ADDP Oncology

Introduction of ADDP capability

Axcelead Drug Discovery Partners, Inc.
Integrated Translational Science, Oncology

Overview of ADDP capability in Oncology

Research step	Target identification	Lead generation	Lead optimization	Study for IND filing	Study for NDA
Biology	☐Pharmacological validation of concept (Proof of concept)	☐Evaluation of lead compounds on target molecule and / or cells (in vitro screening)	☐Evaluation of compounds on target molecule and in disease model in vivo (in vivo/ex-vivo screening, Target engagement, efficacy study)	☐Pharmacological evaluation of candidate on disease model ☐Differentiate from competitor(s) (Create pharmacological data package for IND/NDA filing)	

Target ID/ validation

Multi-Omics analysis
BI analysis
Flow cytometry
Phenotypic screening
scRNA-seq/ VISIUM

in vitro cell-based assay

Co-culture assay (MLR)
Gene KO/KI by CRISPR
Killing assay
Immunocytochemistry
T cell exhaustion assay

in vivo assay

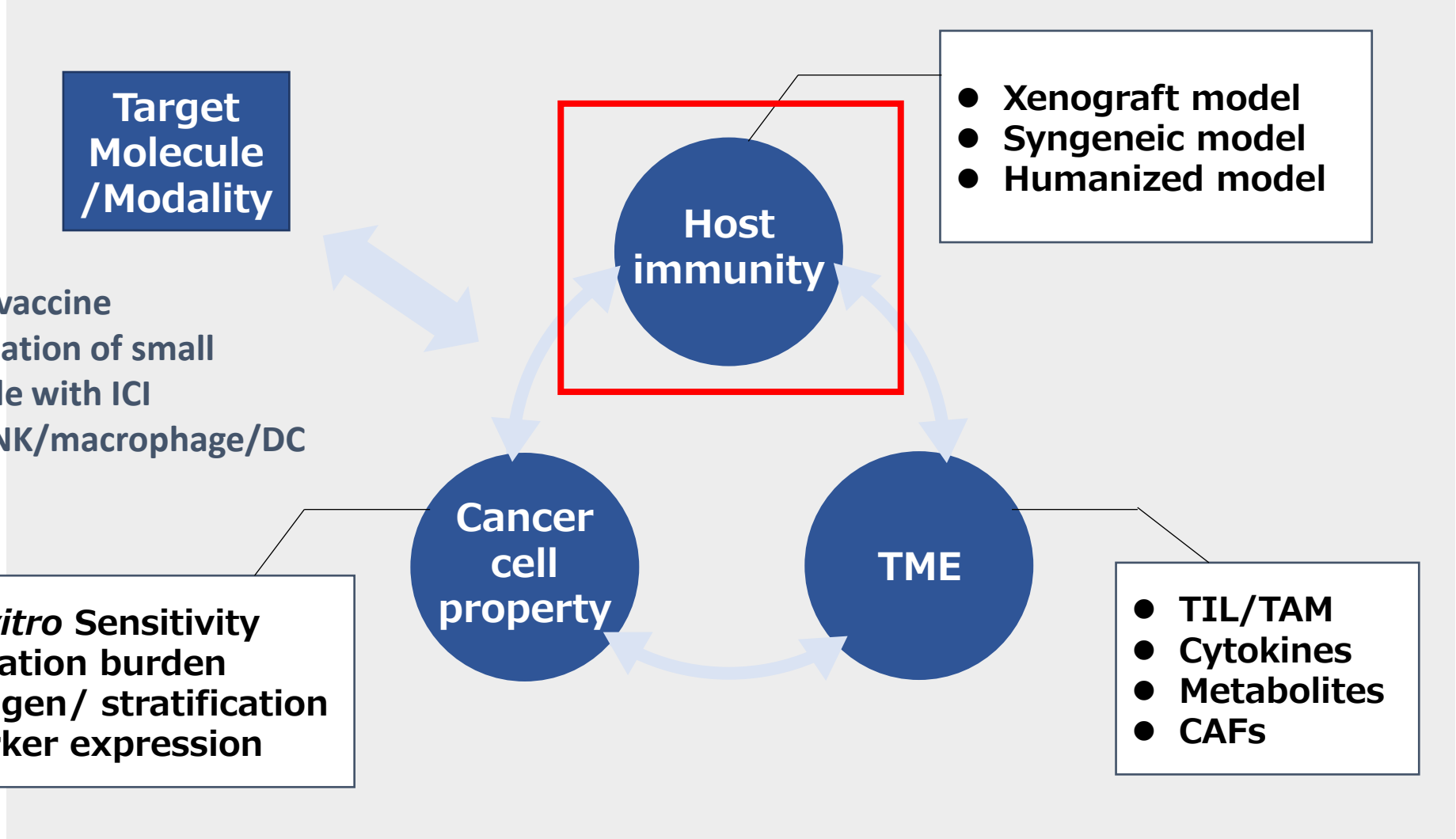
Xeno/ Syn model
Immune-humanized model
IVIS/ x-ray CT/ Echo
Immunophenotyping
Microdyalysis

