

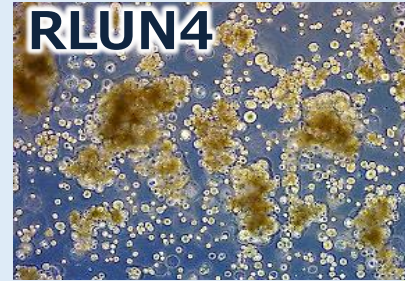
# Establishment and characteristics of F-PDO

# F-PDO



**RLUN3**

**Lung**

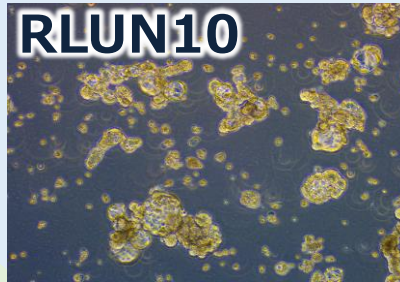


**RLUN4**

**Ovarian**

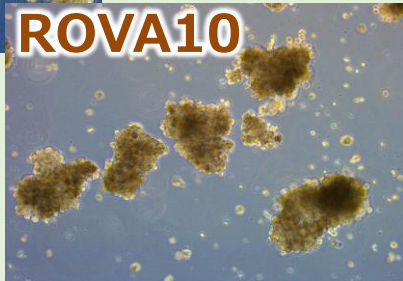


**ROVA3-1**

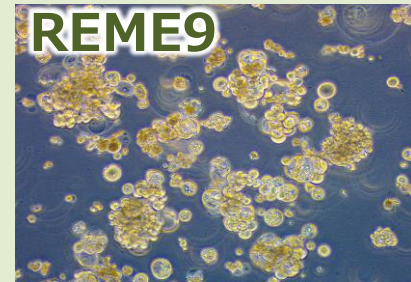


**RLUN10**

**Endometrial**

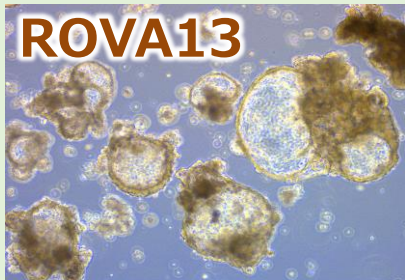


**ROVA10**

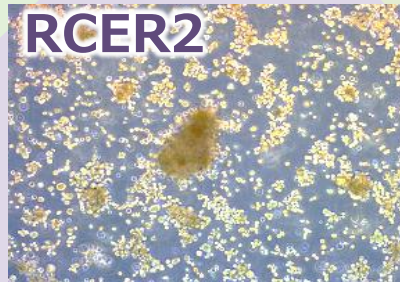


**REME9**

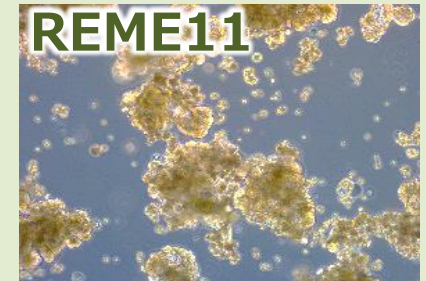
**Cervical**



**ROVA13**



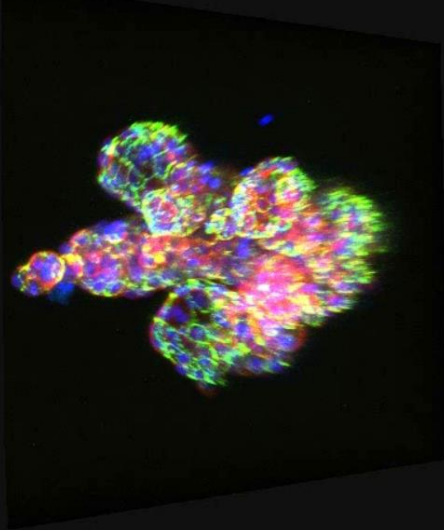
**RCER2**



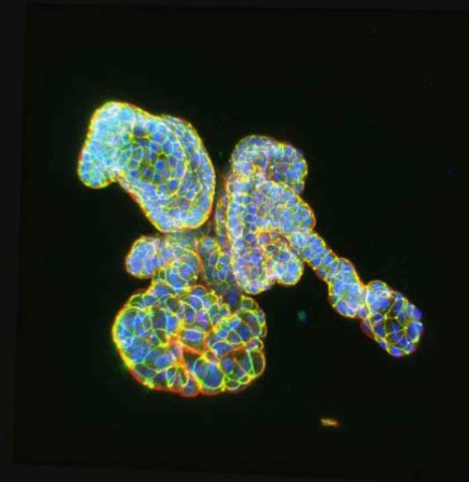
**REME11**

# 3D analysis of morphology of F-PDO

RLUN014: lung tumor



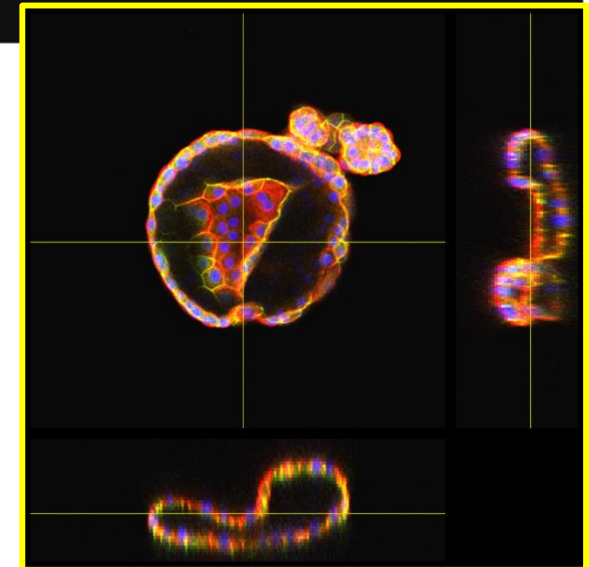
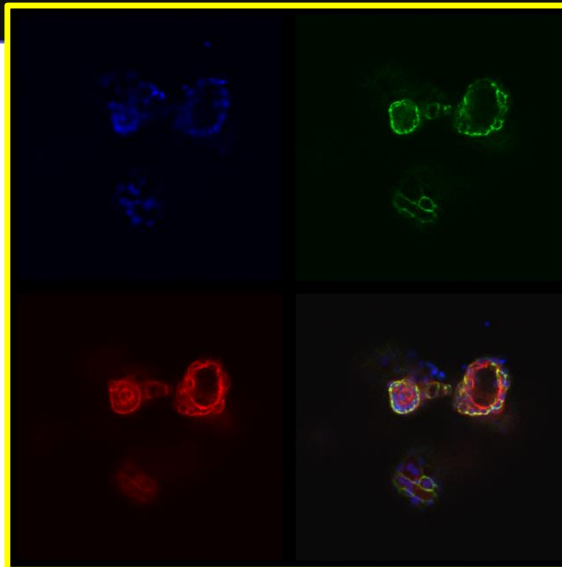
RLUN020: lung tumor



nucleus

actin

zo-1

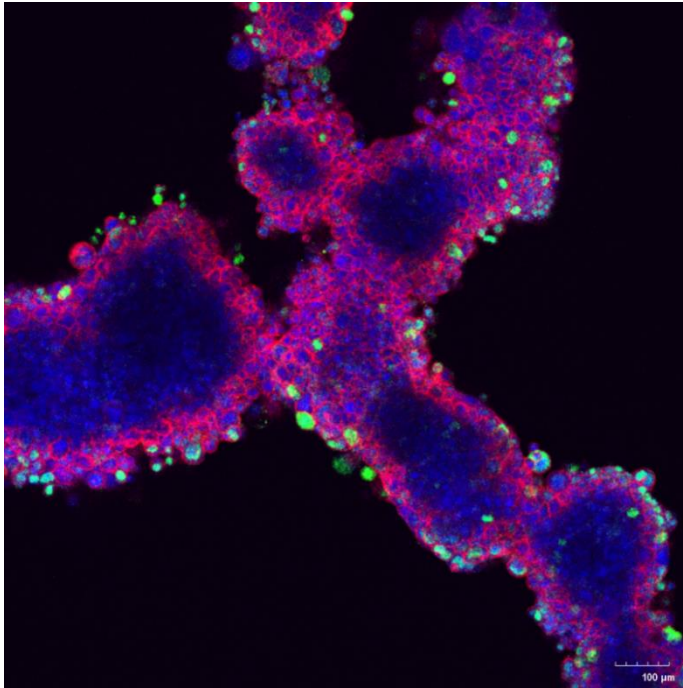


These data were obtained through collaboration with Olympus Corporation.<sup>3</sup>

# Immunostaining of F-PDO by antibody drugs

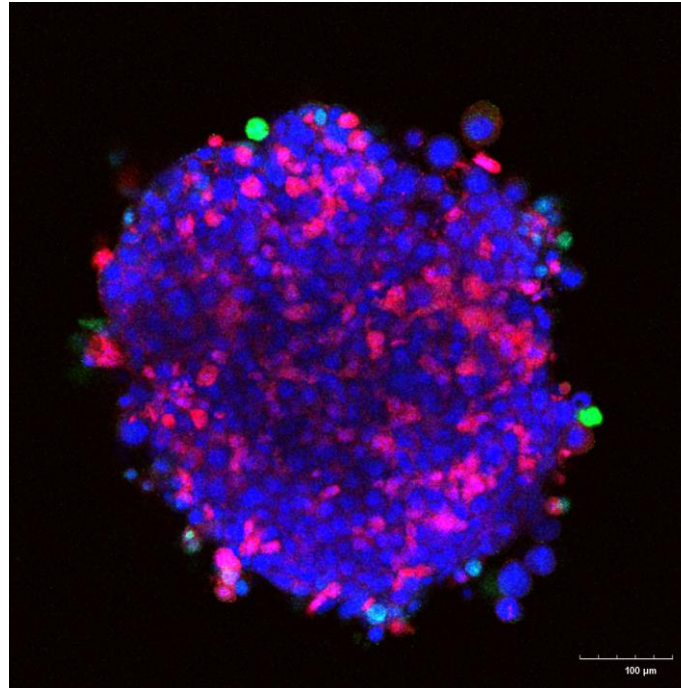
## Lung RLUN021

**Erbix**



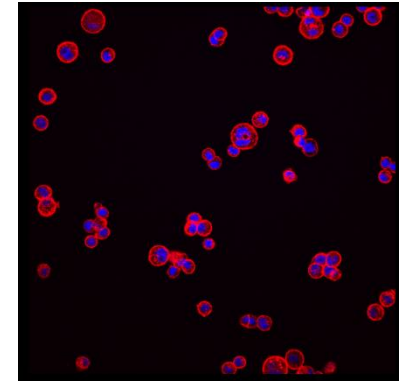
**EGFR detection**

**Herceptin**



**HER2 detection**

Monolayer culture  
ZR-75-30



**HER2 detection**

**nucleus**

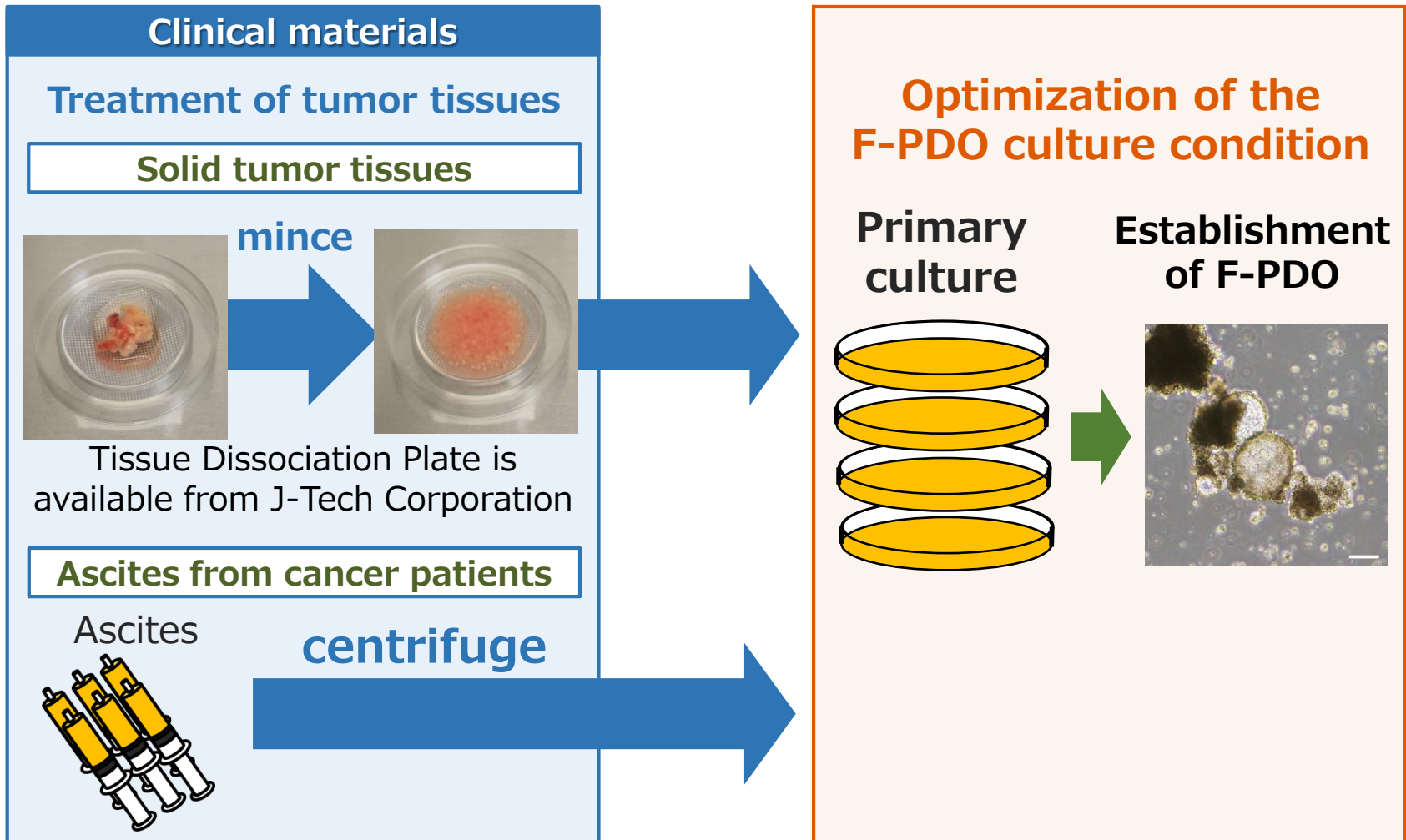
**Ki67**

**antibody**

These data were obtained through collaboration with Olympus Corporation.

