency cardiac-echo apparatus)

Latheta LCT-200 (X-ray CT system)

Oncology

1. Variety of models

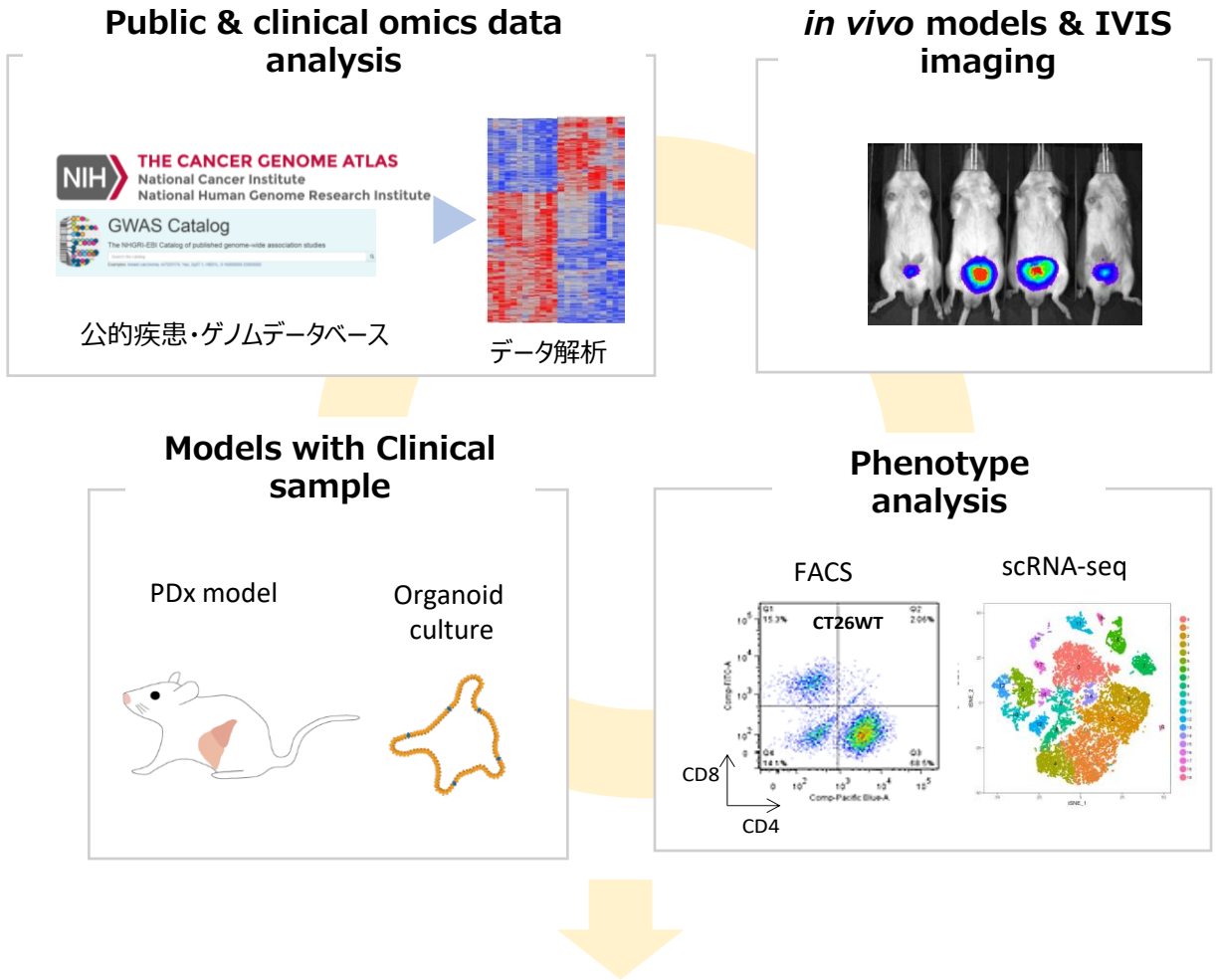
- **Appropriate model selection & development**
  - ➢ Appropriate cell line/ model selection by public & clinical omics data analysis
  - ➢ Development & evaluation of new disease models
    - ☐ In vivo imaging by IVIS for orthotopic and metastasis tumor models
    - ☐ Patient-derived PDx models & Organoids
    - ☐ Syngeneic mouse models

2. Multiple approaches & techniques

- **Multiple approach for target/ compound evaluation**
  - ➢ 3D culture/ invasion/ migration assay
  - ➢ Phenotypic analysis including immuno-profiling of tumors with FACS, IHC, scRNA-seq etc.
  - ➢ Experience on evaluation of small molecule, antibody, nucleic acid and CAR-T etc.
  - ➢ Evaluation of DDS carriers (Liposome, exosome etc.)

3. Support on Translational research

- **Biomarker analysis for clinical study**
  - ➢ Identification of biomarkers by Omics analysis using clinical samples
  - ➢ Validation of PD marker with patient-derived blood
- **Translational research for clinical strategy**
  - ➢ Multi-drug combination to expand target indications
  - ➢ Data for documentation/ publication



❖ **We will provide integrated and appropriate solutions for Oncology drug discovery**

AXCELEAD Drug Discovery Partners

Immunology

1. Wide variety of species and cell types

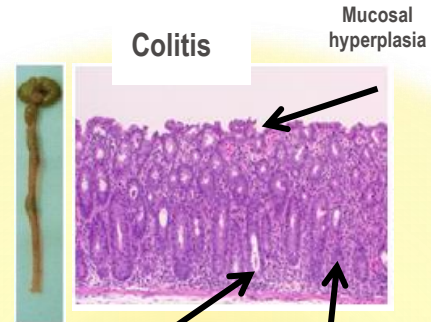
- Cells and biological samples from human, monkey, dog as well as rat, mouse
- In vitro evaluation of differentiation/proliferation/ activation of T cells (Th1, Th17, Treg, CD8 T)、B cells、neutrophils etc.