Translational research

PK/ PD/ efficacy
Tg/KO mouse
Combination panel
Biomarker analysis
Assay w cinical samples

- **Our strength is a variety of capabilities cultivated in small molecule drug discovery**
- ✓ **And now, we are expanding our capability for cell therapy research, especially in Immuno-oncology field**

Key elements for Immuno-oncology research



TME: Tumor Microenvironment

in vivo models, modality and evaluation in Oncology

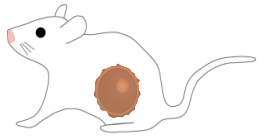
■ Xenograft/Syngeneic/ PDx mouse model

s.c. models, Orthotopic models

PDx model: Lung, Liver, Colon, stomach, glioma etc.

■ Immune-humanized model

T cell/ NK cell /B cell



■ Modalities

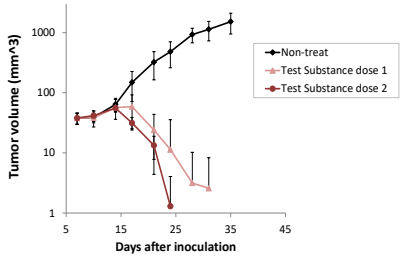
Small molecule, Antibody, CAR-T, ASO, Radiation

Combination, LNPs

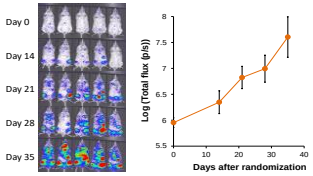
■ Evaluation

Tumor size, IVIS, X-ray CT, Ultrasound Echo

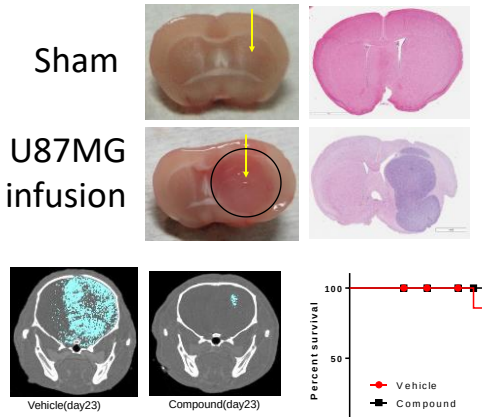
Syngeneic model (tumor size)



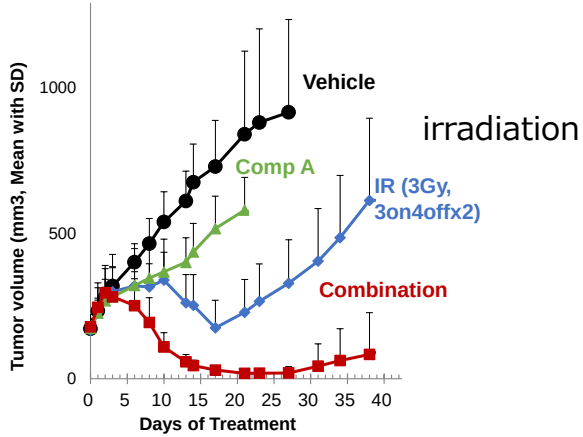
Disseminated model (IVIS)



Glioma orthotopic model (X-ray CT)



In vivo combination (tumor size)



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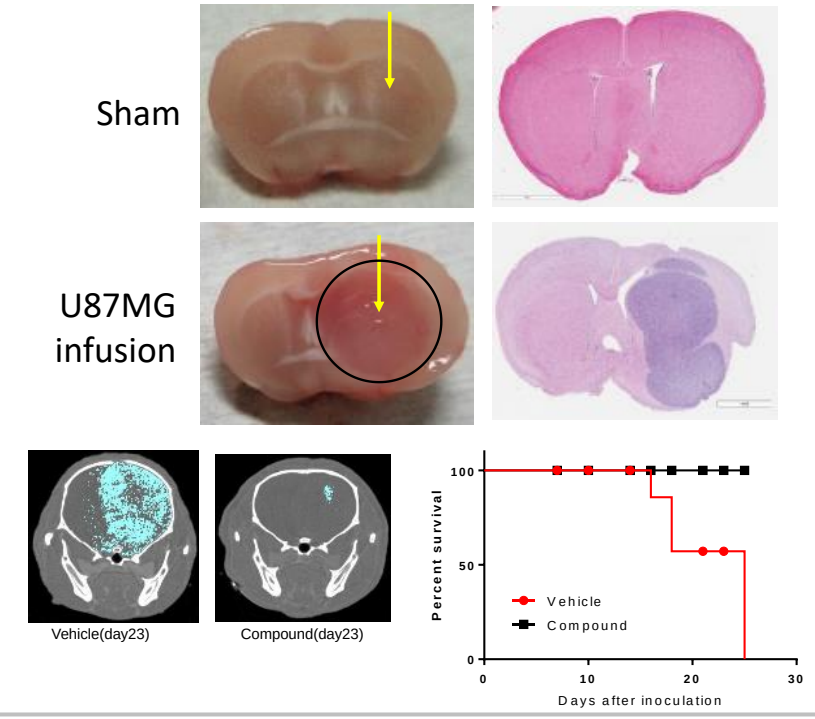
Orthotopic models and non-invasive detection