# How to establish F-PDO

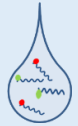
## Establish of the non-adherent organoids from primary tumor tissues



# Characterization of F-PDO

Data collection of F-PDO by various analytical methods

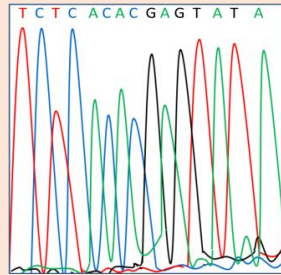
## Gene Expression



Gene expression profiling



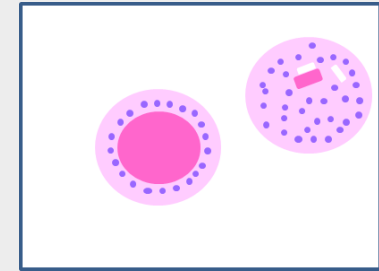
## Genome



Gene expression profiling



## Pathology



Morphological observation



**Compare with the source tissues**

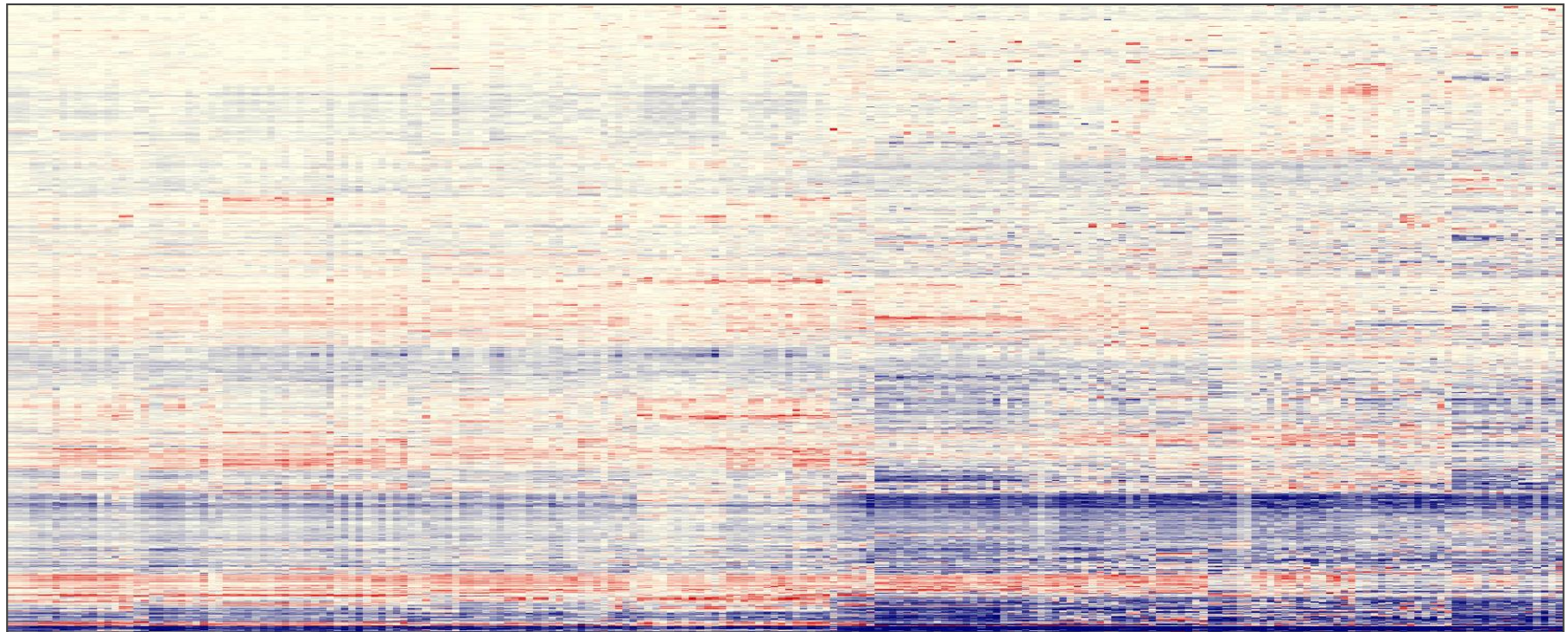
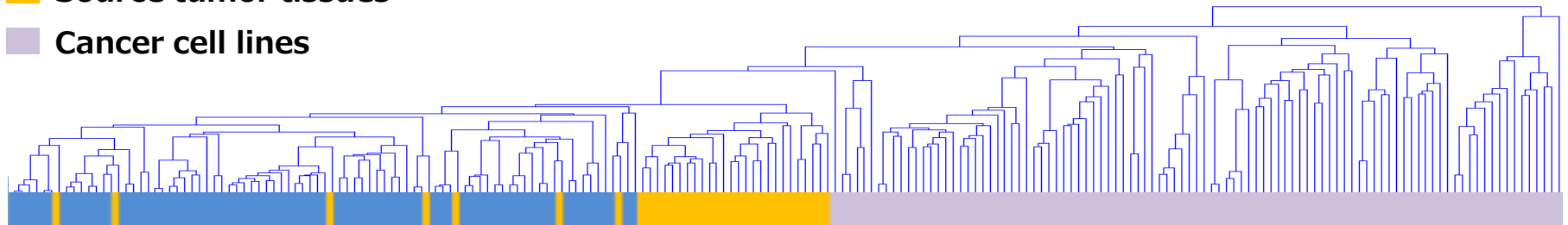
**Well-characterized research material with annotation data is a high value and can be applied to a broad range of research to create valuable results**

# Gene expression profiling of F-PDO

F-PDOs

Source tumor tissues

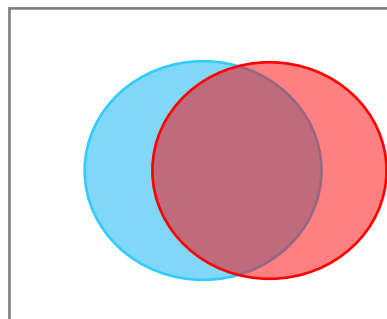
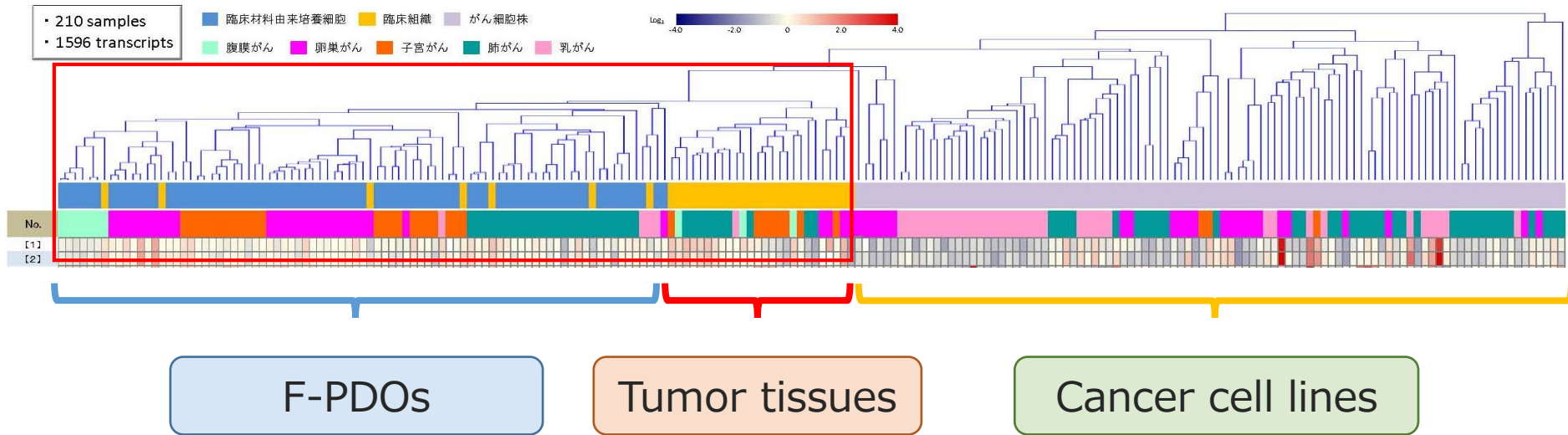
Cancer cell lines



Log<sub>2</sub> -4.0 -2.0 0 2.0 4.0

# Gene expression profiling of F-PDO

## Comparative analysis between F-PDO, tumor tissues and cancer cell lines based on gene expression analysis



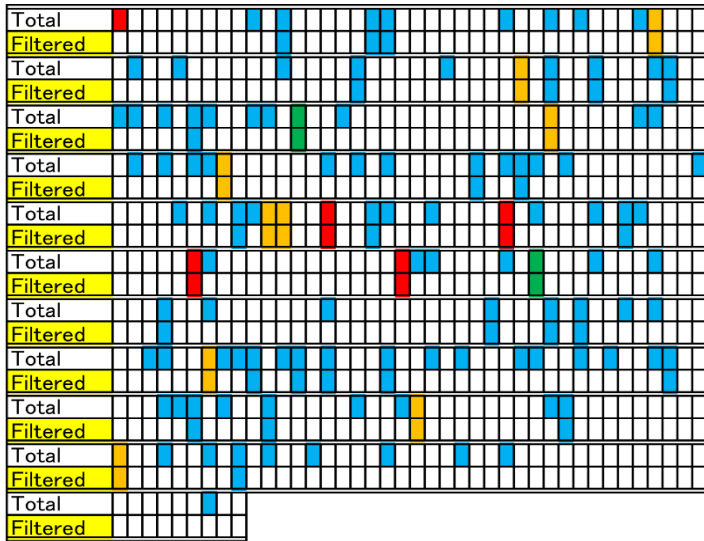
The gene expression profiles from F-PDOs are more closer to the profiles of tumor tissues than those of cancer cell lines.

F-PDO is close to the source tumor tissue profile

# Genome analysis of lung F-PDO

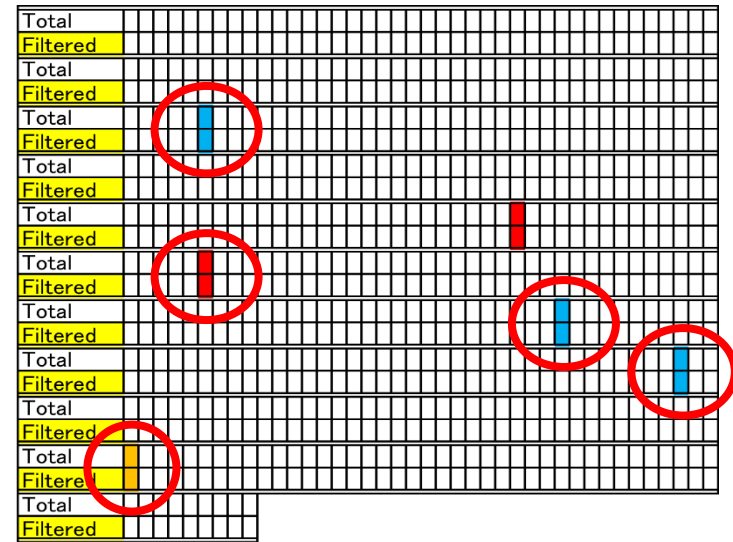
## Lung cancer derived F-PDO: RLUN16

### <Single analysis>



Normalized by comparable normal tissue sample

### <Paired analysis>



- : Missense
- : Non-Frameshift InDel
- : Frameshift InDel
- : Nonsense

Red circles show co-existing somatic mutations between source tumor tissue and the established F-PDO

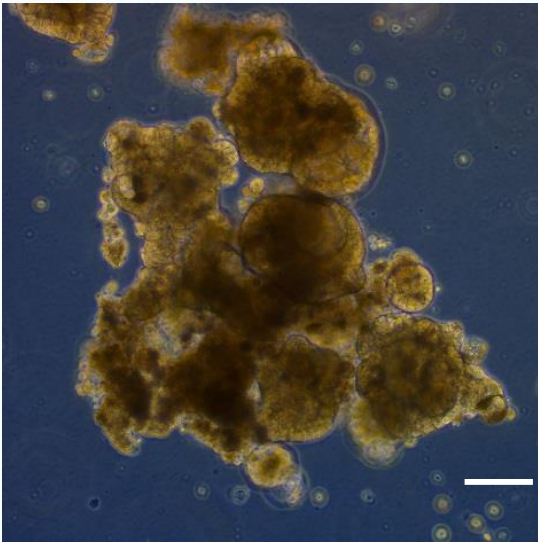
➔ F-PDO is preserving similar genetic profile with source tumor tissue

# Comparison of the morphology of F-PDO and source tumor tissue

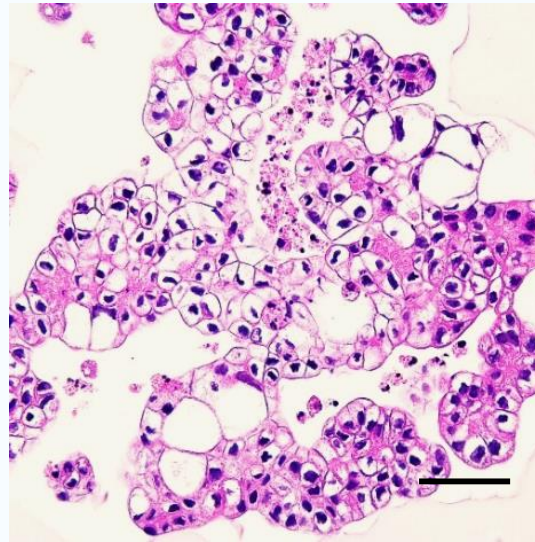
---

## REME011-2 (Uterine tumor source, clear cell carcinoma)

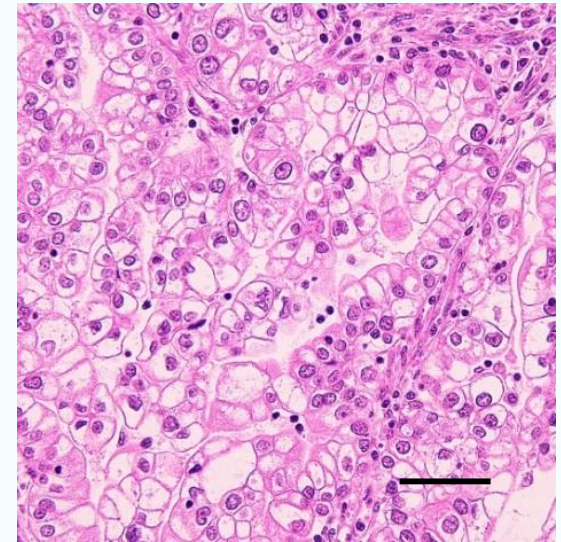
Phase-contrast image  
F-PDO



HE image  
F-PDO



HE image  
Source tumor tissue



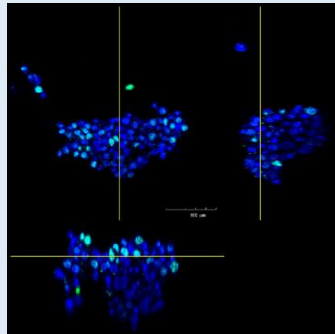
Scale bars: phase-contrast images, 200  $\mu\text{m}$ ; HE-stained images, 50  $\mu\text{m}$ .

F-PDO is similar to the source tumor tissue

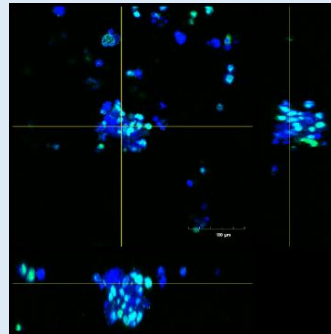
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# Morphological analysis of F-PDO using NoviSight

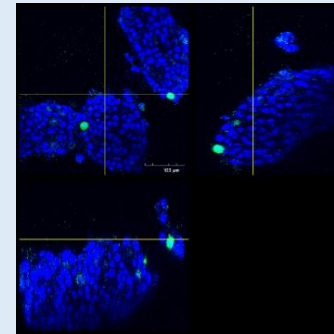
Calculate morphological information  
(number of cells, volume, number of Ki67-positive cells)