2. Multiple approaches

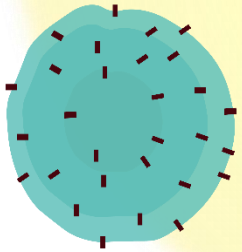
- Immunophenotyping
  - ✓ Quantitative analysis of immune cells in blood and tissues using 5-laser equipped BD LSRFortessa™
- Evaluation of cell-activation
  - ✓ Quantitative analysis of intracellular signal transduction (e.g. protein phosphorylation) using FCM、Western blotting法 etc.
  - ✓ Multiple analysis of biomarkers, such as cytokines, using Bio-Plex and MSD instrument)

3. Wide variety of disease models

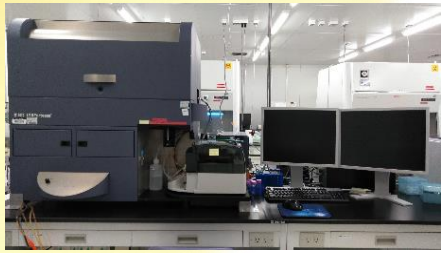
- Two kinds of T cell-transferred colitis models (anti-TNFa antibody responsive and resistant models) and DSS-induced colitis model
- EAE、RA、Psoriasis、allo/xeno-GvHD models etc.



Integrated Research for Immune diseases



Immune cell



BD LSRFortessa™

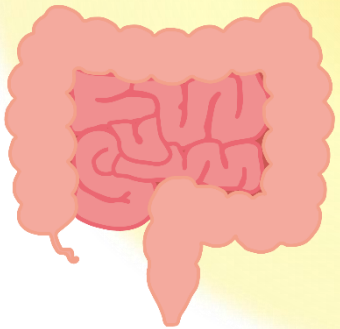
➢ **We can design and carry out the most appropriate in vitro/ in vivo studies to progress your project in inflammation and immunology driven diseases**

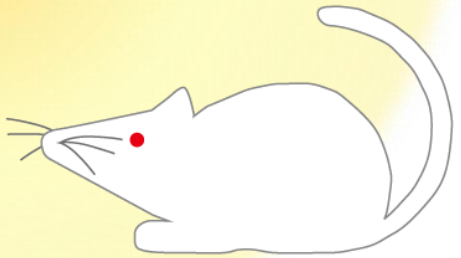
Motility

1. Ex-vivo and in vivo evaluation of gastrointestinal motility

- Evaluation of contractile response of murine/rat gastrointestinal tissues using organ bath (Magnus).
- Qualitative analysis of gastric emptying
- Beas-expulsion assay in mice
- Qualitative analysis of defecation in rats and mice

Integrated Research for GI/Motility





➢ **We will work with you to proof your concept and to progress your program through providing the most appropriate in vivo pharmacology solution in GI-motility disorders.**

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Next Generation Sequencing (NGS) and Bioinformatics

1.Acquisition of AmpliSeq data using NGS

- Global gene expression profile (applicable to frozen cells from 1well of 96-well plate)
- DNA mutation of specific genes （can handle clinical samples）
- Fingerprinting of compounds, ASOs

2.Visualization of omics data, making hypothesis from your data

- Using your data, and public data
  - ✓ Prioritization of chemotypes/compounds
  - ✓ Profiling of your compounds for repositioning
  - ✓ MOA verification of your compounds, ASOs, antibodies
  - ✓ Searching marker candidates
  - ✓ Evaluation of extrapolation to humans using DTC DNA genetic testing data
- Using single-cell RNA-seq data
  - ✓ Understanding cell variety, finding novel cell sub-populations, building differentiation trajectories
  - ✓ Searching cell surface markers

3. Consulting

- Experimental design for omics data acquisition
- Wet validation
- etc.

Samples and omics data

Patients

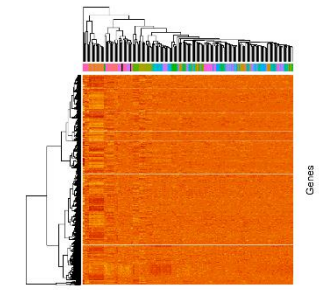
Animal modes

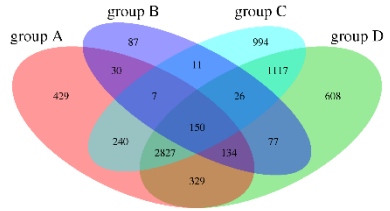
in vitro data

Original data

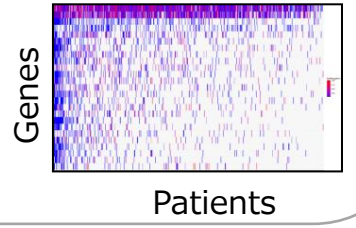
Data@public

Visualization・hypothesis





Heat map of mutations



❖ **We will support you from data acquisition, hypothesis making, through validation steps**

AXCELEAD Drug Discovery Partners

Proteomics/Metabolomics

1. Metabolome and proteome wide-approach

- Our high-end platforms assess about **600 metabolites, 30 lipid classes, 7000 proteins and 15000 phosphorylation sites** from a single biological sample.
- This approach enable **a promising discovery of biomarker or mechanism of interest with time- and cost-saving** compared with the traditional narrow-target approach.