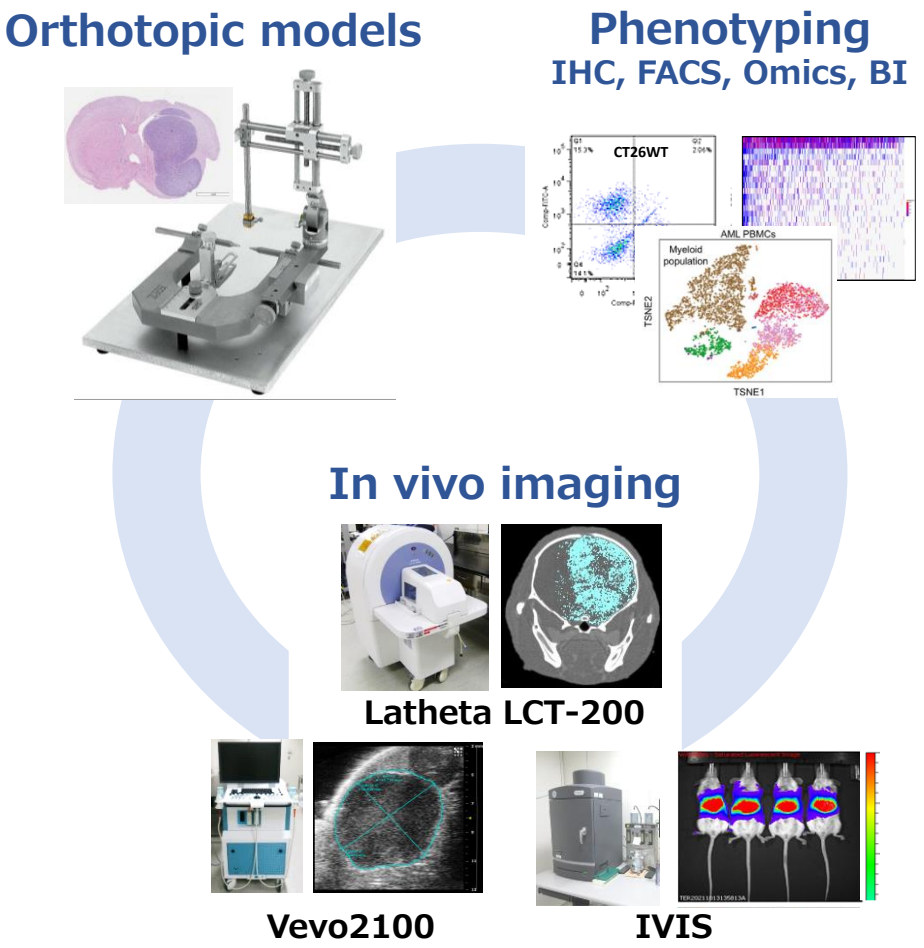
Orthotopic models

- **Glioma** : cell infusion into specific part of the brain
- **Ovarian cancer** : cell transplantation into ovary under microscope
- **Renal cancer** : cell transplantation into kidney capsule
- **Others**

Ex.) Glioma model (U87MG cell)