RLUN5

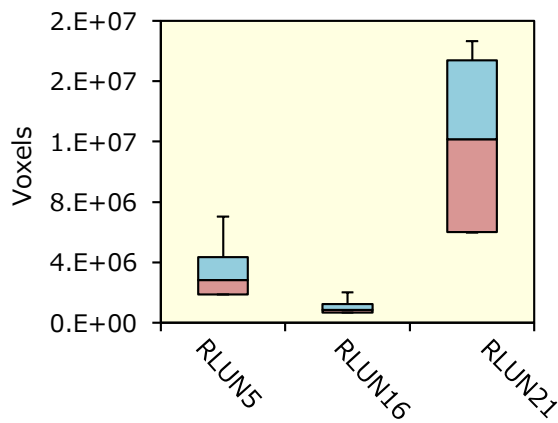


RLUN16

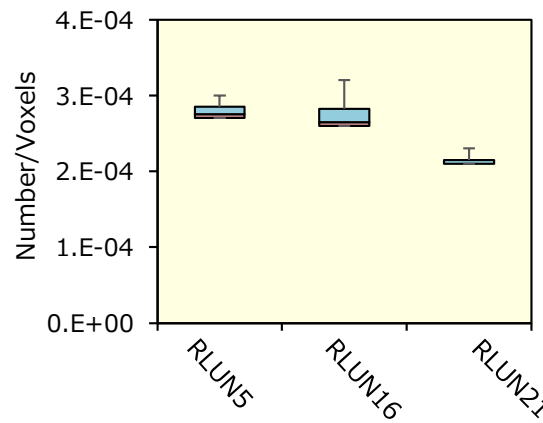


RLUN21

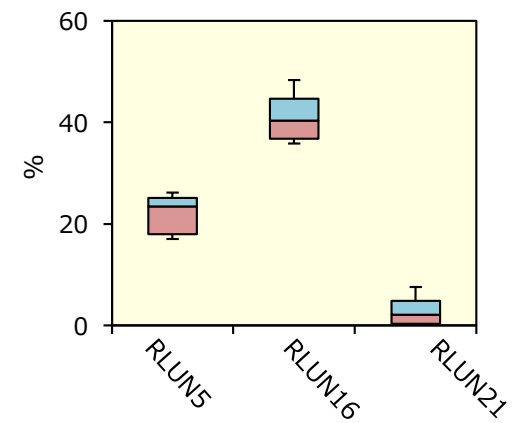
Cell volume



Cell density

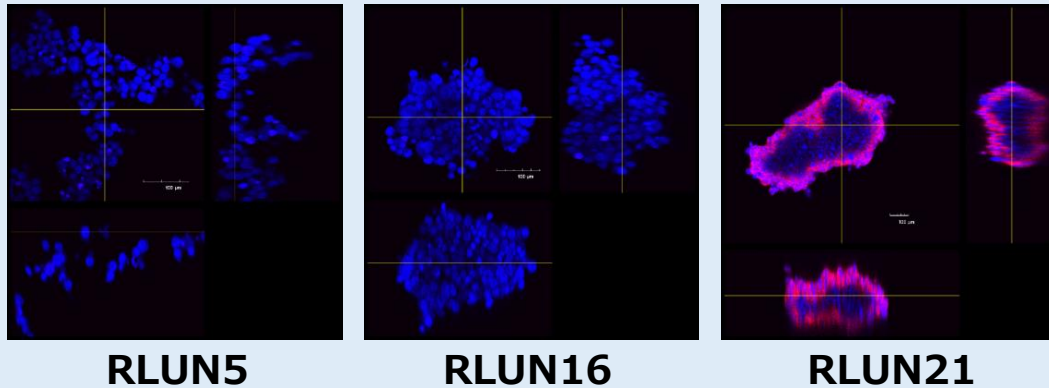


Ki67-Positive ratio

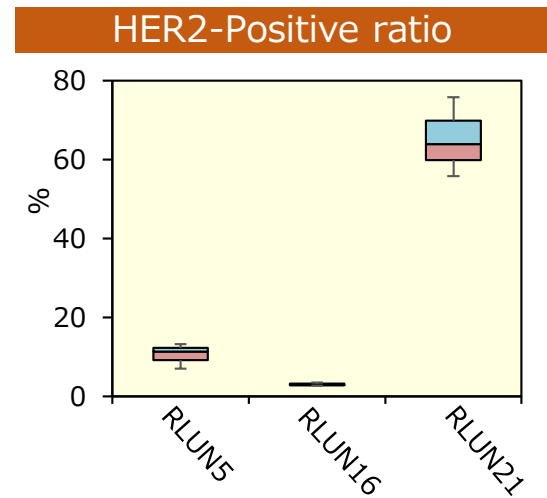
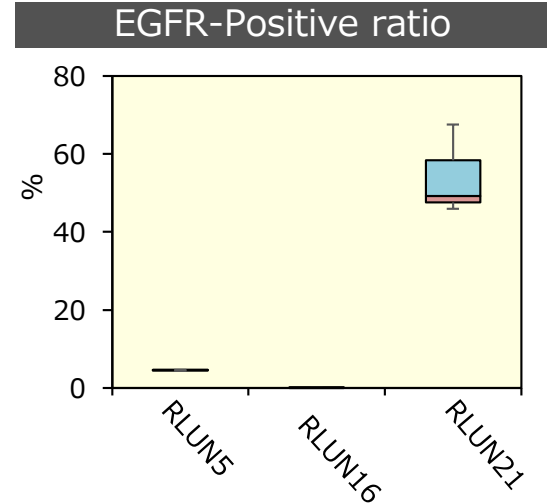
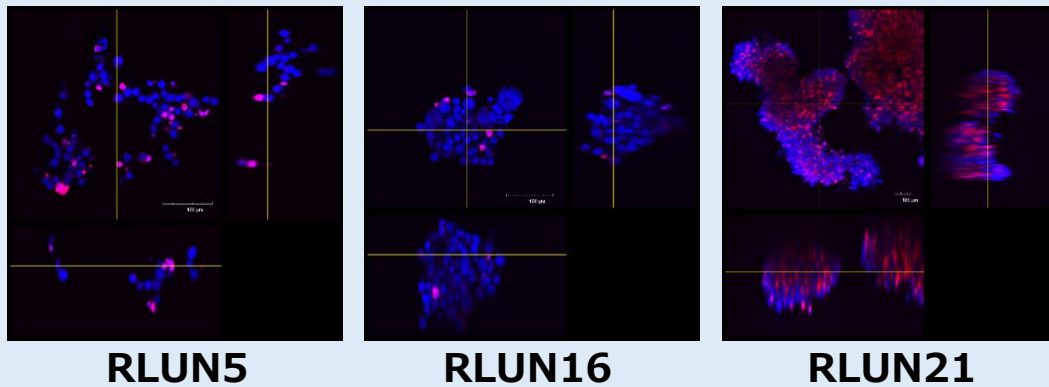


# Morphological analysis of F-PDO using NoviSight

Calculate morphological information  
(volume of antibody-positive cells)



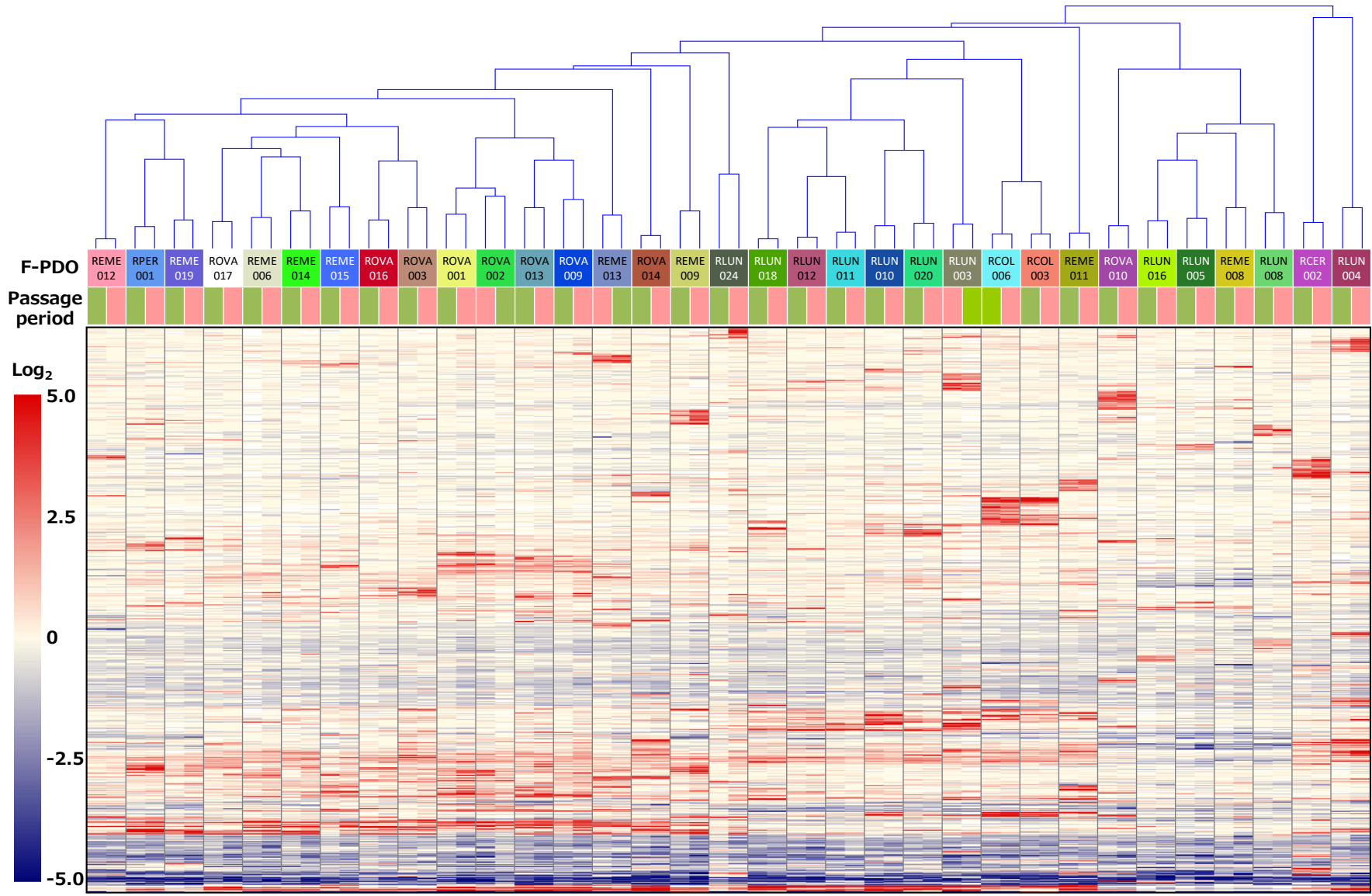
D



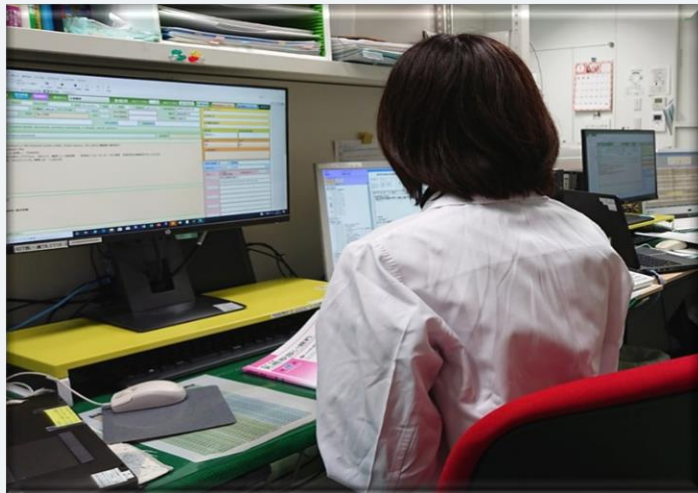
These data were obtained through collaboration with Olympus Corporation.

# Changes in gene expression profiles of F-PDO by passaging

■ Immediately after establishment   ■ After several months of culture



# Clinical data of F-PDO



Collect clinical information from electronic medical records

Lung tumor			
Sampling site	Lung	Metastasis	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
(Lung tumor) L2.2 <input type="checkbox"/> Squamous cell carcinoma L2.2 <input type="checkbox"/> Adenosquamous cell carcinoma L2.3 <input type="checkbox"/> Small cell carcinoma L2.5 L2.6 ( <input type="checkbox"/> Carcinoid tumor L2.6.1 <input type="checkbox"/> Large cell neuroendocrine carcinoma (LCNEC) L2.6 ) (Lung tumor) L2.7 Adenomatoid or sarcomatous elements L3 L3.1 <input type="checkbox"/> Spindle cell carcinoma L3.2 <input type="checkbox"/> Giant cell carcinoma L3.3 <input type="checkbox"/> Pulmonary blastoma L3.5 <input type="checkbox"/> Adenomatoid tumor L4.1 <input type="checkbox"/> Malignant mesothelioma L4.2			
Size		28x22 mm	
<input checked="" type="checkbox"/> pT1b	<input type="checkbox"/> pT2a	<input type="checkbox"/> pT2b	<input type="checkbox"/> pT3 <input type="checkbox"/> pT4
<input type="checkbox"/> pN1	<input checked="" type="checkbox"/> pN2	<input type="checkbox"/> pN3	
<input type="checkbox"/> pM1 ( <input type="checkbox"/> pM1a <input type="checkbox"/> pM1b )			
<input type="checkbox"/> IB	<input type="checkbox"/> IIA	<input type="checkbox"/> IIB	<input checked="" type="checkbox"/> IIIA <input type="checkbox"/> IIIB
<input type="checkbox"/> G2	<input type="checkbox"/> G3	<input type="checkbox"/> G4	<input type="checkbox"/> GX
<input checked="" type="checkbox"/> No	<input type="checkbox"/> Unknown	Vascular invasion <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> Unknown	
<input type="checkbox"/> No	<input type="checkbox"/> Unknown	Bronchovascular invasion <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> Unknown	
<input checked="" type="checkbox"/> No	<input type="checkbox"/> Unknown	Pleural effusion <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> Unknown	
<input type="checkbox"/> No	<input checked="" type="checkbox"/> Unknown	Washing cytology <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> Unknown	
<input type="checkbox"/> No	<input type="checkbox"/> Unknown	Pulmonary metastasis <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> Unknown	



Database of clinical information

	Pleural plaque	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		
	Pleural invasion	<input checked="" type="checkbox"/> pI0	<input type="checkbox"/> pI1	<input type="checkbox"/> pI2 <input type="checkbox"/> pI3
	Intrapulmonary metastasis	<input checked="" type="checkbox"/> pm0	<input type="checkbox"/> pm1	<input type="checkbox"/> pm2 <input type="checkbox"/> pm3
Immunohistochemical staining	MIB1	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Not yet	TTF-1 <input type="checkbox"/> Yes
	Napsin	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Not yet	ER <input type="checkbox"/> Yes
	PgR	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Not yet	
Genetic diagnosis	EGFR	<input type="checkbox"/> Exon19 del <input type="checkbox"/> G719X <input type="checkbox"/> S768I <input type="checkbox"/> L858R <input type="checkbox"/>		
		<input type="checkbox"/> Exon20 int <input checked="" type="checkbox"/> T790M <input type="checkbox"/> Other ( )		
		<input type="checkbox"/> wild type <input type="checkbox"/> Not yet		
	ALK	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Not yet	PD-L1 <input type="checkbox"/> Yes
Medical history	<input checked="" type="checkbox"/> Diabetes	<input checked="" type="checkbox"/> Coronary artery disease	<input type="checkbox"/> Hypertonia	<input type="checkbox"/> Cerebral infarction
	<input type="checkbox"/> Other ( )			
Radiation therapy	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Chemotherapy	<input type="checkbox"/> Amrubicin	<input type="checkbox"/> Bevacizumab	<input type="checkbox"/> Carboplatin	<input type="checkbox"/> Cisplatin
	<input type="checkbox"/> Etoposide	<input type="checkbox"/> Gefitinib	<input type="checkbox"/> Gemcitabine	<input type="checkbox"/> Irinotecan
	<input type="checkbox"/> Paclitaxel	<input type="checkbox"/> Pemetrexed	<input type="checkbox"/> Tegafur-Gimeracil	<input type="checkbox"/> Oteracil
	<input type="checkbox"/> Other ( )			
Smoking	<input type="checkbox"/> Yes (present) <input checked="" type="checkbox"/> Yes (past) <input type="checkbox"/> No			
Drinking	<input checked="" type="checkbox"/> Yes (practice) <input type="checkbox"/> Yes (opportunity) <input type="checkbox"/> No			
Notes				

# F-PDO lineup

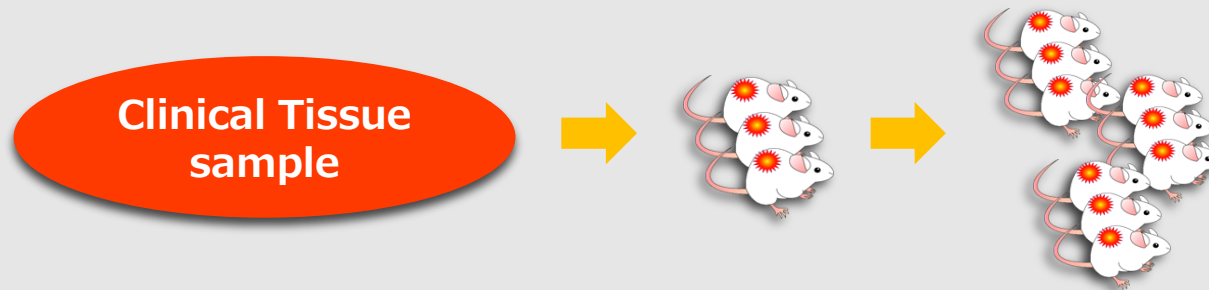
## Status of establishment of F-PDO (Oct. 2022)

Tissue	Number
Uterine cancer	34
Lung cancer	23
Ovarian cancer	13
Colorectal cancer	5
Brain neoplasms	2
Peritoneal cancer	4
Malignant melanoma	6
Soft tissue tumor	3
Breast cancer	2
Pancreatic cancer	2
Kidney cancer	2
Fallopian tube cancer	1
Bile duct cancer	1
Gastric cancer	1
Bone tumor	1
Esophageal cancer	1
Leukemia via PDX	3

Total: **104** types

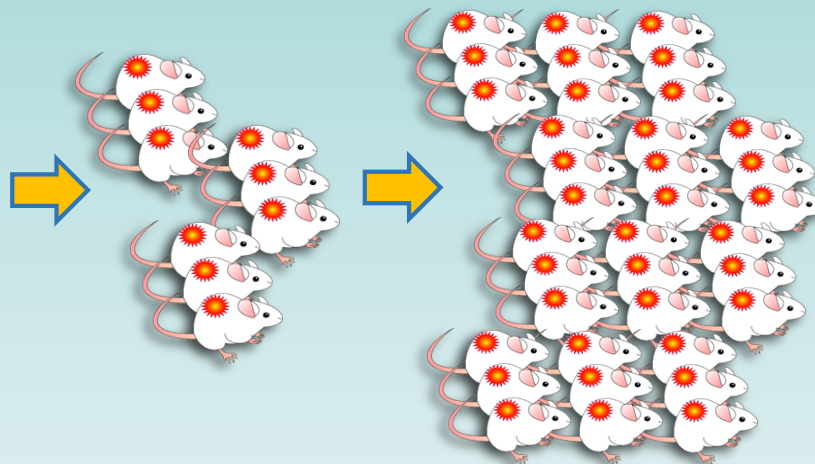
# Advantages of F-PDO on PDX mouse development

PDX mouse model development using clinical tissue sample



PDX mouse model development using F-PDO

F-PDO



- ✓ Well-characterized, tumor-like properties
- ✓ Quick expansion
- ✓ Enriched cancer cells
- ✓ Plenty starting material compared to primary tissue sample