2. Our multi-omics platforms using mass spectrometry technology

- **Metabolomics platform** focuses on major metabolic pathways including carbohydrate, energy, lipid, nucleotide and amino acid metabolism.
- **Lipidomics platform** covers various simple and complex lipids by combination of unique one-step lipid extraction, liquid chromatography and high-resolution mass spectrometry technologies.
- **Proteomics platform** detect alteration of protein expressions and phosphorylation profile with high quantitativity by stable isotope labeling and peptide fractionation technologies.

❖ **Our omics technologies offer the efficient biomarker discovery and MoA analysis through metabolome- and proteome-wide approaches.**

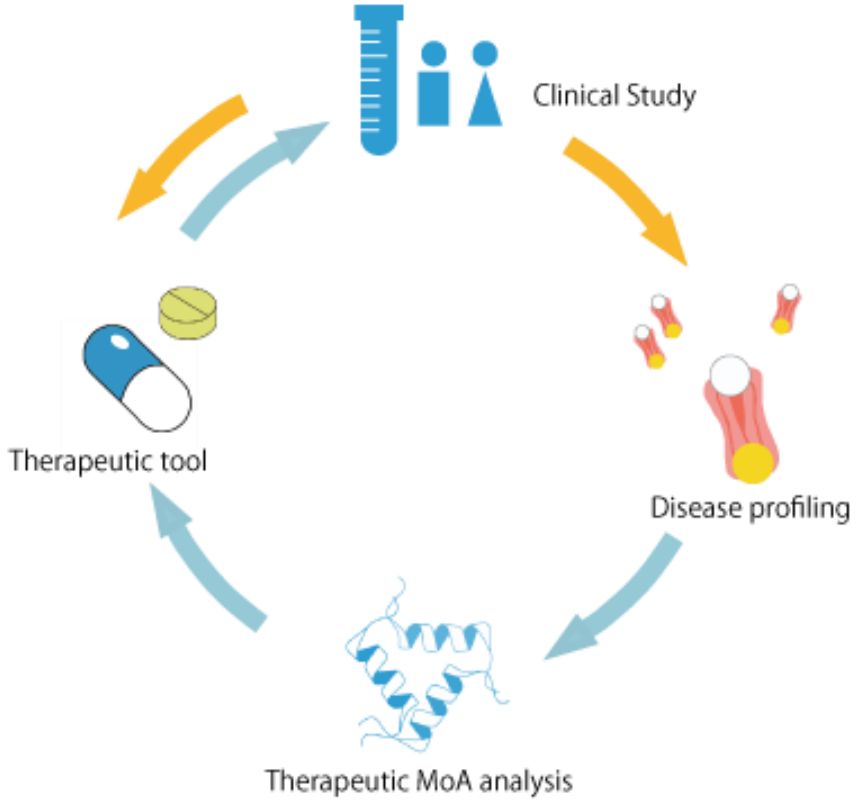


Fig. Applications of omics technologies for Drug Discovery and Development

・Disease profiling
・Therapeutic MoA analysis
・Discovery of clinical biomarkers
・Biomarker assessment in clinical study
・Profiling of therapeutic response

Genetically modified mouse/rat

1. High-speed and high-efficiency KO mice/rats creation.

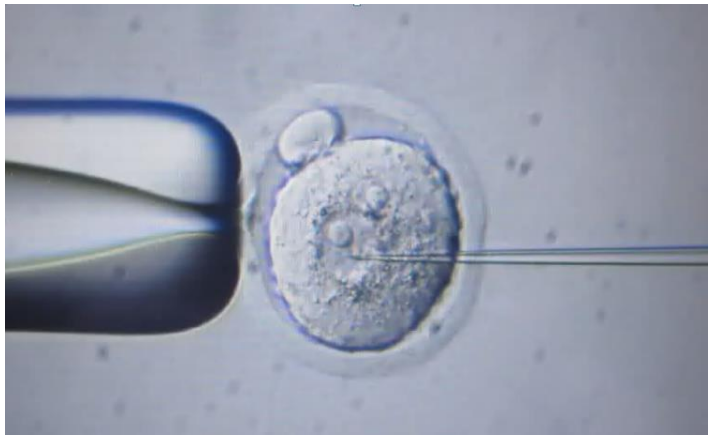
- F0 homo KO mice can be obtained within 2 months from pronuclear injection of CRISPR/Cas system. Therefore, the test using homo KO mice can be started within 3 months from project start.

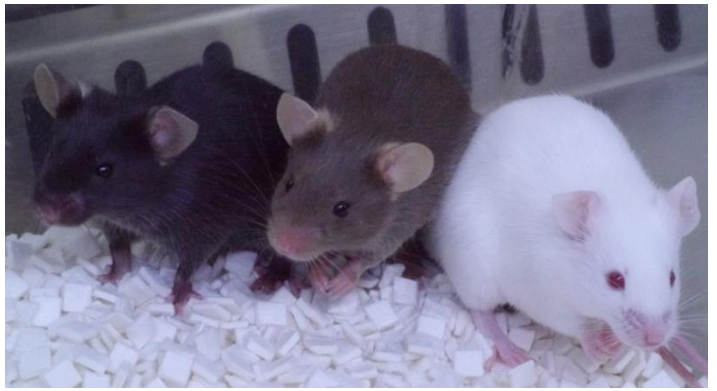
2. High-speed KI mice/rats creation.