Visualization of orthotopic tumor



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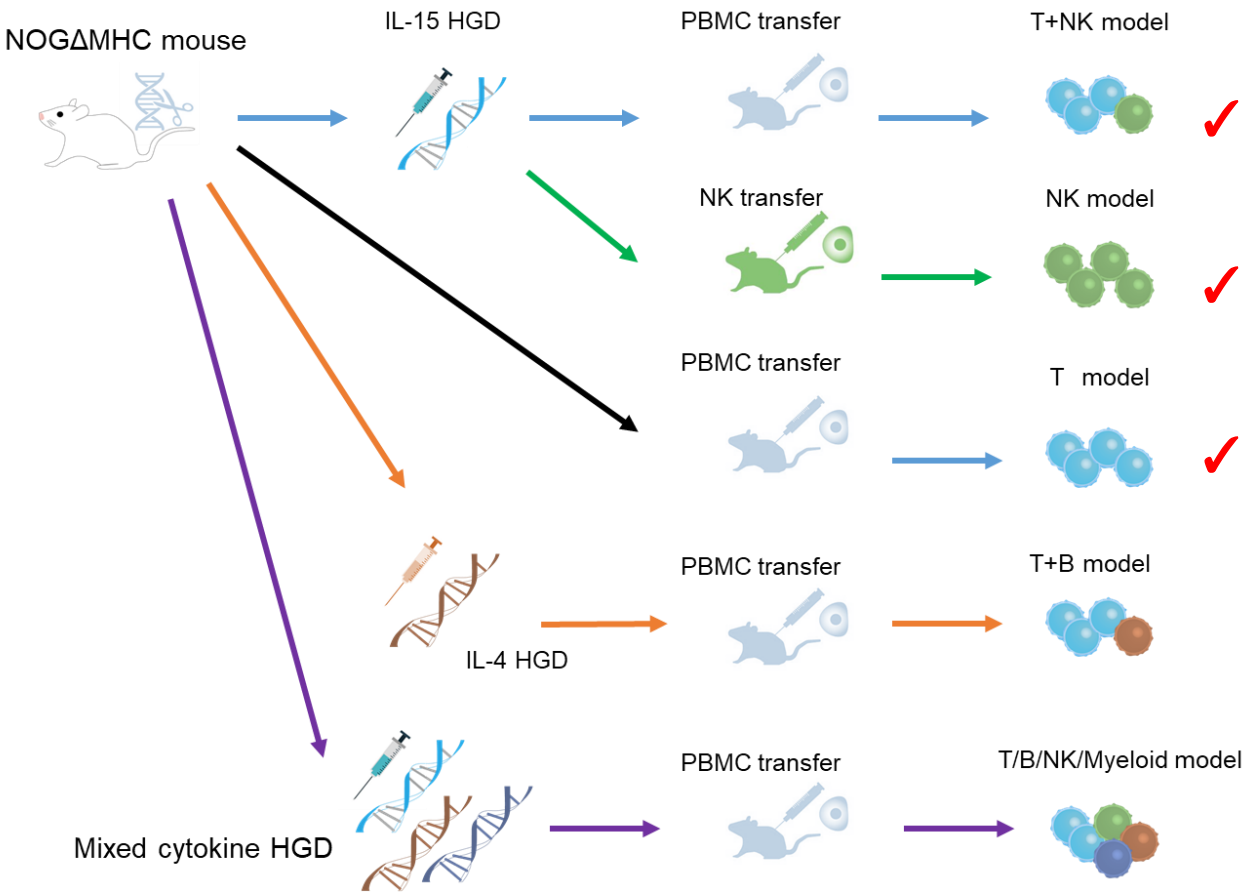
ADDP's next generation humanized mouse model

We are developing next generation humanized models



- Allogeneic CAR-T cell rejection model

- Vaccination induced human antibodies models
- Autoimmune disease models
- Non-clinical safety assessment models

We can collaborate to generate new models you are interested in !

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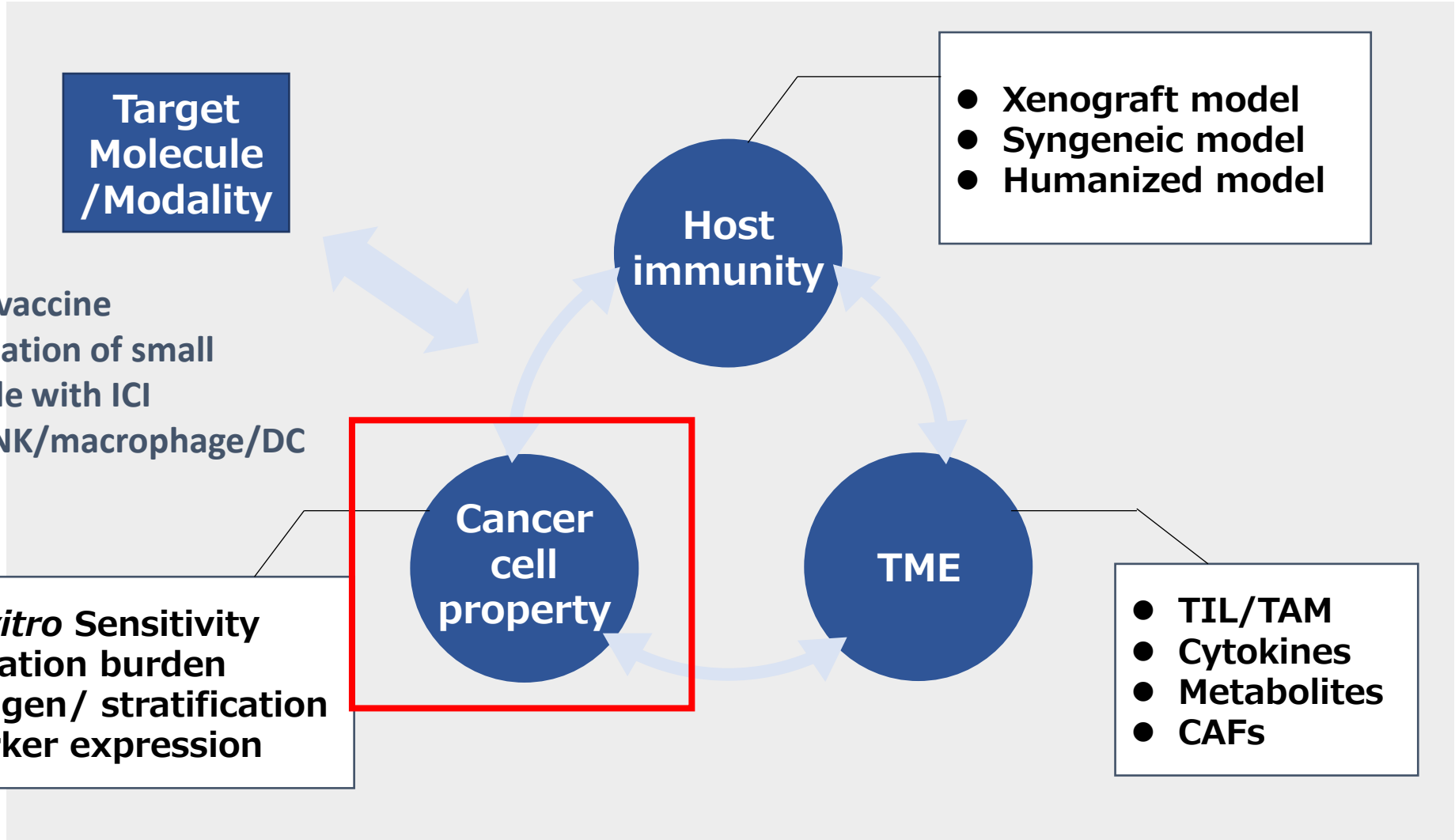
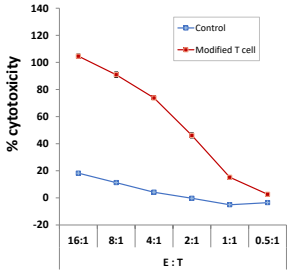
Key elements for Immuno-oncology research