# **Difference between F-PDO and other PDOs**

# Features of F-PDO

## *F-PDO*

- No enzyme treatment of tissue, suspension culture, heterologous cell spheroid
- Confirmation of features using morphologic, genomic, gene expression analyses

Completely different established and cultures methods

A

- **Establishment from PDX**
- **Using enzyme for tissue treatment**
- **Limiting analytic data**

B

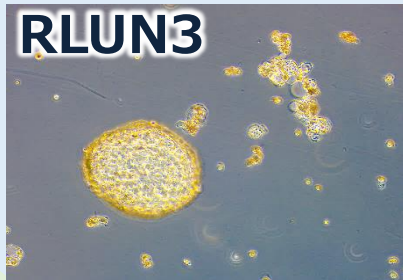
- **Culture in matrigel**
- **Using enzyme for tissue treatment**
- **Limiting analytic data**

C

- **Similar to F-PDO ?**
- **Finite proliferation ?**
- **Limiting number of lines**
- **Limiting analytic data**

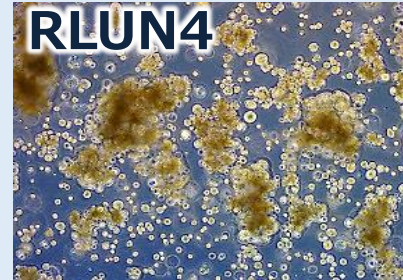
# Construction of assay systems using F-PDO

# F-PDO



**RLUN3**

**Lung**

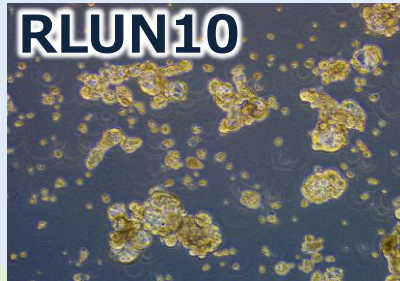


**RLUN4**

**Ovarian**

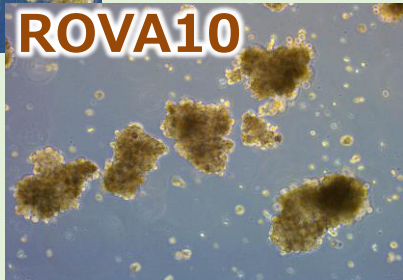


**ROVA3-1**

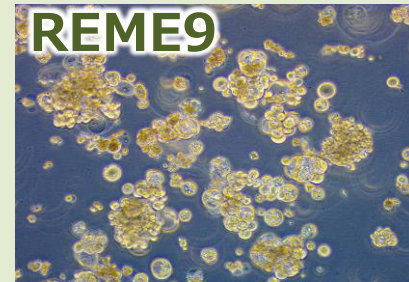


**RLUN10**

**Endometrial**

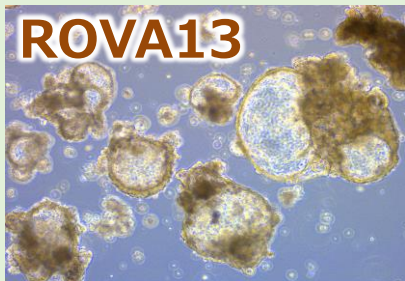


**ROVA10**

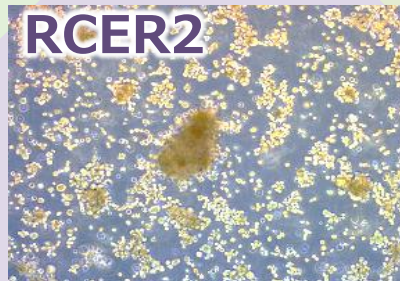


**REME9**

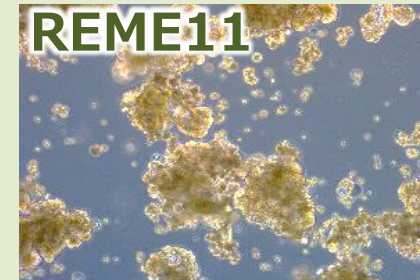
**Cervical**



**ROVA13**



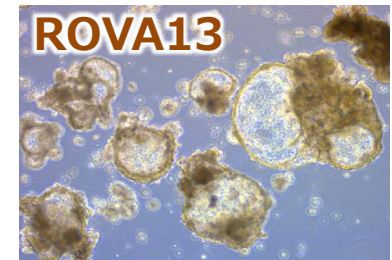
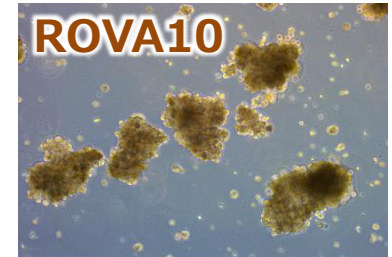
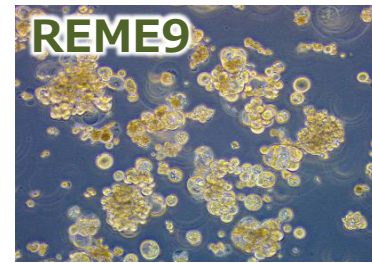
**RCER2**



**REME11**

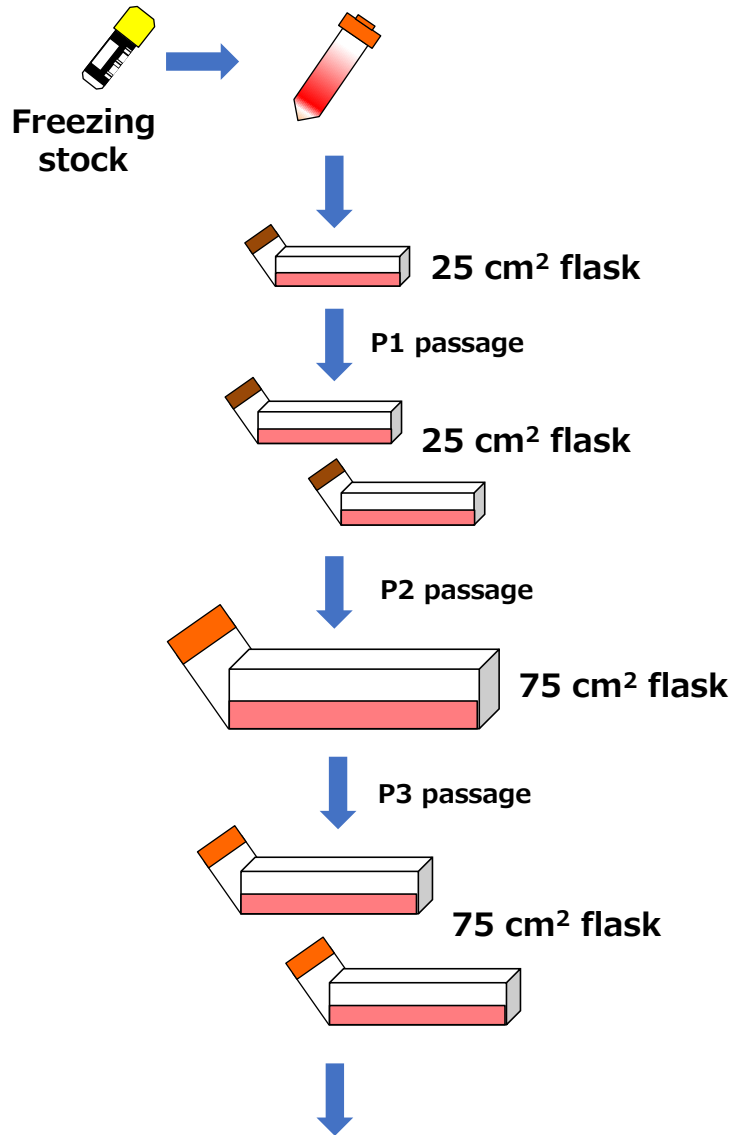
# Properties of F-PDO

- ✓ Slow proliferation (compared to cell lines)
- ✓ Morphological variety
- ✓ Forming cell clusters

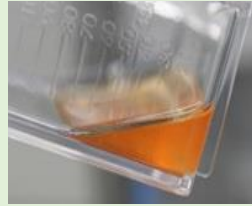


**Additional technics are required to culture and analysis of F-PDO**

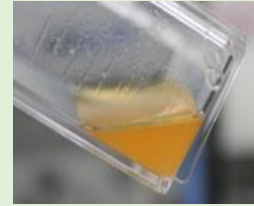
# Standard culture method of F-PDO



## Medium change twice/week



50~70%  
change



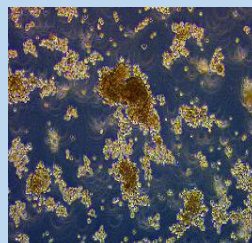
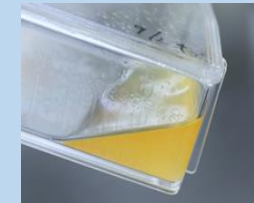
70~80%  
change

## Timing of passage

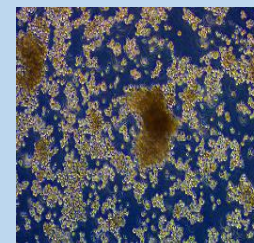
### Change of medium color



Increase of single cells and debris



Immediately  
after passage



Saturation  
density

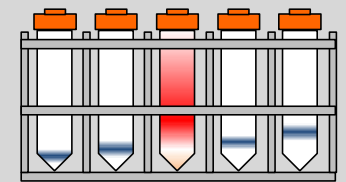
## Measurement of pellet volume

15 mL tube



↓ **Centrifuge**  
100 x g, 2 min

## Move the tube to a stand



50  $\mu$ L    **pellet**    200  $\mu$ L  
100  $\mu$ L    150  $\mu$ L

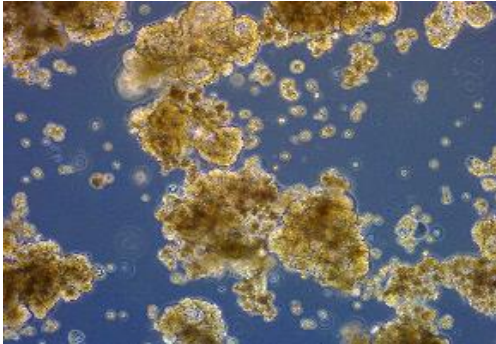
Compare with 15 mL tube marked at heights of 50~150  $\mu$ L

# Standard culture method of F-PDO



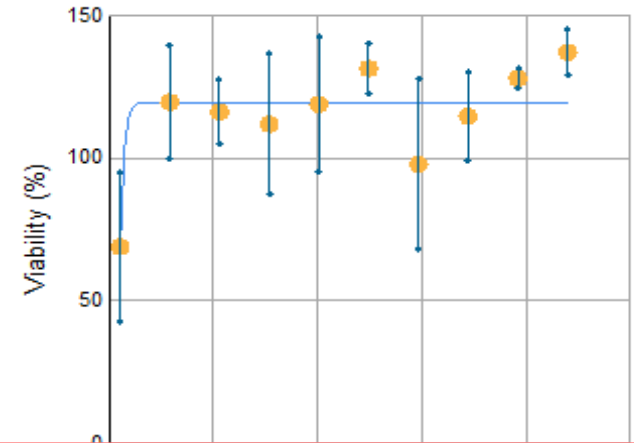
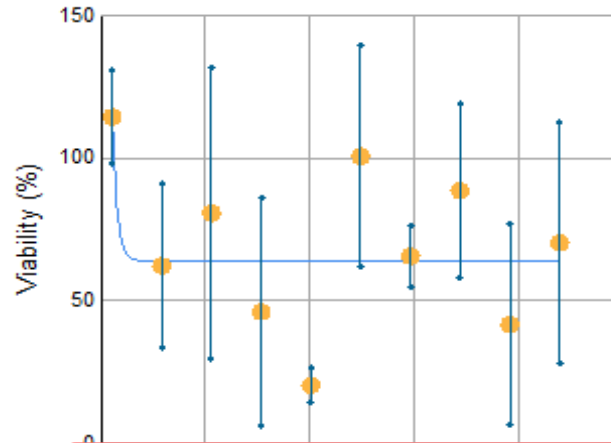
# An issue on assay system of F-PDO

## Endometrial F-PDO

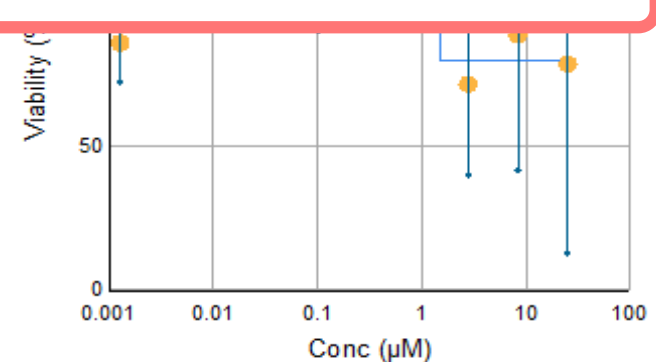
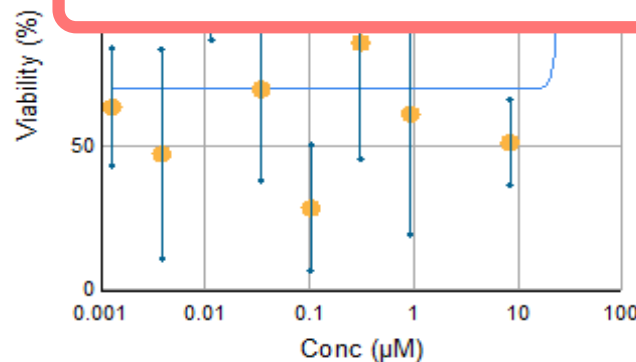


Typical Suspension  
& Plating

## Anti-proliferation assay: seeded into 384-well plate



An issue: statistical instability of data caused by non-uniform seeding

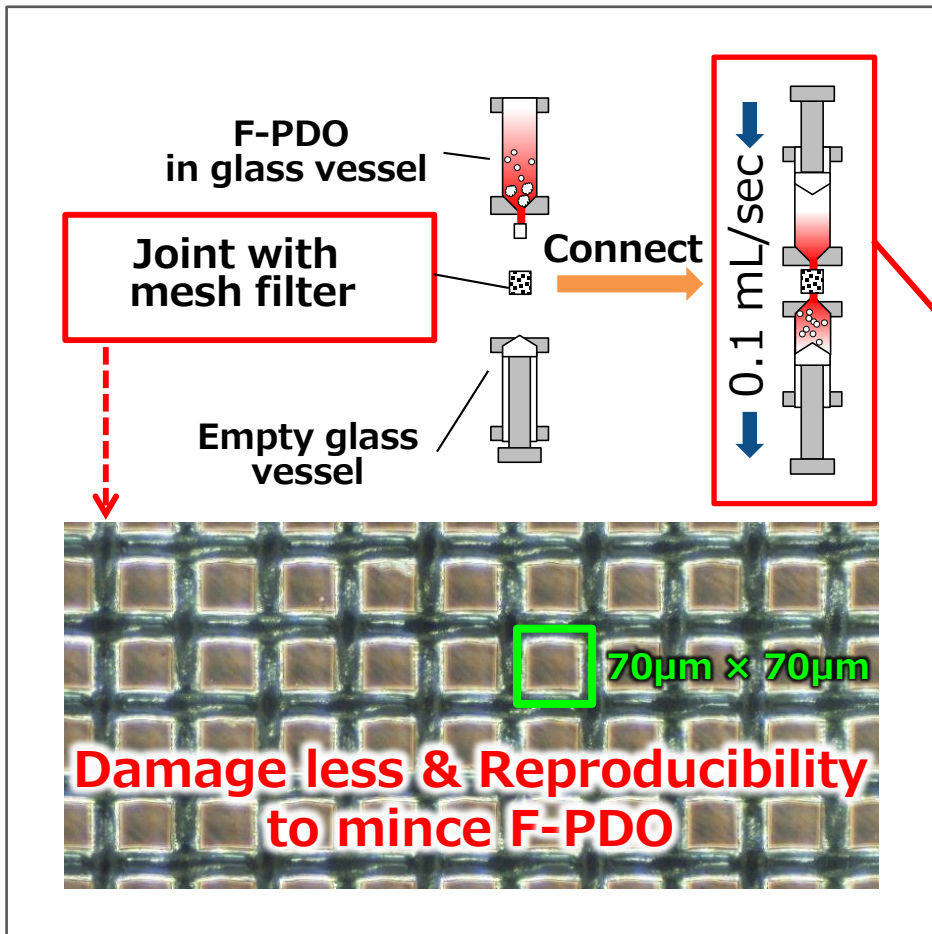


# Fragmentation of F-PDO

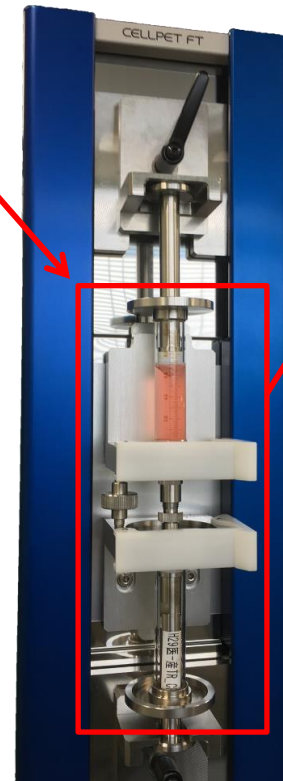
## Auto cell spheroid-mincing device: CellPet FT