- By pronuclear injection of CRISPR/Cas system with donor vector, several Kbp fragment KI(knock in) mice/rats can be created. Therefore, homo KI mice/rats can be created within 9 months.

3. Increase the number of mice in a short time by IVF.

- A number of genetically modified mice required for the test can be prepared in a short time by using superior IVF (In Vitro Fertilization) technique.





- In addition to the creation of genetically modified animals in a short time, pharmacological analysis and pathological analysis can be performed in a consistent system within Axcelead. Therefore, test results using GM animals can be acquired in a shorter time in the AAALAC certified animal facility.
- Complex genetically modified mice such as additional genetic modification to model mice can be created.
- Holding CRISPR/Cas9 basic patent license (ERS genomics).

Integrated Pathology (Histopathology/Pathology)

1. Pathological analysis of various animal species & organs

- Pathological evaluation supported by pathologists (JSTP/JCVP) experienced in a variety of study types and therapeutic areas (Toxicology, Oncology, Neuroscience, Inflammatory & Metabolic disease, etc.)
- Tissues from a wide variety of species (Rodents, Dogs, Non-human Primates, Pigs, etc.)
- Evaluation of toxicological study and pharmacological study by means of scoring system arranged for disease models, morphometric methods.
- HE stain, various special stain, Immunohistochemistry, *in situ* hybridization
- Support interpretation of clinical pathology data & identification of biomarkers for clinical trials

2. Comprehensive modalities for Pharmacological/Toxicological evaluation

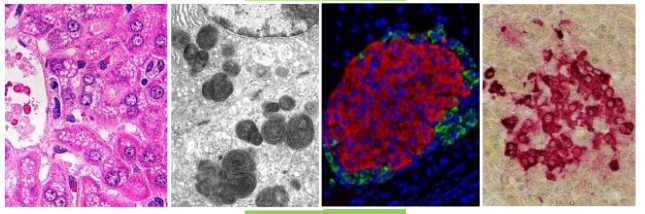
- Suitable evaluation for Small molecules, Antibodies, Gene therapies, Cell-based therapies
- Analysis of target expression in tissue using Tissue Microarray, Laser microdissection, IHC, ISH
- Evaluation of tissue distribution of administered antibodies/Oligonucleotides by Immunohistochemistry & *in situ* hybridization
- Evaluation of target molecule expression in cultured cells, identification/tissue distribution of graft human cells, host reaction to graft cells

3. Proposal/Establishment of appropriate image analyses

- Efficient, Reproducible, little biased quantitative image analysis using autostainers (BOND/ Ventana), slide scanners (Nanozoomer/Aperio), and image analysis platforms (Halo/Volocity)
- 3D-tissue structure and cultured cells by confocal microscopy
- Ultrastructural investigation by electron microscopy (TEM/SEM)

Autostainer (Left : BOND RX/Leica Biosystems;
Middle : Ventana discovery XT/Roche; Right: Ventana BMK-SS/Roche)

Whole Slide Scanner (Left : Nanozoomer S60/Hamamatsu Photonics;
Right : Aperio scan scope/Leica Biosystems)



Our services offer the optimal solutions for various problems on morphological evaluation

AXCELEAD
Drug Discovery Partners

Integrated Pathology (Clinical Pathology)

1. Clinical examination of various animal species

- **Various tests are available**
  - Hematology, Coagulation tests, Myelogram, Blood chemistry, Isozyme tests, Urinalysis, Hemolytic test, Platelet aggregation test, Complement tests
- **Reliable data**
  - Select the optimum sampling method that suits the test and analysis with appropriate sample condition.

Hematology:
ADVIA2120i/Siemens

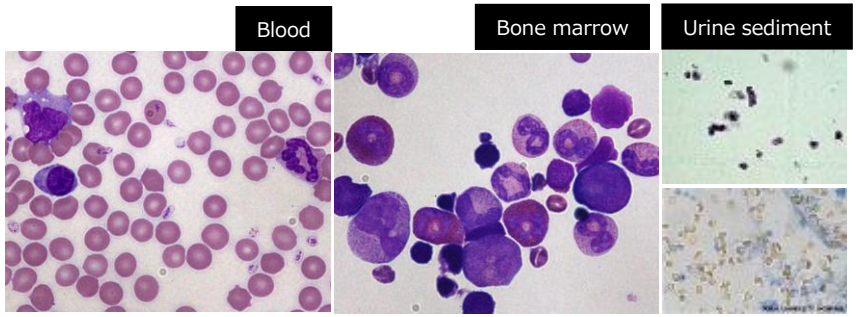
Coagulation:
CS2000i/Sysmex

Biochemistry:
LABOSPECT008/HITACHI

Urinalysis:
Cliniteck/Siemens

2. Proposal/Evaluation of appropriate analyses method

- **Proposal appropriate tests**
  - Injury marker analysis for kidney ,liver and heart
  - Blood parameter analysis for disease model animals
  - Support interpretation of pathology data & identification of biomarkers
  - In Vitro method to evaluate hemolysis, inhibition of coagulation or platelet aggregation
- **Analysis evaluation**
  - Clinical pathological evaluation supported by clinical technologists experienced in a variety of test types and understanding parameters for each species