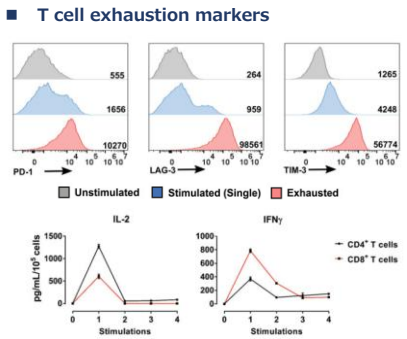
TME: Tumor Microenvironment

Immunology-related *in vitro* capability

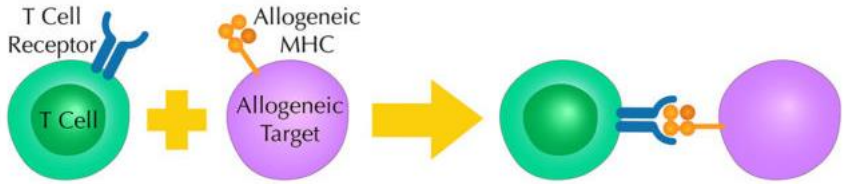
Killing assay



T cell exhaustion assay



Mixed lymphocyte reaction (MLR)



T cell proliferation & NK cell activation

✓ CFSE labeled PBMC x Mitomycin-treated HLA-mismatched PBMC or x cells investigated for their immunogenicity (iPS-derived cell, human)

In vitro capability in immunology

Function	Cell	Output	Species
Activation & Function	BMDC	LPS-stimulated cytokine production (ELISA)	Mouse
	Splenocyte	Cytokine production (ELISA)	Mouse
	Th1 & Th17	Cytokine production (ELISA)	Mouse / Human
	Treg & iTreg	Inhibition of Teff proliferation (FACS), CFSE+ cell (FACS) & cytokine production (ELISA)	Mouse / Human
	B cell	IgM-stimulated CD86 (RT1B) & Apoptosis (sub G1 population)	Rat / Mouse / Human
	CD8 T cell	Granzyme, perforin, IFNg (ELISA / FACS)	Human / Mouse
	Basophil	b-hexosaminidase release	Rat
	Fibroblast-Like Synoviocytes (HFLS)	Cytokine production (ELISA)	Human
	PBMC (T cell & NK cell)	TLR-stimulated cytokine production (ELISA), one-way MLR	Human
	Whole blood	Cytokine production (ELISA)	Mouse / Rat / Monkey/Human
	Cell lines & Whole blood	Phospho flow with FACS & Multi-Plex (p-p38, p-STAT, p-p65, p-ERK)	Mouse / Rat / Human
Differentiation	Th1, Th17 and iTreg from naive T cells	Intracellular IFNγ, IL-17, FoxP3 (FACS) & cytokine production (ELISA)	Mouse / Human
	Th17 from memory CD4+ T cells	Intracellular IL-17 (FACS) & cytokine production (ELISA)	Human
	CD8 T cell	CFSE assay (FACS)	Human
Migration	Migration of Splenocyte, Thymocyte, B cell, Neutrophil, Bone marrow cell, HL-60 cell etc.	Number of migrated cells toward CXCL12, S1P, CCL20, fMLP	Mouse / Rat / Human