F-PDO

Transfer to  
glass vessel



Set to  
device

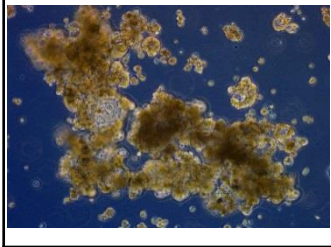


0.1 mL/sec

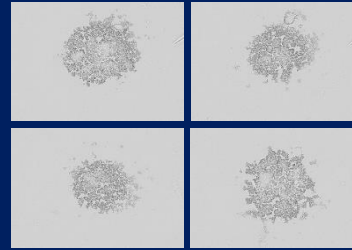
# Diminishment of experimental error by CellPet FT

**REME11**

Endometrial F-PDO

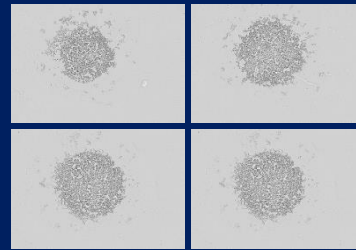
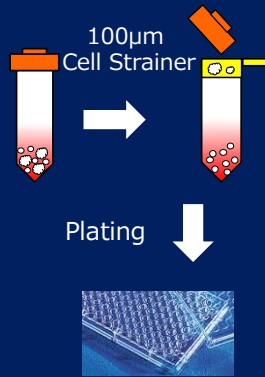
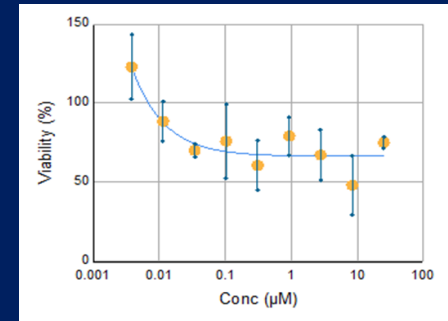


**CellPet FT**



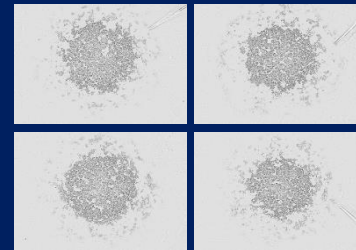
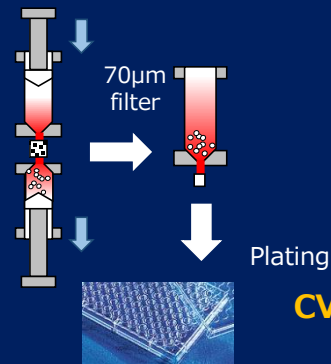
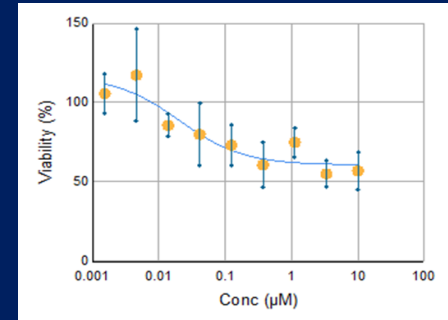
**CV value (0h) ON test: 34.9%**  
**Proliferation rate: 1.88**

**Anti-proliferation assay (Paclitaxel)**



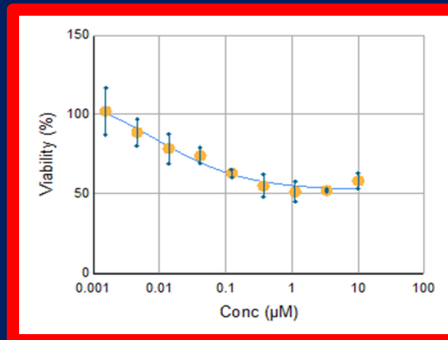
**CV value (0h) TH test: 27.0%**  
**Proliferation rate: 3.81**

**Anti-proliferation assay (Paclitaxel)**

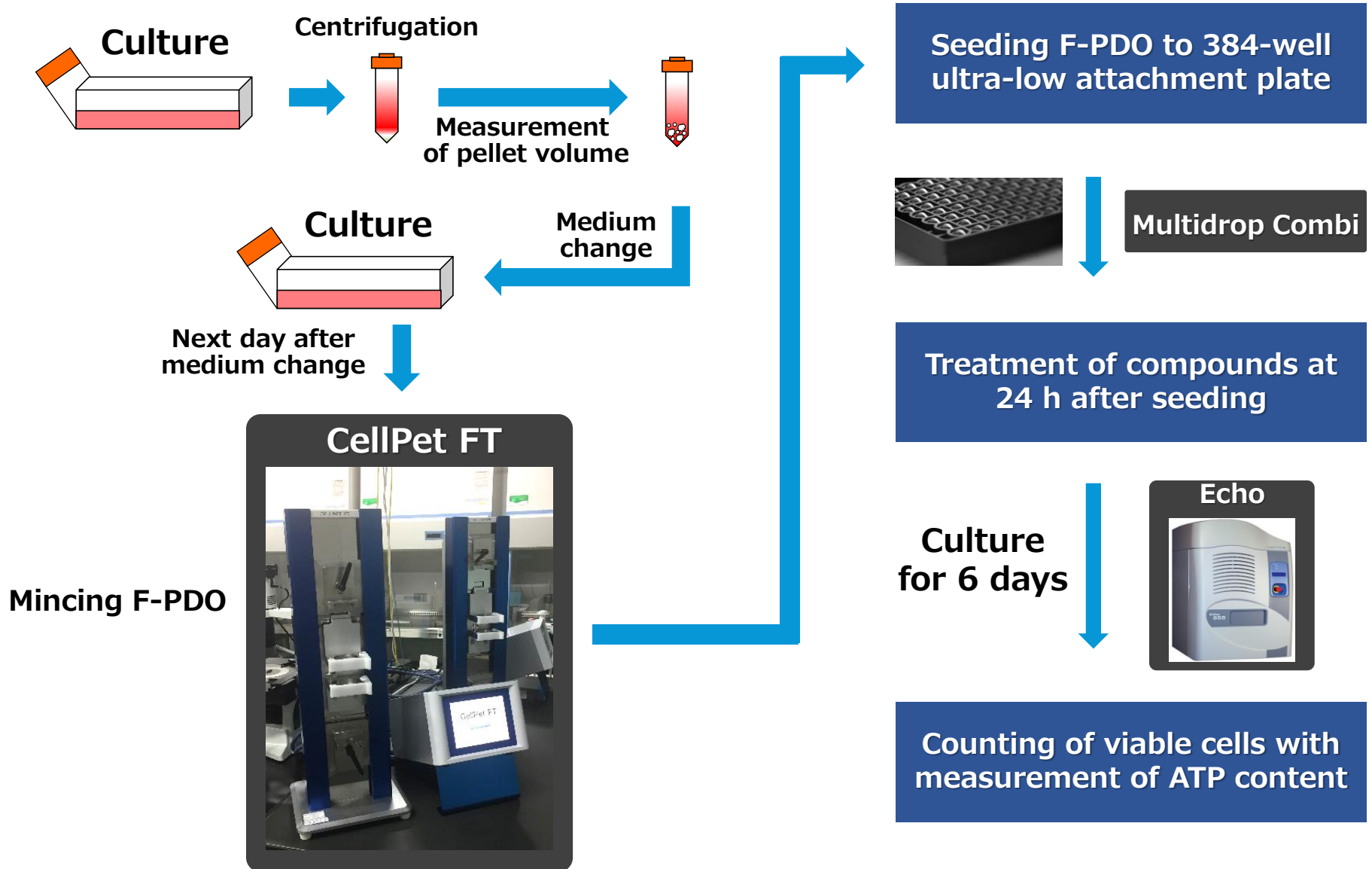


**CV value (0h) TH test: : 14.4%**  
**Proliferation rate: 3.29**

**Anti-proliferation assay (Paclitaxel)**



# HT assay for F-PDO



# Cell growth inhibition assay (384-well plate)

## DAY 1 Seeding of F-PDO

- Plate: 384-well plate
- Vendor: Corning ultra-low attachment plate



37°C, 24 h after seeding

## DAY 2 Addition of test substance

- N: 3
- Solvent: DMSO
- Concentration: Dilution 3 fold, 9 dose  
\* Available upon request
- Volume: 40 nL [Solvent con. 0.1% (vol. %)]



37°C

144 h after addition of test substance

## DAY 8 Measurement of the amount of ATP in living cells

- Reagent: Promega CellTiter-Glo 3D

### Analysis

IC<sub>50</sub> (50% inhibition concentration)

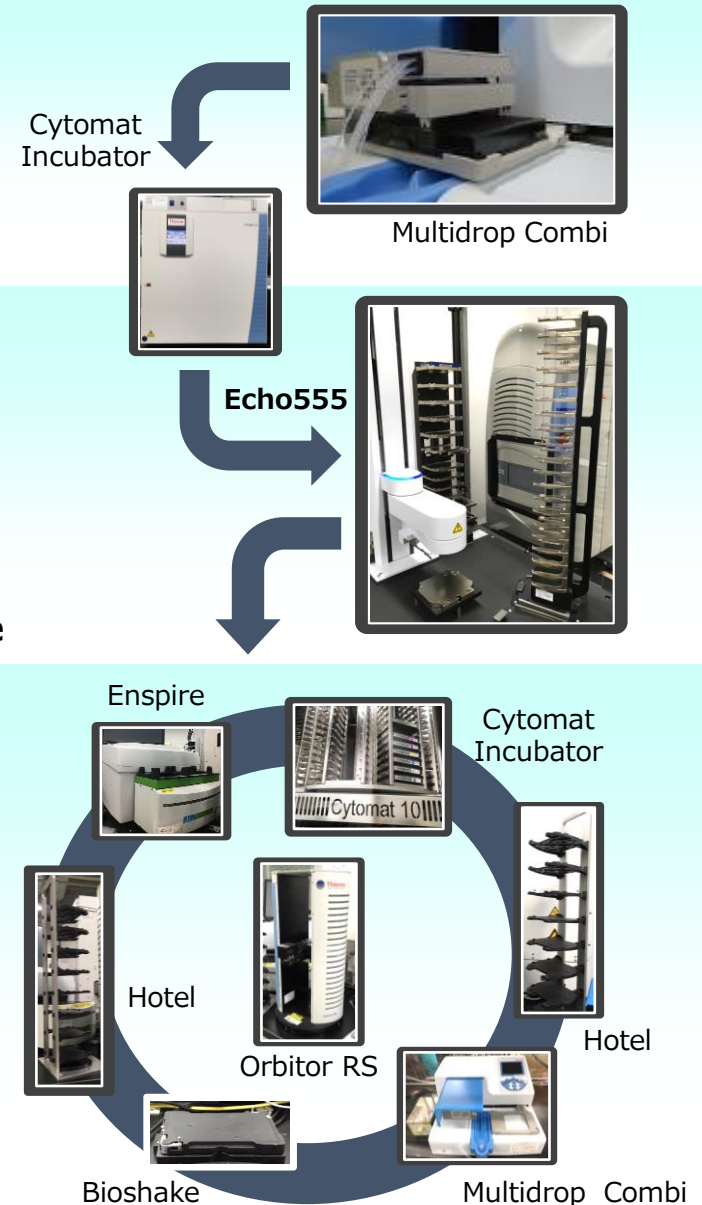
AUC (area under the curve)

Opition:

EC<sub>50</sub> (50% effective concentration)

GI<sub>50</sub> (50% growth inhibition concentration)

imaging, detection of apoptosis etc.



**Anti-cancer drug evaluation using F-PDO**  
**Differences in sensitivity between F-PDO and**  
**cancer cell lines**

# Panel assay using F-PDOs

**Lung 11 lines**

RLUN005, RLUN010, RLUN012, RLUN014, RLUN015, RLUN016, RLUN017, RLUN018, RLUN021, RLUN022, RLUN023

**Uterine 6 lines**

REME009, REME011, REME012, REME013, REME016, REME017

**Cervical 1 lines**

RCER003

**Ovary 3 lines**

ROVA002, ROVA009, ROVA014

**Peritoneal 1 lines**

RPER001

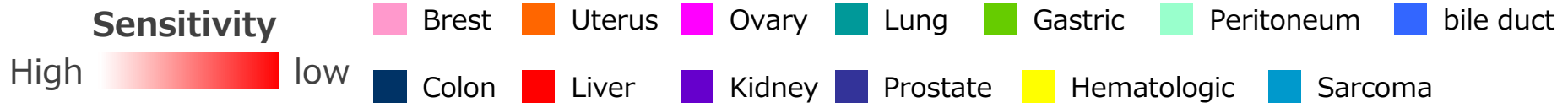
**Colon 4 lines**

RCOL003, RCOL004, RCOL005, RCOL006

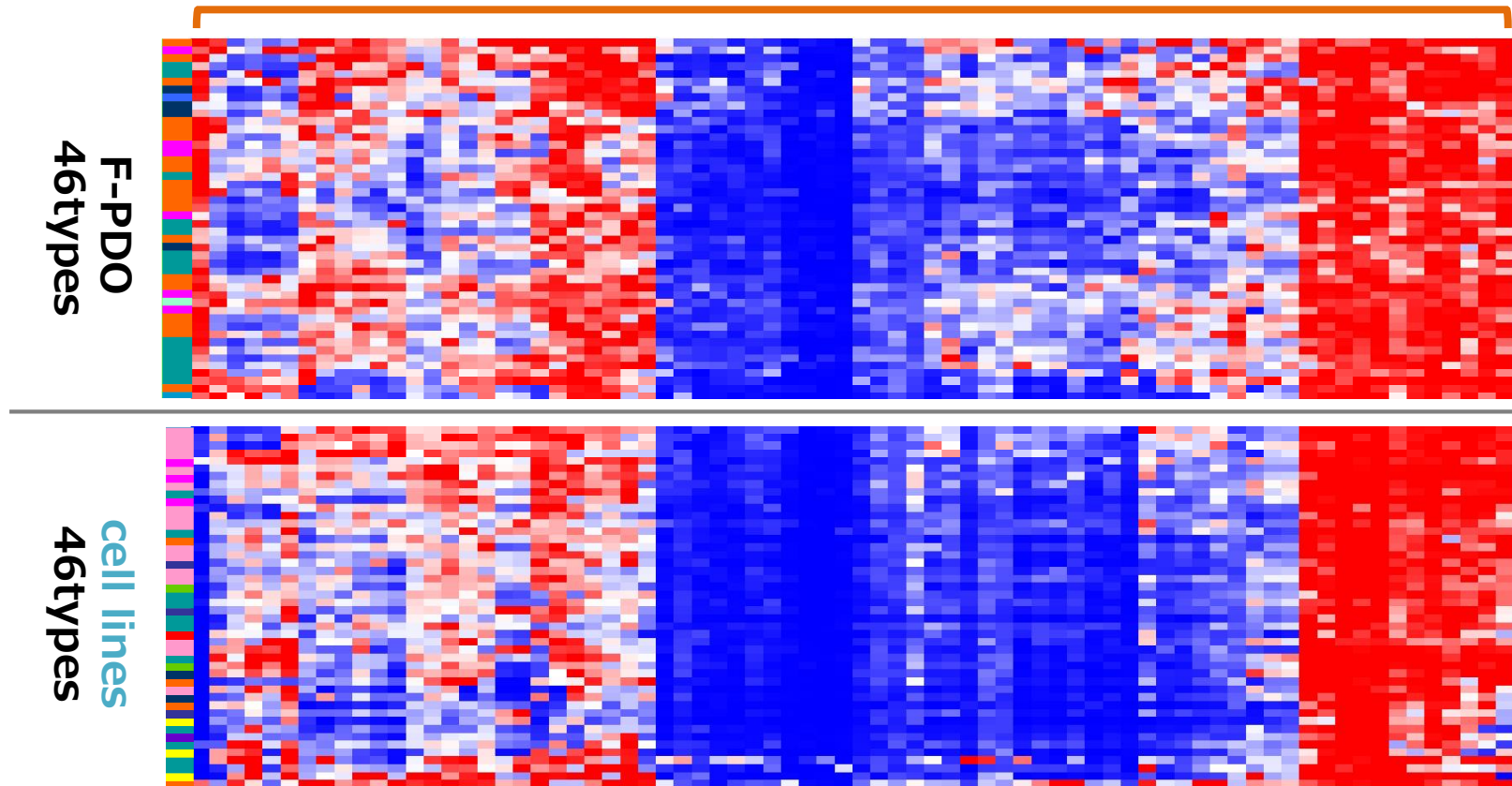
► **74 anti-cancer drugs**

Compound name				
Afatinib	Carfilzomib	Foretinib	Nilotinib	RO-4929097
AGI-6780	Ceritinib	Ganetespib	Nutlin-3	Ruxolitinib
Alvespimycin	Crizotinib	GDC-0068	NVP-BKM120	Sunitinib
Aminoglutethimide	Dabrafenib	GDC-0980	Obatoclox	Tacrolimus
AZD 0530	DCC-2036	GSK126	Olaparib	Tamoxifen
AZD 4547	Decitabine	Ibrutinib	OSI-906	Tandutinib
AZD 6244	Dexamethasone	Idelalisib	PAC-1	Temozolomide
Belinostat	Dinaciclib	Lapatinib	Paclitaxel	Tivantinib
Bicalutamide	Elesclomol	Leflunomide	Panobinostat	Tubastatin A
Binimetinib	Entinostat	Lenalidomide	PCI-34051	Varlitinib
BMS-754807	Entrectinib	Melphalan	Pentostatin	Vemurafenib
Bortezomib	Erlotinib	Methotrexate	PHA-793887	Vindesine
Brivanib	Etoposide	Mitomycin C	Ponatinib	Vismodegib
BX-795	Everolimus	MLN-4924	Rapamycin	Volasertib
Carboplatin	Fluorouracil	Mycophenolic acid	Regorafenib	