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Axcelead's value: Target identification & validation

1. Multiple approaches

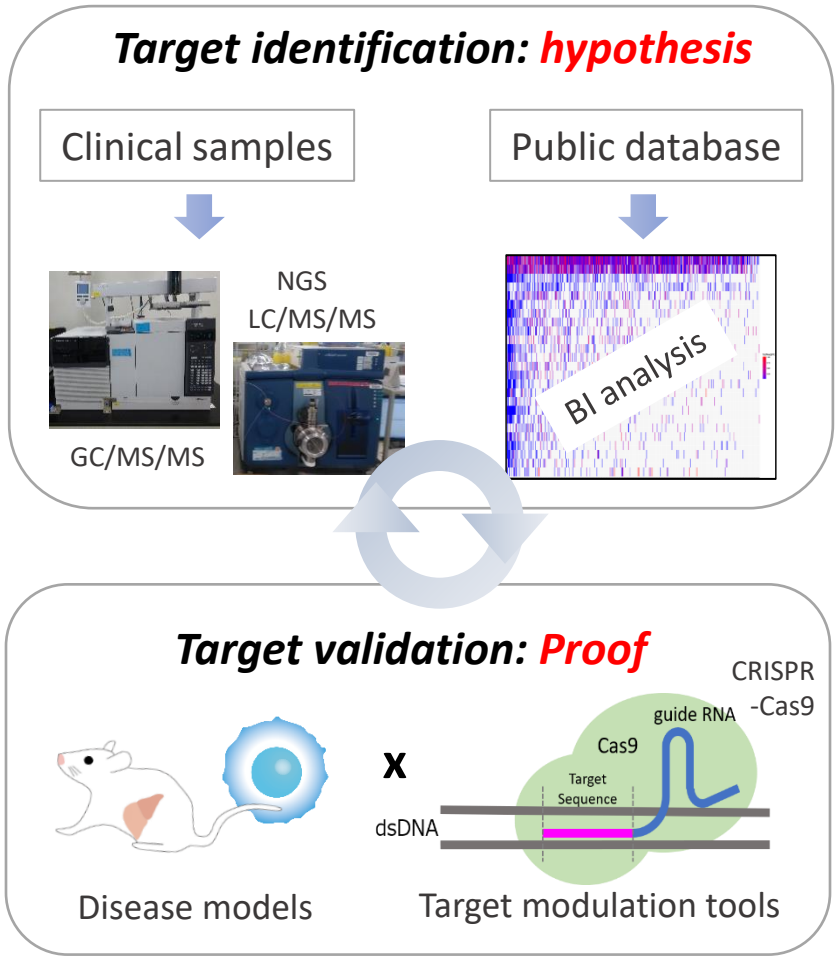
- **Clinical sample/data analysis**
  - Multi-omics analysis & Bioinformatics
  - Studies using blood, tissues, CSF, iPSC etc.
- **Model & tool for validation**
  - Tg/KO cells and animals & iPS-derived models
  - CRISPR-Cas9, shRNA/siRNA, tool compounds
- **Target assessment by various aspects**
  - Druggability analysis
  - On-target toxicity risk assessment
- **Takeda legacy asset for validation**
  - Accumulated data & tool compounds

2. Solid experience/ performance

- Over 20-yrs experience on target ID & validation
- Project launch in over 10 therapeutic area and more than 500 HTS for variety of targets
- Generation of >600 Tg/KO strains

3. One-stop shop solution

- All functions are co-located in the iPark and work as a project team



❖ **Axcelead provides one-stop/ integrated solutions to identify and validate better targets by flexible and efficient approaches**

High-throughput ADME / HT-ADME

1. Proposal of target ADME profiles and assay process design

- ADME profiling of your present molecules and proposal of research plan for lead optimization
- Strategic planning of assay process and decision tree for *in vivo* further evaluation and lead optimization

2. Fast and continuous improvement

- Automated screening system to provide ADME data within several days
- Refined skills and robust assay data over 20 years

3. Assay customization / Problem-solution fit

- Bioanalysis of more than 60,000 molecules
- Bioanalysis method development of new modalities（LC/MS/MS and ELISA）
- Metabolic profiling for chemical design and biological mechanism analysis
- Compound-specific issue identification and assay customization

ADME Screening

- PAMPA
- Metabolic Stability
- MDR1 substrate screening
- CYP inhibition
- CYP induction
- Plasma Protein Binding
- Cassette Dosing Study

Lab Automation & HTS

Rapid Customization of Bioanalysis Method

❖ **Our HT-ADME team can enhance your activities for drug discovery and lead optimization with our efficient screening systems & effective solutions.**

Drug Disposition and Analysis & Translational Research (DDA&TR)

1. Compound optimization at discovery stage

- Clarification of pharmacokinetic (PK) issues with ADME data
- Optimization of compounds with solving the PK issues: setting of screening workflow, in vitro-in vivo correlation, structure-property relationship analysis, metebolite identification, etc.