✓ We can support development of new in vitro model

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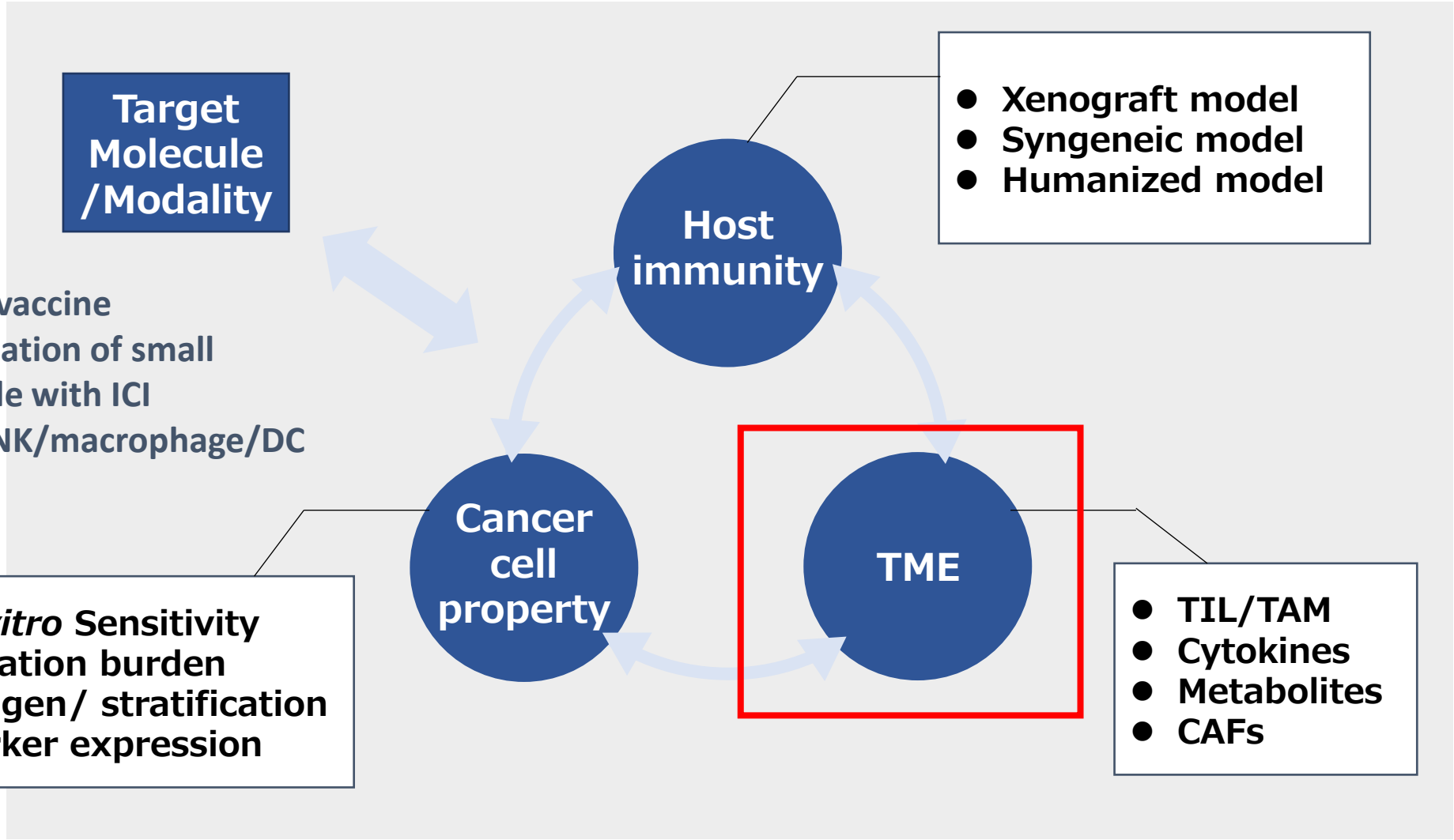
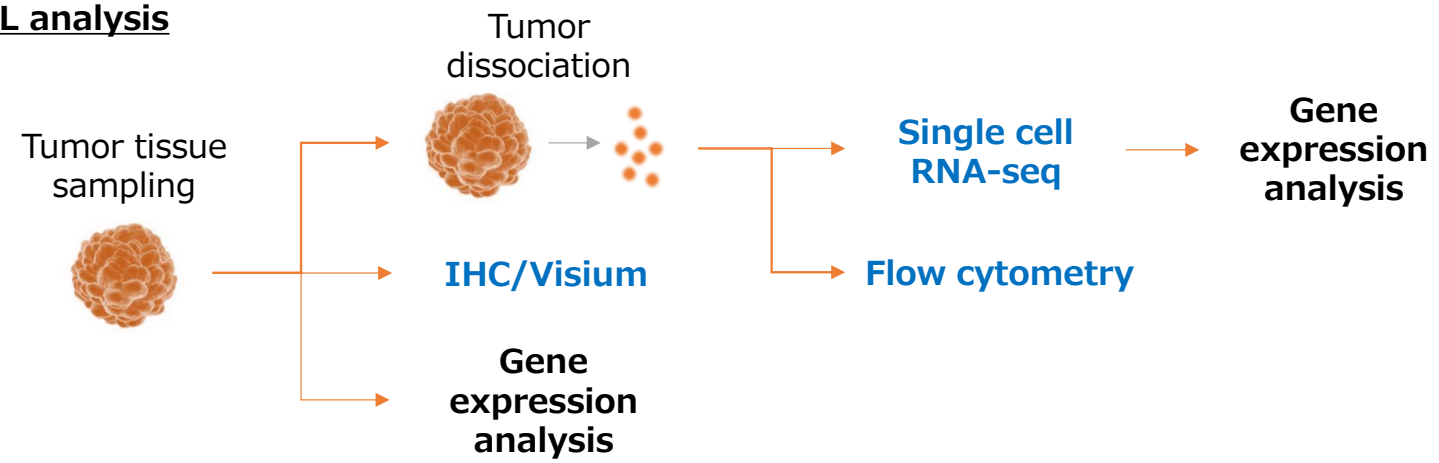
Key elements for Immuno-oncology research