# Differences in sensitivity between F-PDO and cancer cell lines



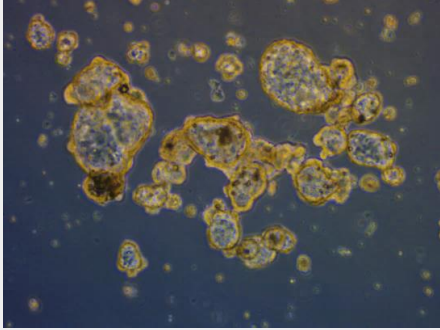
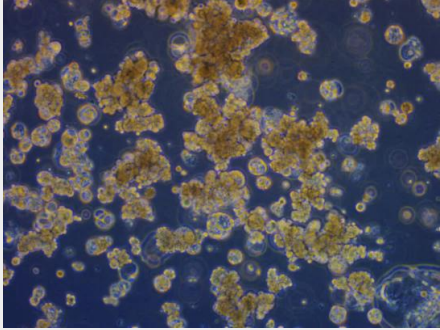
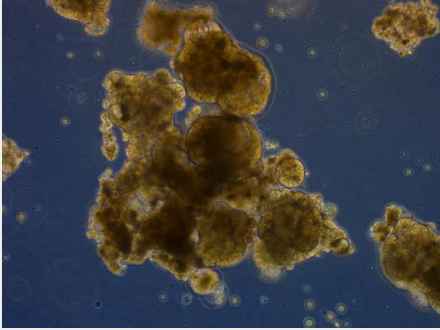
## 74 anti-cancer drugs



**F-PDO has diverse patterns of drug sensitivity**

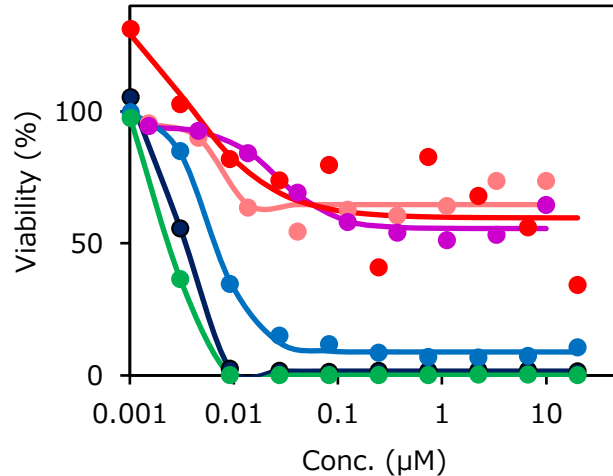
# **Sensitivity to chemotherapeutic agents**

# Sensitivity of gynecological F-PDO to chemotherapeutic agents

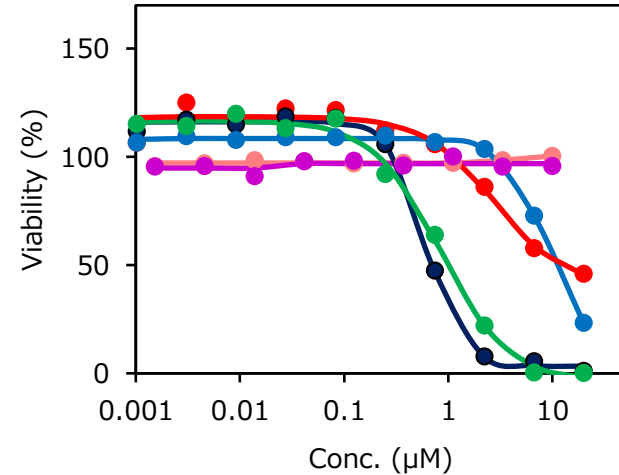
	<b>ROVA14</b>	<b>REME9</b>	<b>REME11</b>
	<b>Ovarian</b>	<b>Uterine</b>	<b>Uterine</b>
<b>Phase contrast image</b>			
<b>Diagnosis</b>	Serous adenocarcinoma	Endometrioid adenocarcinoma with squamous differentiation	Clear cell adenocarcinoma
<b>Clinical drug sensitivity</b>	Tolerant to Paclitaxel & Carboplatin	Tolerant to Paclitaxel & Carboplatin	Tolerant to Paclitaxel & Carboplatin

# Drug sensitivity: F-PDOs vs Cell lines

## Paclitaxel



## Carboplatin



Cells	IC <sub>50</sub> (µM)	
	Paclitaxel	Carboplatin
● Ovarian ROVA14	> 10	> 10
● Uterine REME9	> 10	> 10
● Uterine REME11	> 10	> 10
● Ovarian OVCAR-3	0.003	0.712
● Ovarian SK-OV-3	0.007	10.33
● Uterine SK-UT-1B	0.003	0.988

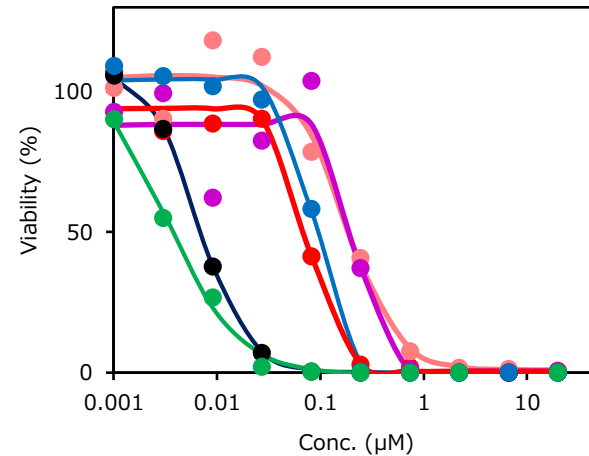
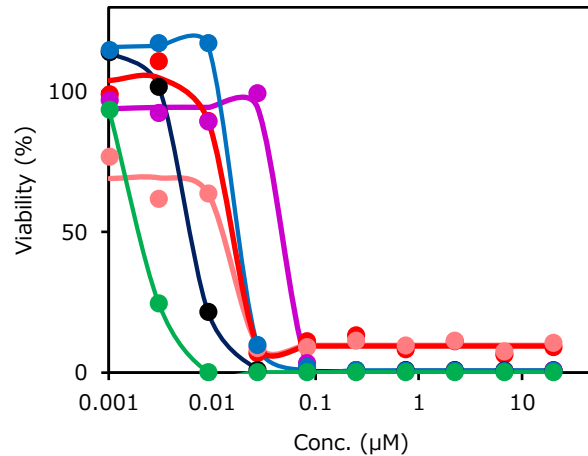
- F-PDO showed tolerance to anti-cancer drugs like drug-resistant patient

# Drug sensitivity: F-PDOs vs Cell lines

Drugs in clinical stage: showing high efficiency

CDK inhibitor: Dinaciclib

HDAC inhibitor: Panobinostat

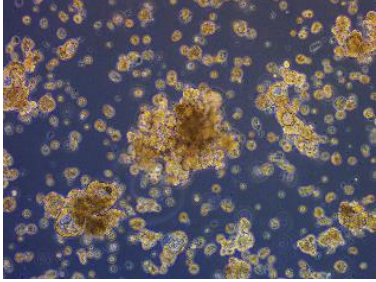
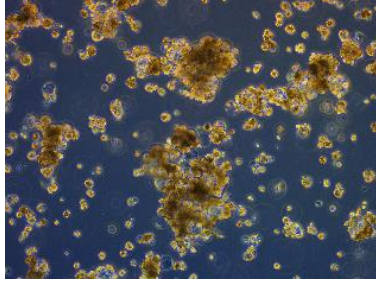
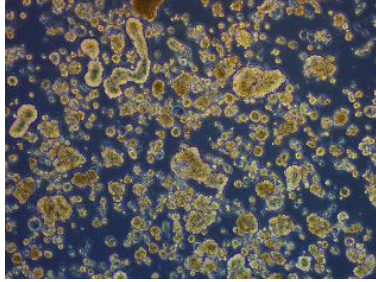
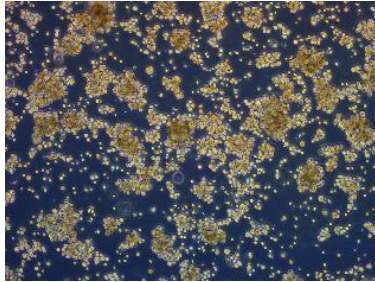


Cells	IC <sub>50</sub> (µM)	
	Dinaciclib	Panobinostat
● Ovarian ROVA14	<b>0.010</b>	<b>0.073</b>
● Uterine REME9	<b>0.010</b>	<b>0.190</b>
● Uterine REME11	<b>0.069</b>	<b>0.238</b>
● Ovarian OVCAR-3	<b>0.006</b>	<b>0.007</b>
● Ovarian SK-OV-3	<b>0.024</b>	<b>0.090</b>
● Uterine SK-UT-1B	<b>0.003</b>	<b>0.004</b>

# **Sensitivity to molecular target drugs**

# EGFR-inhibitor sensitivity for Lung F-PDO

## EGFR inhibitor sensitive mutant

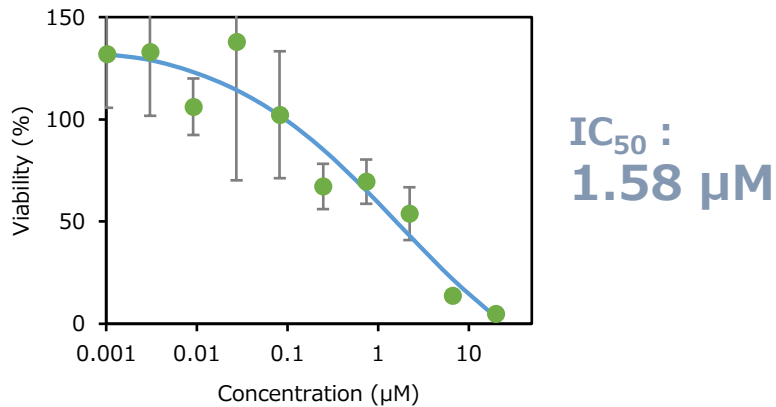
EGFR gene	L858R	E746_A750del	Wild type	
Cell	RLUN14-2	RLUN23	RLUN15-2	RLUN16-2
Phase contrast image				
Diagnosis	Papillary adenocarcinoma	Papillary adenocarcinoma	Pleomorphic carcinoma	Squamous cell carcinoma

# EGFR-inhibitor sensitivity for Lung F-PDO

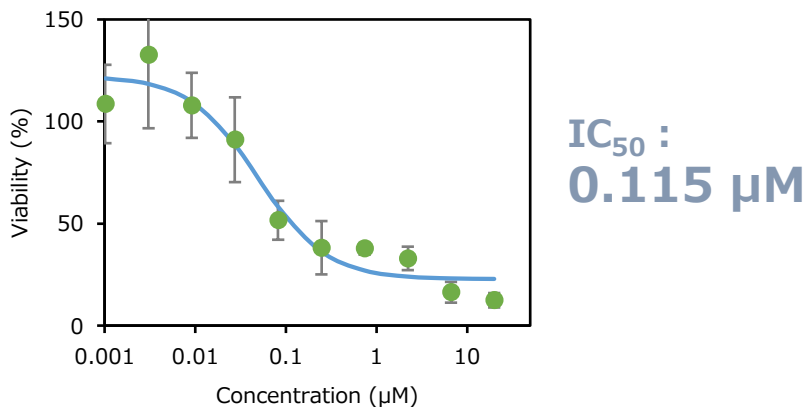
## Erlotinib (EGFR inhibitor) sensitivity