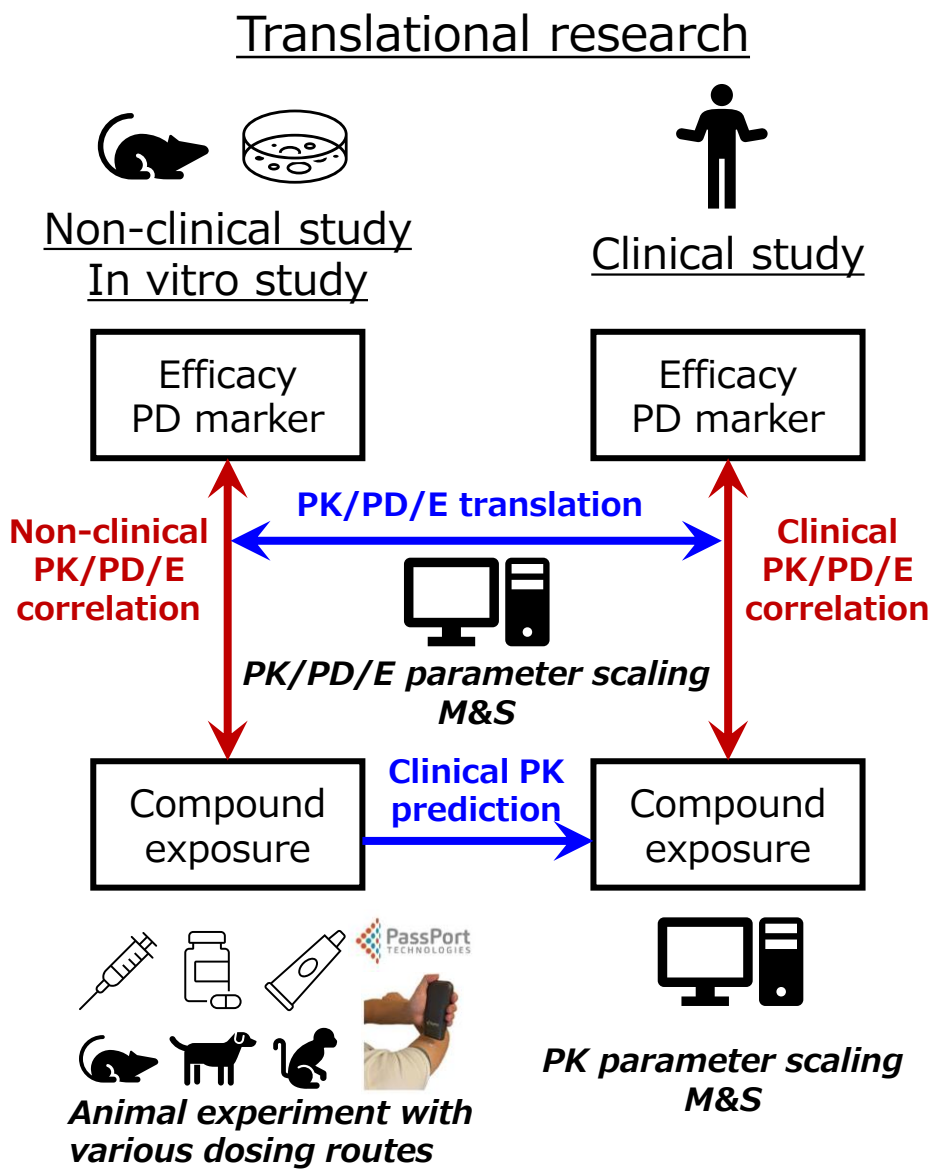
2. Translational PK/PD/E analysis

- Acquisition of POM/POC by the analysis of the relationship among compound exposure, PD marker, and efficacy (PK/PD/E)
- Estimation of clinical efficacious concentration and dose for the candidates by clinical PK prediction and translational PK/PD/E analysis

3. Technology for translational research

- Animal experiments with various dosing routes
  - ✓ Chimeric mouse with a humanized liver (PXB mouse)
  - ✓ Active transdermal drug delivery technology (PassPort® System)
- Quantification for various modalities (peptides, oligonucleotides, etc.)
- Prediction of oral absorption by a simulator (in preparation)
  - ✓ Exploration of issues and proposal of the solutions
- Modelling & simulation (M&S)
  - ✓ Extraction of issues from PK and PK/PD/E model analysis, and sensitivity analysis
  - ✓ Cooperation with Leiden Experts on Advanced Pharmacokinetics & Pharmacodynamics Consultants BV (LAP&P), the authority of M&S

LAPP Leiden https://lapp.nl/



Physicochemistry & Preformulation (PP-G)

1. Physicochemical property evaluation is essential for drug discovery

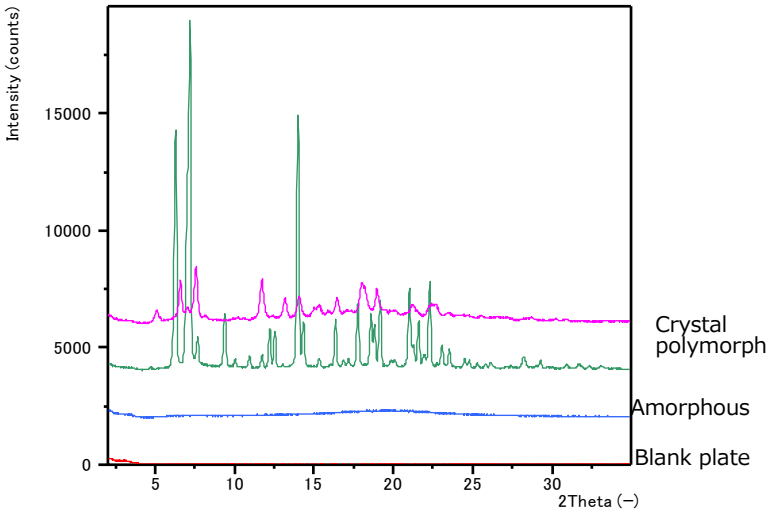
Function	Activity of PP-G
HTS	Purity check for Hit compounds
Chemistry	Quality check for synthesized compounds (Purity, Crystal form, Particle size etc.)
In vivo efficacy	Optimize dosing formulation for exposure enhancement
DMPK	Evaluate property for factor analysis of pharmacokinetics (Solubility etc.)
Toxicology	Test materials management, Preparation and Optimize dosing formulation for exposure enhancement