TME: Tumor Microenvironment

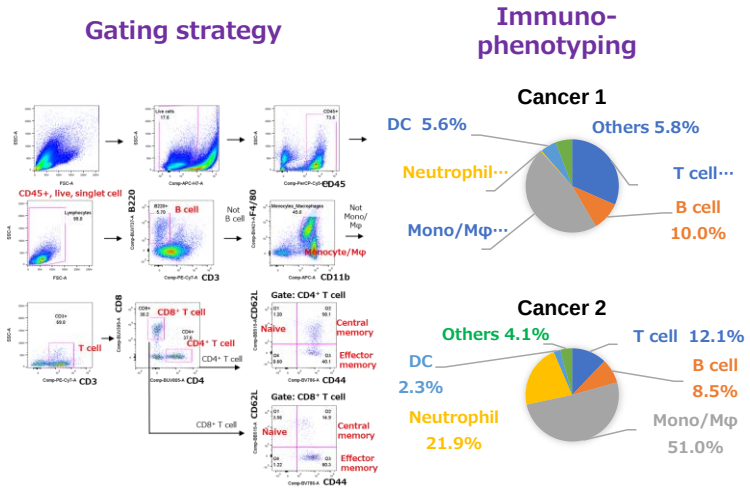
Capability for detection and evaluation of cells in *in vivo* samples

TIL analysis

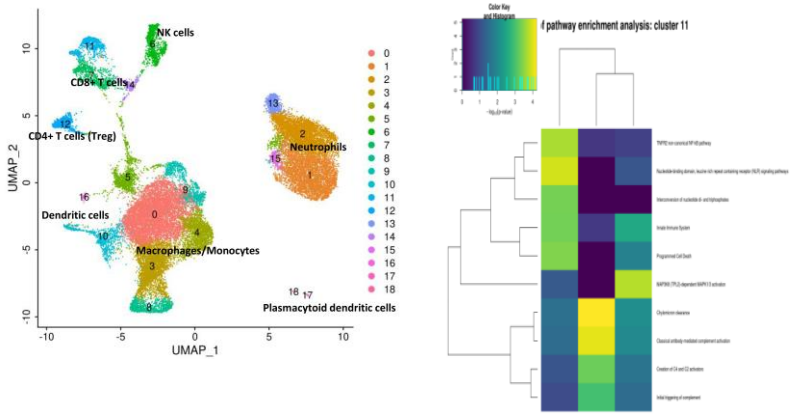


- ✓ **We can evaluate distribution and effect of cells in tumor and in organs**
- ✓ **Omics and pathological analysis are also in ADDP capability**

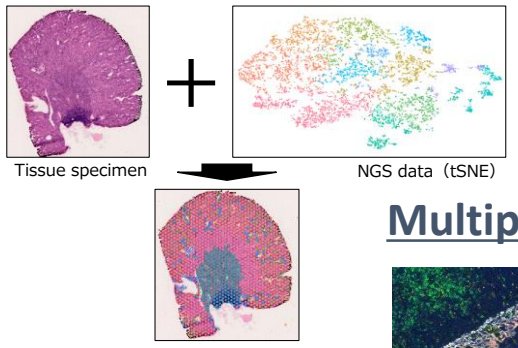
Flow cytometry



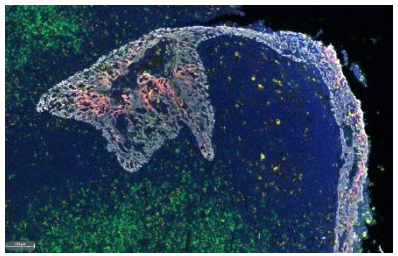
Single cell/ nuclear RNA-seq



Spatial transcriptomics



Multiple-IHC



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ADDP can support cell therapy project as a project member

✓ **Not only conduct fixed studies for cell therapy, but we will also support model development and POC studies etc. discussing with customer**