### EGFR inhibitor sensitive mutant

#### RLUN14-2 (EGFR L858R)

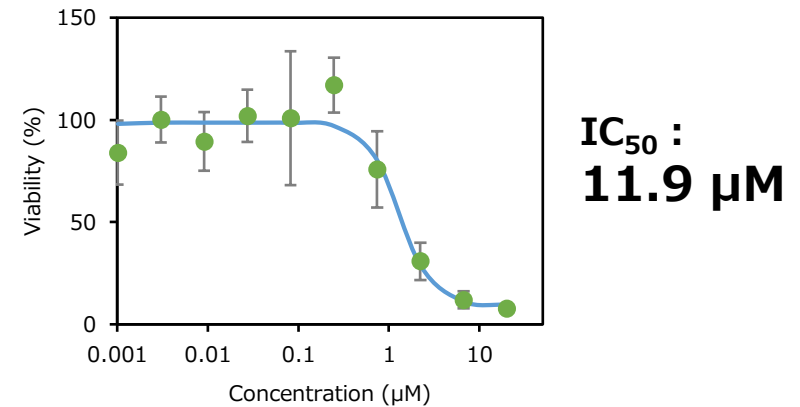


#### RLUN23 (EGFR E746\_A750del)

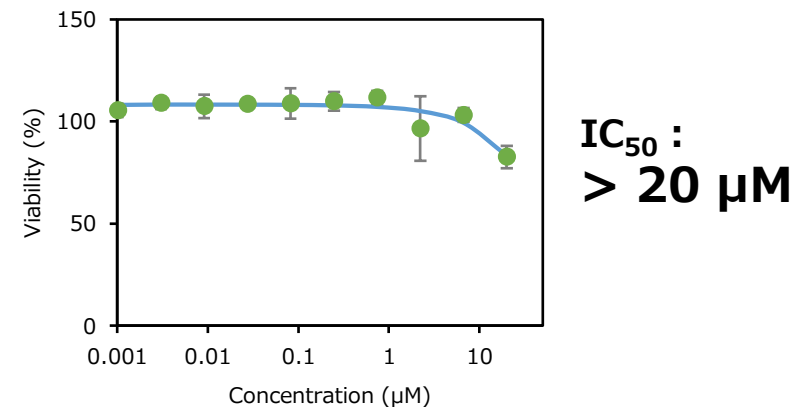


### Wild

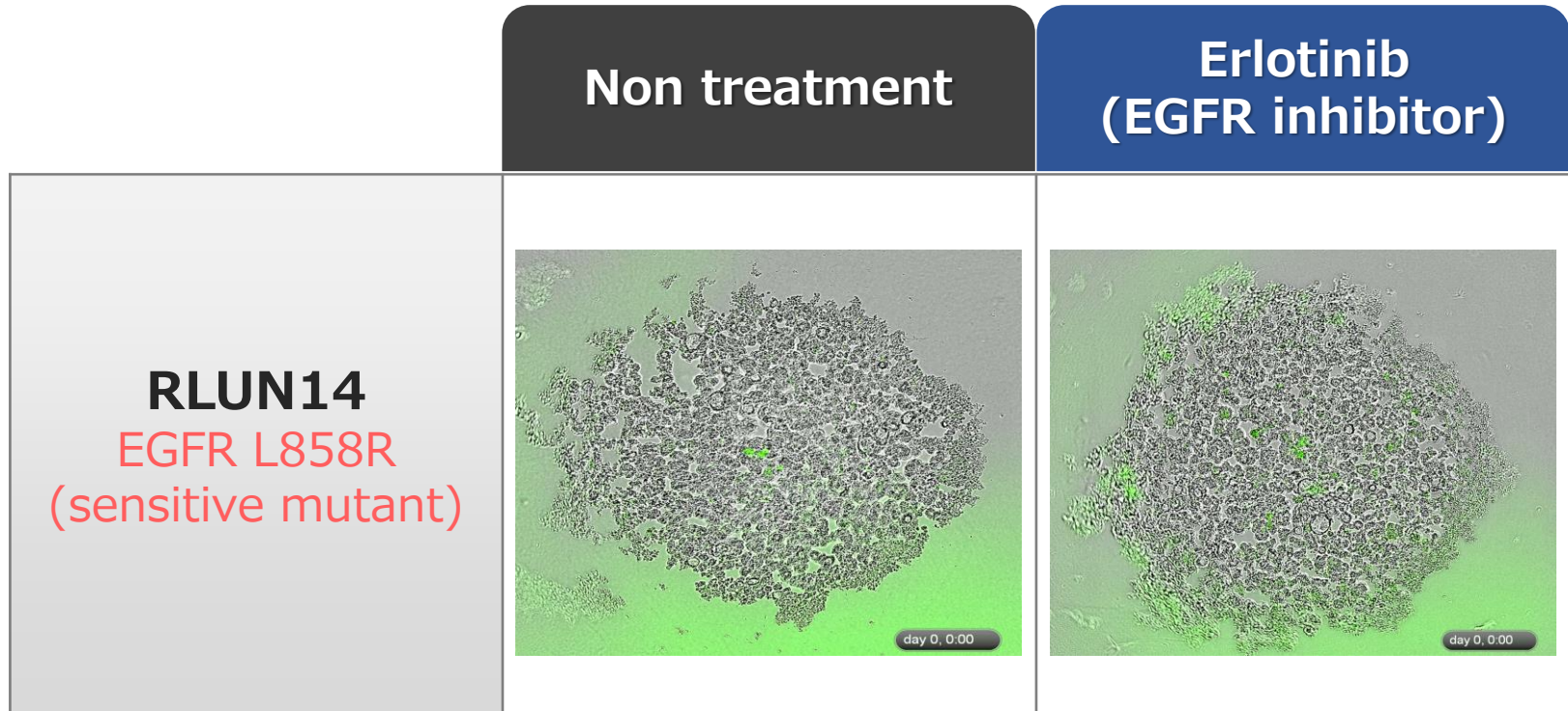
#### RLUN15-2



#### RLUN16-2



# Apoptosis induction by Erlotinib



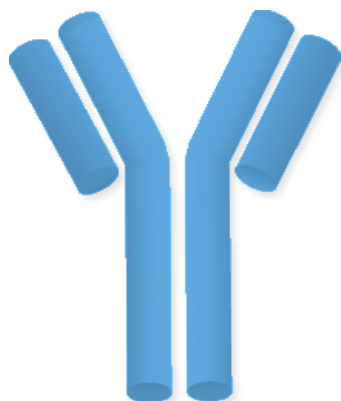
Showing response mimic clinical data

# **Evaluation of antibody-drug conjugate (ADC)**

# Anti-HER2 antibody and ADC

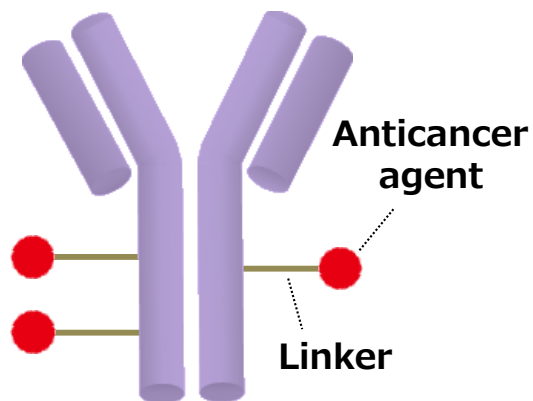
## Anti-HER2 antibody

### Herceptin 《Trastuzumab》



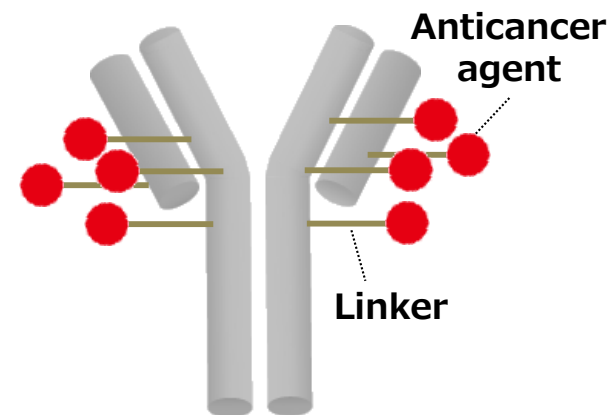
- ◆ Anti-HER2 antibody
- ◆ ADCC activity
- ◆ Approved in 2001

### Kadcyla 《Trastuzumab Emtansine》



- ◆ Conjugate of trastuzumab and emtansine
- ◆ Inhibition of tubulin polymerization
- ◆ Approved in 2014

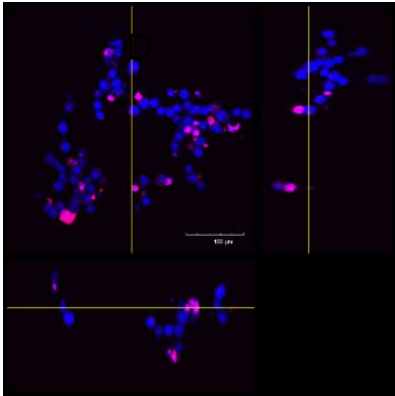
### Enhertu 《Trastuzumab Deruxtecan》



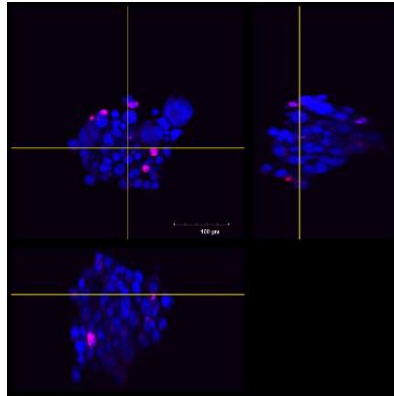
- ◆ Conjugate of trastuzumab and deruxtecan
- ◆ Inhibition of Topoisomerase I
- ◆ Approved in 2020

# Evaluation of ADC using lung F-PDO

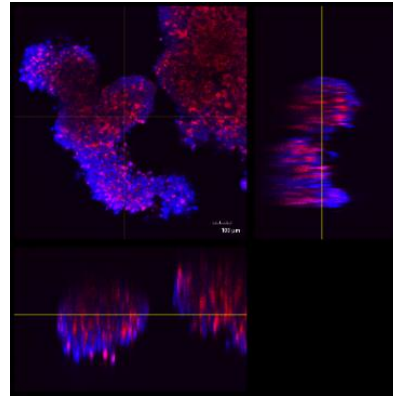
Calculate the volume and HER2 positivity of F-PDO



RLUN5

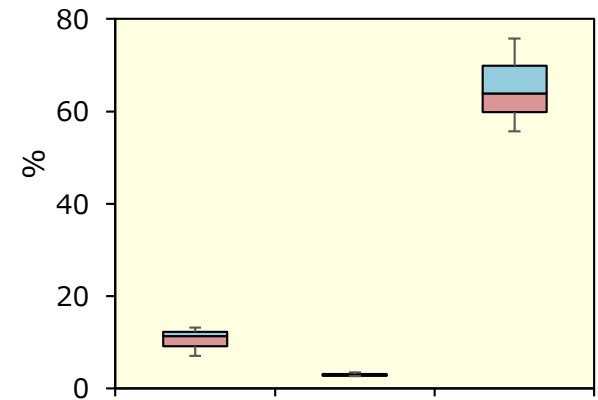


RLUN16

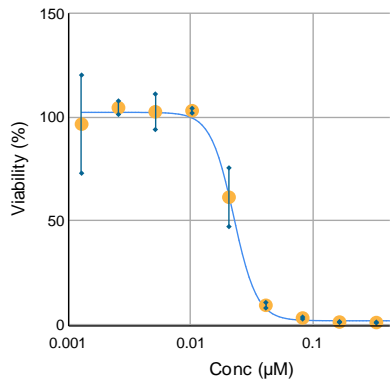


RLUN21

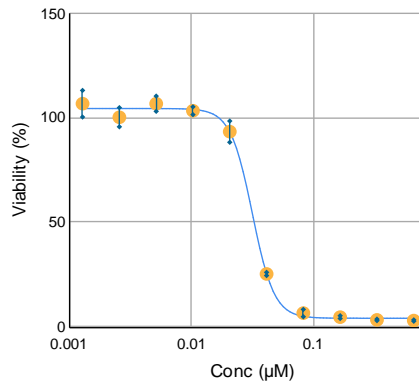
Ratio of HER2-Positive cells



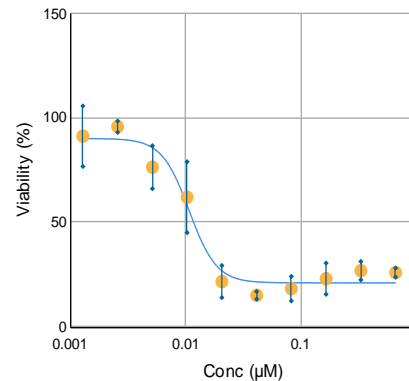
IC<sub>50</sub> 3.4 µg/mL



IC<sub>50</sub> 4.9 µg/mL



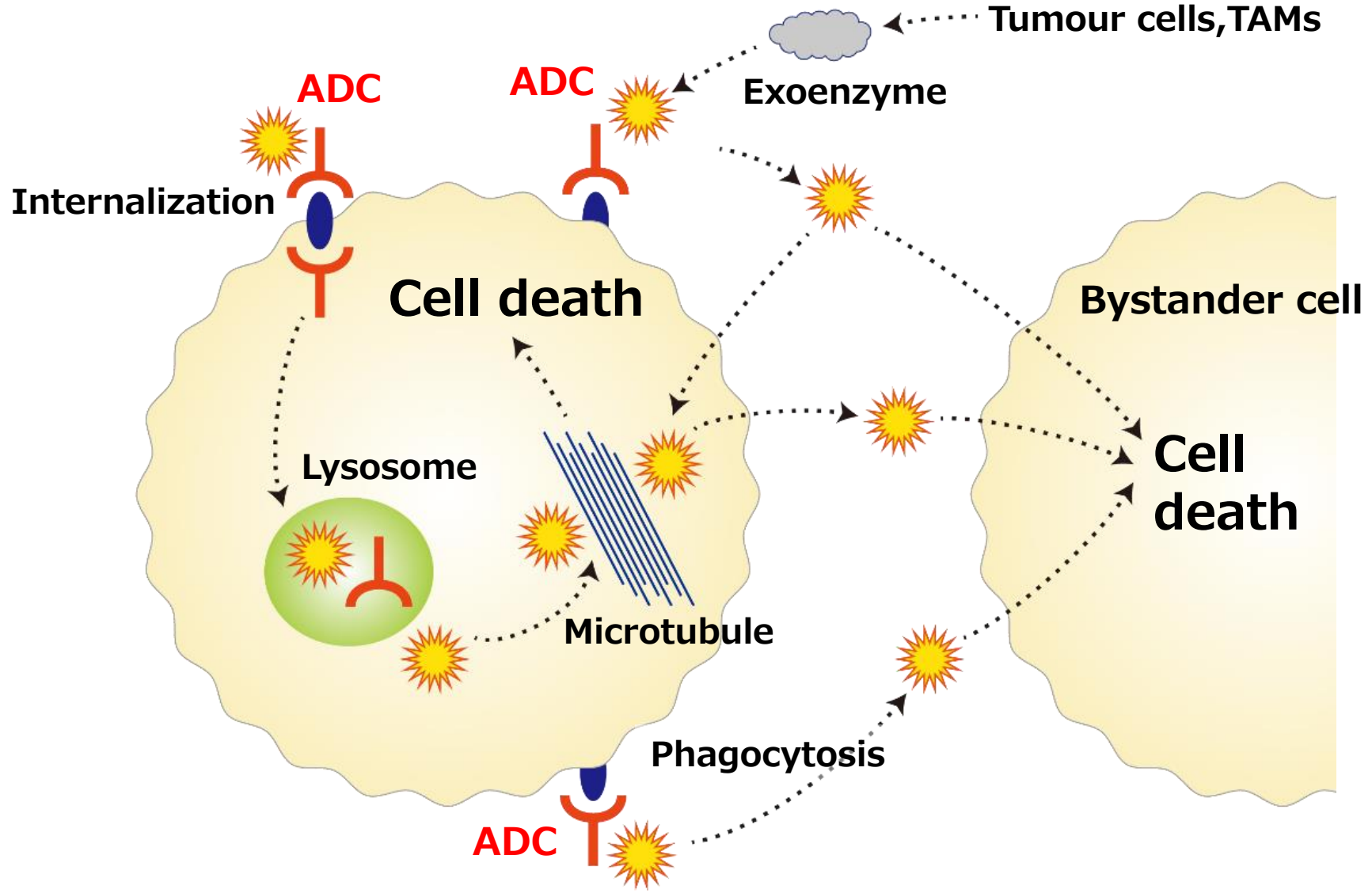
IC<sub>50</sub> 1.7 µg/mL



**ADC also shows efficacy in F-PDO with low HER2 expression.**

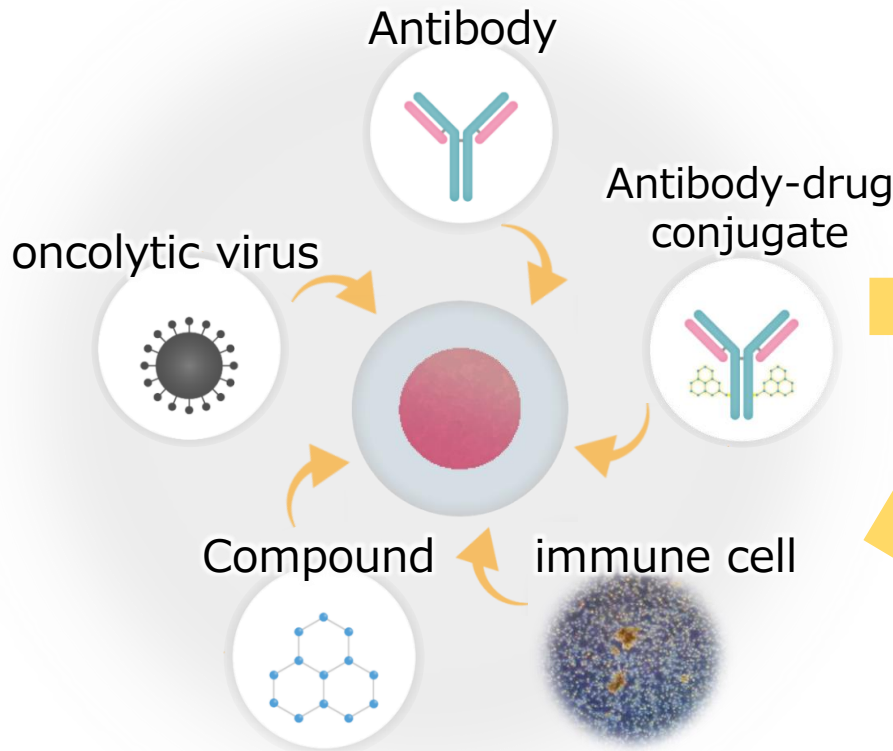
These data were obtained through collaboration with Olympus Corporation

# Bystander effect of ADC

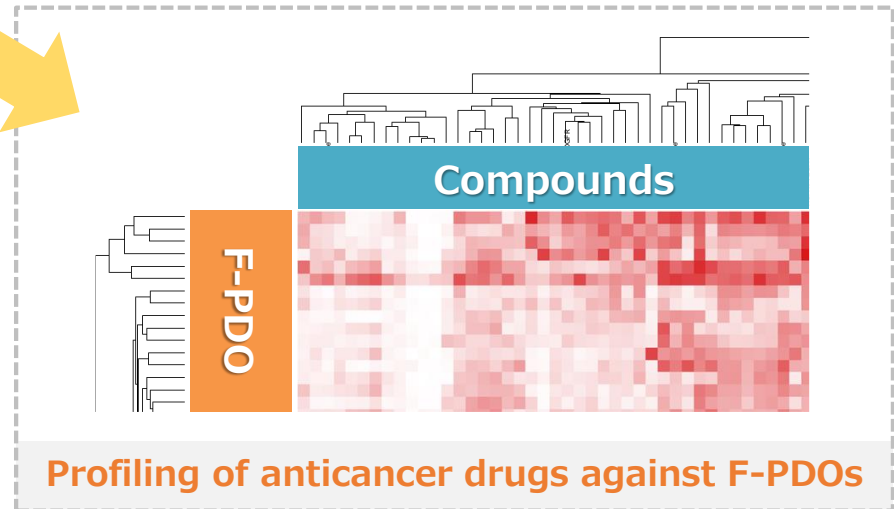
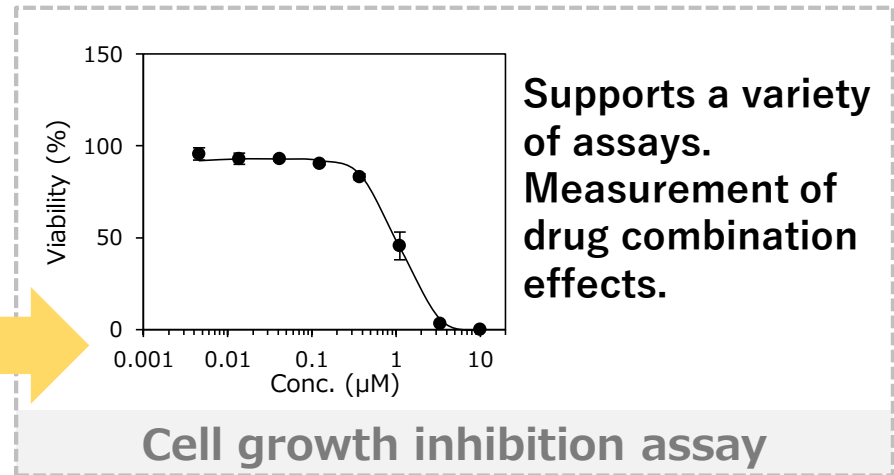