2. Evaluate the physicochemical profile for lead optimization

- **High throughput assays**
  - Kinetic solubility (JP2nd, 320 cmpds/w)
  - logD (HPLC method, 320 cmpds/w)
  - Purity (UPLC-PDA-CoronaCAD-MS method, 640 cmpds/w)
- **Assays for powder materials**
  - Physicochemical profiling(Powder X-ray diffraction, Thermal analysis, Thermodynamic solubility, 15 lots/w)

3. Decide nomination form of candidate compounds

- **The equipment needed to select the nomination form**
  - Powder X-ray diffract meter, Thermal analyzer (TG and DSC), Dynamic gravimetric vapor sorption analyzer
- **Knowledge and experience to select an optimal crystal form**
  - Possess methods to compare energy levels between polymorphs
  - Possess the skills to select the nomination form from the obtained results



Select an optimal crystal form

Integrated Safety Assessment (*in vitro* tox)

1. On- & Off-target Safety Assessment

- ◆ Data mining of on-target safety & proposal of safety risk solution
- ◆ Off-target safety risk evaluation by receptor binding/enzyme assay (8-target panel)

2. Cardiotoxicity Assessment

- ◆ ECG QT/Proarrhythmic potential assessment by multi-ion channel assay (hERG, Nav, Cav)
- ◆ Integrated cardiac E-C coupling assessment using human iPS-cardiomyocytes (MEA & Ca transient)

3. Genotoxicity Assessment

- ◆ Mutagenicity assessment (Ames)
- ◆ Chromosome aberration assay (TK6-MN/Umu)

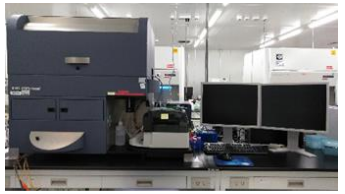
4. Miscellaneous Assessment

- ◆ Cytotoxicity assay (Glu or Gal medium)
- ◆ Phospholipidosis assay (PLsis)
- ◆ Phototoxicity assay (UV/Vis abs./NRU)

On- & Off-Target Safety Assessment
- ➢ **Target Safety Assessment**
- ➢ **Promiscuity Panel Assay**

Cardiotoxicity Screening
- ➢ **Multi-ion CH assay (SyncroPatch 384PE)**
- ➢ **MEA & Ca transient assay**

Genotoxicity Screening
- ➢ **Ames (Micro-Ames)**
- ➢ **MN (Micro-Flow)**
- ➢ **DNA damage (Multi-Flow/Umu)**



BD LSRFortessa™

Miscellaneous Screening
- ➢ **HepG2 Glu/Gal, 24/72h cytotoxicity**
- ➢ **HepG2 PLsis (NBD-PE accumulation)**
- ➢ **3T3 Phototoxicity (UV/Vis abs./NRU)**

We support your new drug development as a best partner by virtual and in vitro safety screening, with better time- & cost-performance.

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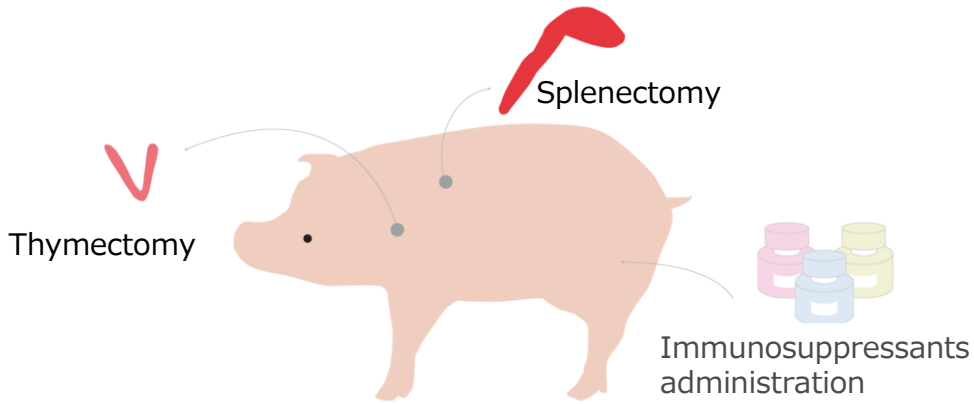
Pharmacological study using large animal