Ex.) Studies ADDP supported for CAR-T project

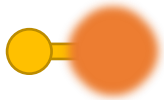
Model development

- **Host/Cell line selection**
  - Xenograft/ Syngeneic
  - Antigen expressing cell (FCM/IHC)
  - CAR-T sensitivity (killing assay)
  - Dev. of Gene-modified cell clone

- **Model development & profiling**
  - Yield/ tumor growth rate
  - Antigen expression (FCM/IHC)
  - TIL profiling (FCM/scRNA)

CAR-T preparation

- **CAR-T production**
  - Gene introduction (LNP, Retrovirus)
  - CAR-T expansion



- **CAR-T evaluation**
  - CAR expression (FCM)
  - In vitro killing assay

Evaluation

- **Efficacy/Distribution/Toxicity**
  - Tumor size (Caliper/IVIS)
  - Distribution of CAR-T in mouse (FCM/IHC)
  - GVHD (IHC/T cell infiltration into organs)
- **Combination study**
- **MoA study**
  - Tumor microenvironment analysis
    - FCM
    - scRNA-seq
    - VISIUM
  - Proteomics/Metabolomics
  - Microdyalysis

In vitro/in vivo combination study

■In vitro screening for exploring combination partner

① High throughput combination study

② Combination matrix analysis

* Select best combination partner by synergy evaluation algorithm (bliss score/CI)

■In vivo combination study

✓Efficacy study after dose setting study

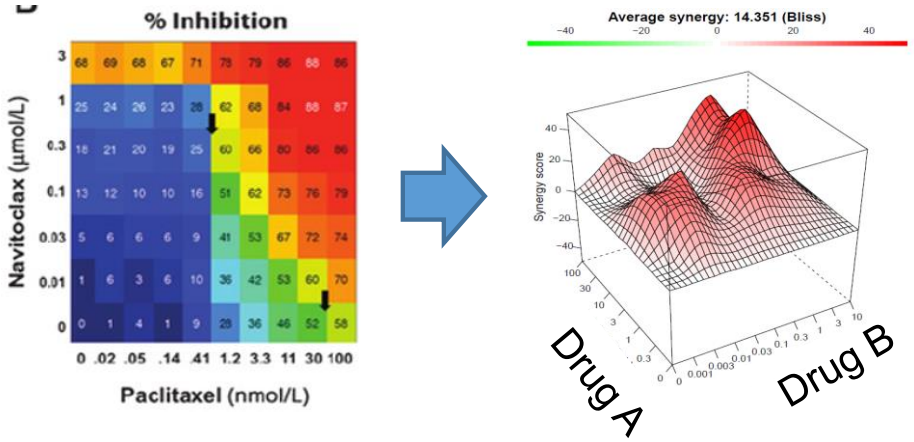
✓Toxicity evaluation (body weight, H&E stain, IHC, observation etc.)

■MoA analysis

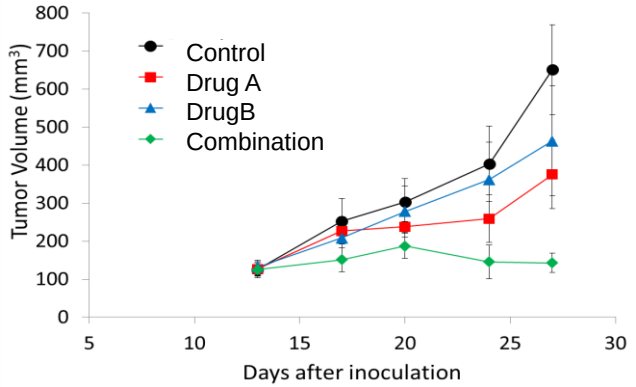
✓Various omics analysis & BI analysis

✓Validation study

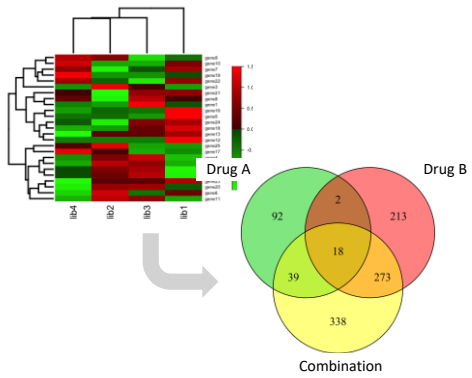
□ In vitro combination study & BI analysis for prioritization of synergistic candidates



□ In vivo combination study



□ MoA analysis



ADDP platform supports your research

■ **Our strength is a variety of capabilities cultivated in small molecule drug discovery**
And now, expanding our capability to other modalities such as LNP and CAR-Ts

Model

- In vitro system
- Animal disease model
- Tg/KI mouse model
- Humanized mouse model

×

Modality

- Degrader/RNA modulator
- Nucleotide/ Peptide
- Cell therapy
- DDS carrier

×

Measurement

- Pharmacology/ Toxicology
- IVIS/ X-ray CT/ Echo
- Immuno-profiling
- Multi-Omics analysis

Flexible evaluations by the combination of our various platform