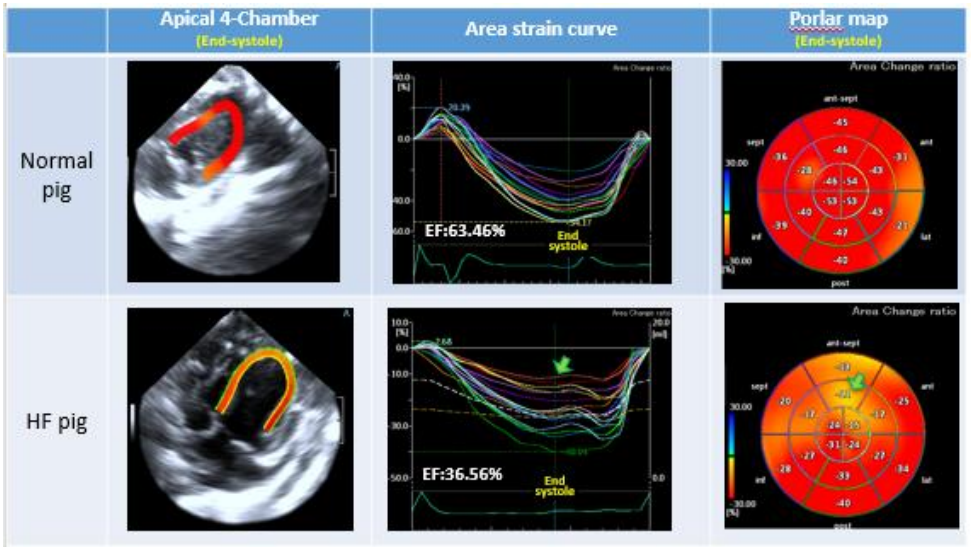
1. Advantages in regenerative medicine

- **Immunosuppressive model (pig, monkey)**
  - Establish immunosuppressive pig/monkey model engrafted by human iPS-derived cells
  - Taylor-made immunosuppressive model
    (Set targeted-dose by proliferation study in peripheral blood mononuclear cells → PK measurement in animals given immunosuppressants → Determine dosing of immunosuppressants)
  - Long-term breeding of immunosuppressive animals (more than half a year)
- **Large-scale surgery**
  - Perform multiple surgeries, including thoracotomy, on the same animal
  - Establish care for post-operative animals
- **Several disease models**
  - Establish heart failure pig/monkey and type 1 diabetes pig, etc.
  - Establish evaluation method (echocardiography, intravenous glucose tolerance test, etc.)
  - Combination of immunosuppression and disease models
- **Facility strength**
  - Certified by AAALAC
  - Biosafety level (BSL)-2 animal facility

Immunosuppressive pig model



Echocardiographic evaluation using 3D echo



In Vivo Platform Technology

Safety

- General Toxicity　(GLP)
- Safety Pharmacology (GLP)
- Reproductive Toxicity

  Embryo-fetal development toxicity, Juvenile rat toxicity
- In vivo Phototoxicity
- Ocular Toxicity

Pharmacology

- Digestive Endoscopy
- Antiemetic Action
- Compound Muscle Action Potential
- Cardiac Function by Echo BP and HR in Conscious Rat

Regeneration Medicine

- Biopsy

  Axillary lymph nodes, Skeletal muscle, Thymus
- Operation for Immunosuppressive Animals –Extraction of spleen/thymus, Gastric fistula

We support your studies of toxicology, pharmacology and pharmacokinetics using our available capability with over 55 special technique!

Special Technique	mouse	rat	rabbit	dog	monkey
intrathecal administration		○		○	
intranasal administration	○	○	○		○
intratympanic administration		○			
sublingual administration	○	○	○		○
intratracheal administration	○	○			○
dermal administration	○	○	○	○	○
intraarticular administration			○	○	
rectal administration		○	○		
intravitreous administration		○	○	○	○
CSF sampling	○	○	○		○
DRG sampling	○	○			
microsampling from jugular vein	○	○			

Ophthalmologic evaluation

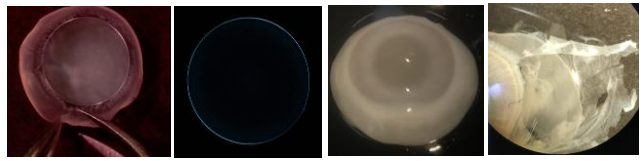
◆Ophthalmoscoy
◆Introcular pressure
◆Electroretinogram（ERG）

Standard evaluation of morphological and functional condition

◆Fluorescein angiography
◆Fluorescein corneal observation

Evaluation of lesion in cornea and retina

◆Organ culture of rat lens
◆Fractionated sampling of eye

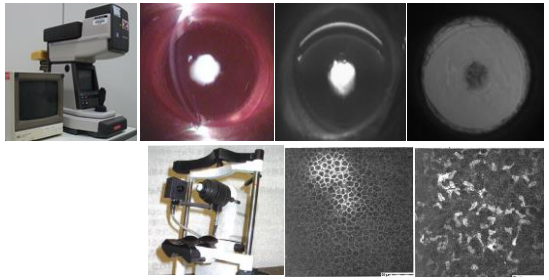


In vitro assessment and assay for each ocular organ

◆Schirmer tear test
◆Preparing dry eye model animals
◆Tear break up time measurement (coming soon)

Evaluation of dry eye

◆Anterior eye imaging
・Scheinpflug camera (lens)
・Conforcal laser microscope（cornea）



In-life assessment of corneal cells and cataract quantification

Diplomate of Japanese Society of Fundamental Ophthalmologists will provide you with total support including protocol for pharmacological and safety studies!