## Support for a variety of modalities

Capable of evaluating chemotherapeutic agents, molecular targeted agents, immune checkpoint inhibitors, and cancer immunotherapy



Search for drug efficacy markers using gene expression and gene mutation data



# Evaluation of immune response using immune cells

# History of cancer immunotherapy

## Non-specific cancer immunotherapy

Boosts systemic immune function

## Specific cancer immunotherapy

Enhance immune function against cancer

## Immune checkpoint therapy

Restores immune function

Vaccine  
Anticancer agent

### 1<sup>st</sup> generation (1970s-)

**Biological response modifier (BRM)**

- Krestin
- Picibanil
- BCG

### 2<sup>nd</sup> generation (1980s-)

**Cytokine therapy**

- Interferon
- Interleukin

### 4<sup>th</sup> generation (1990s-)

- Peptide vaccine therapy
- **Antibody therapy**

### 3<sup>rd</sup> generation (1980s-)

**Activated lymphocyte therapy**

- LAK cell therapy
- CAT cell therapy
- NK cell therapy
- $\gamma\delta$ T cell therapy

### 5<sup>th</sup> generation (2000s-)

**Dendritic cell Vaccine therapy**

### Latest research (2010s-)

**CAR-T therapy**

- CD19 CAR-T cell
- CD269 CAR-T cell

### Latest research (2014-)

**Immune checkpoint inhibitor**

- anti-PD-1 antibody
- anti-PD-L1 antibody
- anti-CTLA-4 antibody

Immune cell therapy

**Activation of immune cells  
Enhanced the accelerator of immunity**

**Blocking immunosuppression  
Release the brake on immunity**

# Immuno-cell therapy: activated lymphocyte

## Therapies

LAK cell therapy: Return activated lymphocytes to the body  
 CAT cell therapy: Return activated **cytotoxic T lymphocytes (CTLs)** to the body  
 NK cell therapy: Return activated **NK cells** to the body  
 $\gamma\delta$ T cell therapy: Return activated  $\gamma\delta$ T cells to the body

## MOA

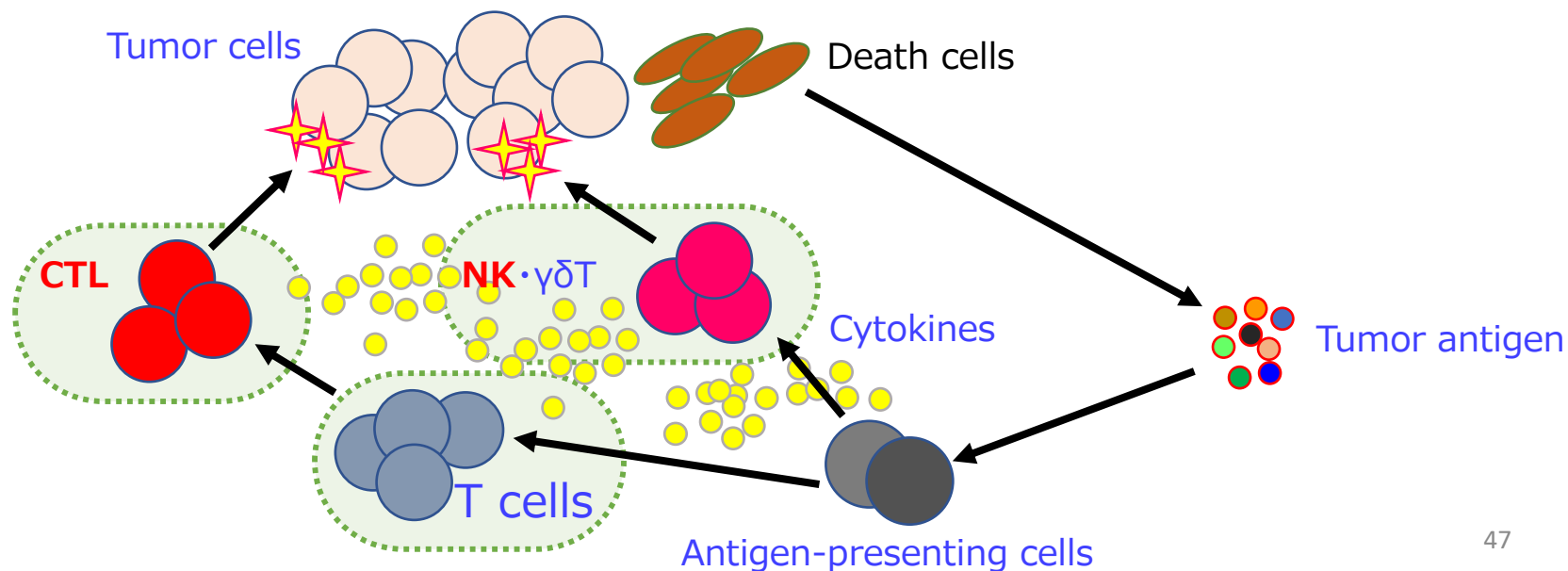
Enhances immune response by returning to the body the immune cells proliferated and activated with cytokines.

## Benefits

Because immune cells are activated outside the body, the side effects by large doses of cytokines can be reduced

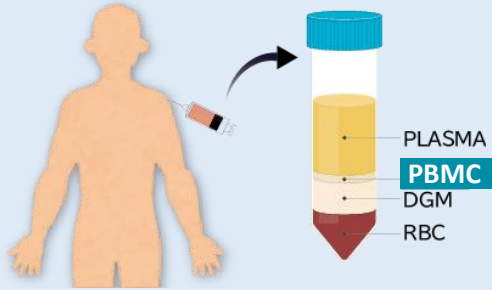
## Disadvantage

Induced CTLs do not always attack cancer cells

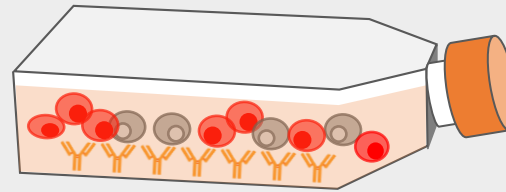


# Lymphokine-activated killer cells

## 1. PBMC

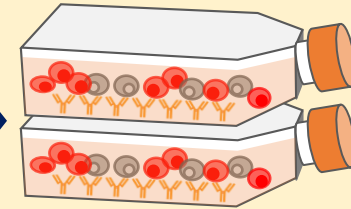


## 2. Culture



▲ Flask coated with anti-CD3 antibody  
Seeding of PBMC into medium containing IL-2

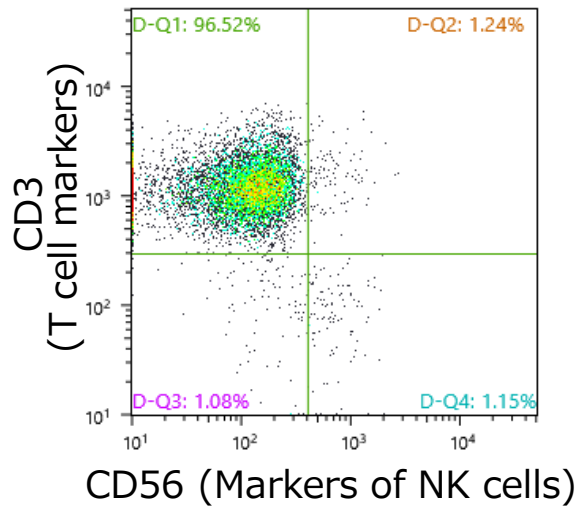
## 3. Expansion



collection

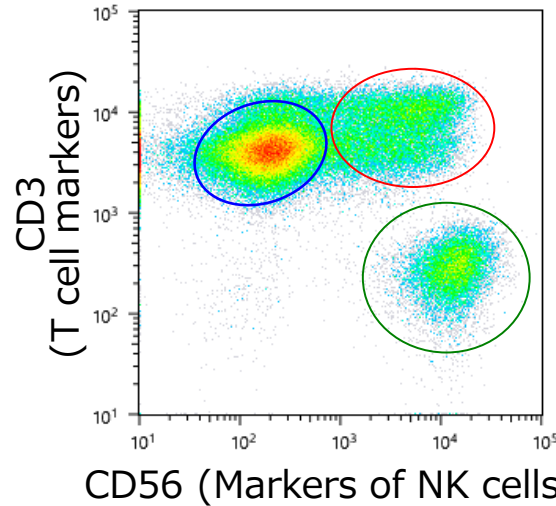
Up the culture scale

## Cytotoxic T cells (CTLs)



CTLs : 96.5%

## NK cells



NK cells stained with anti-CD3 and anti-CD56 antibodies were sorted.

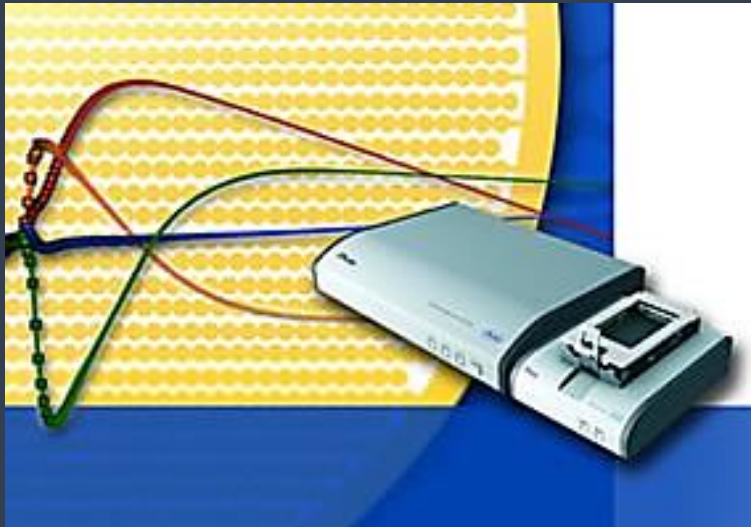
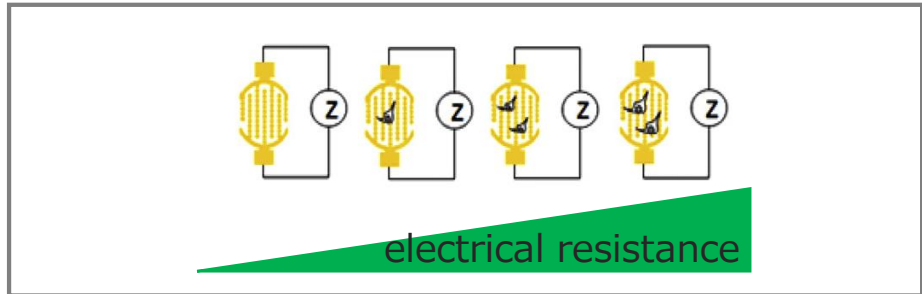
CD3+/CD56- (CTLs) : 52%  
CD3+/CD56+ (NKT cells) : 29%  
CD3-/CD56+ (NK cells) : 18%

# Measurement of cell proliferation using xCELLigence

The response of cells to special stimuli such as growth (proliferation), elongation, morphological change, and death can be determined in a label-free manner by measuring electrical resistance (10 KHz, 25 KHz, 50 KHz).



Bottom of the well is covered with gold electrodes



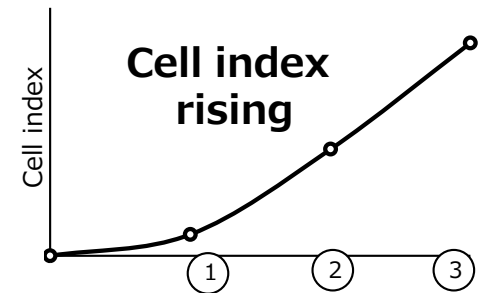
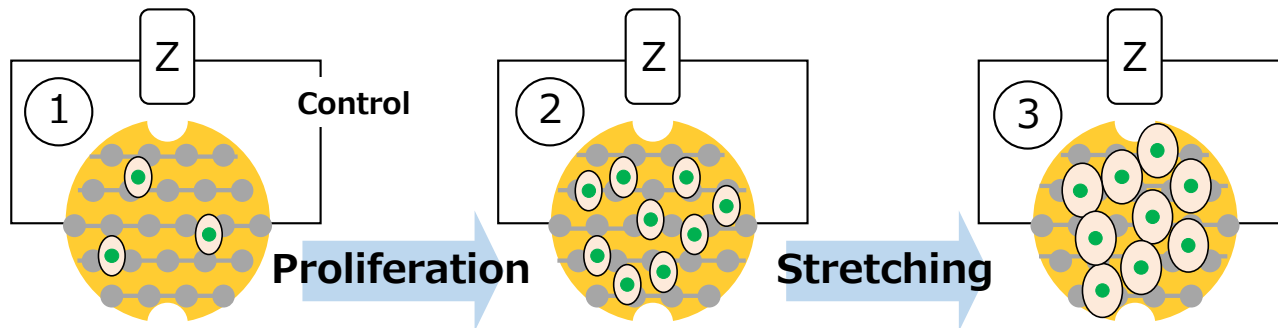
## Advantage

- Simultaneous measurement of multiple samples
- No need for labels or reporters
- Real-time monitoring
- Long time measurement in incubator

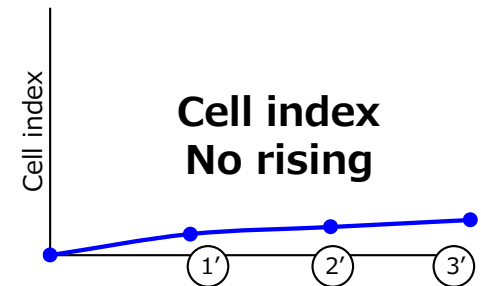
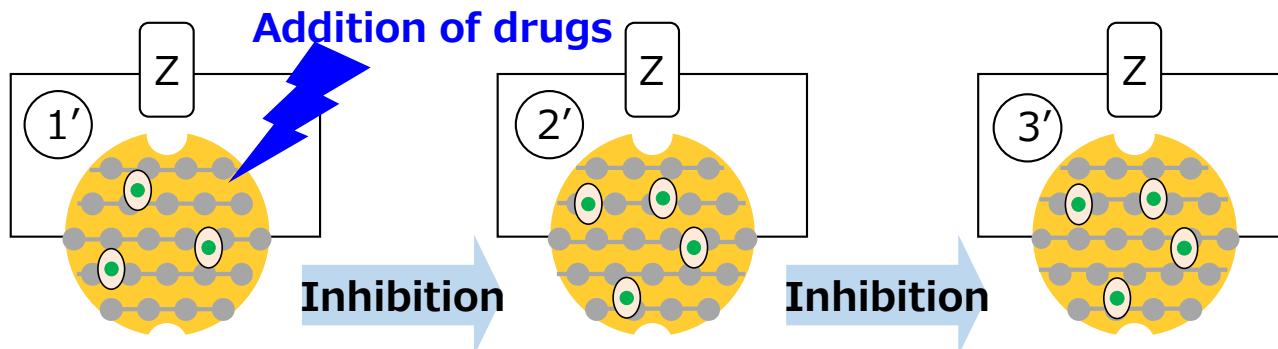
# Measurement of cell proliferation using xCELLigence

## Evaluation of cell growth inhibition by electrical resistance value

### Electrical resistance



### No electrical resistance



# Cytotoxicity assay by immune cells using F-PDO protocol

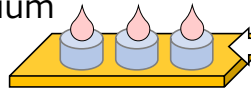
## Electrical resistance measurement using xCELLigence

F-PDO



E-Plate 96

Medium



Measurement of background

Fibronectin-coated wells

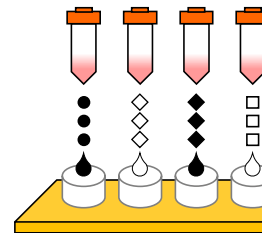
Seeding  
( $2 \times 10^4$  cells/well)



Start Cell Index measurement using xCELLigence

24 h

NK cells T cells



Without effector cells

Addition of effector cells  
effector cells : F-PDO

= 1:5 or 1:10

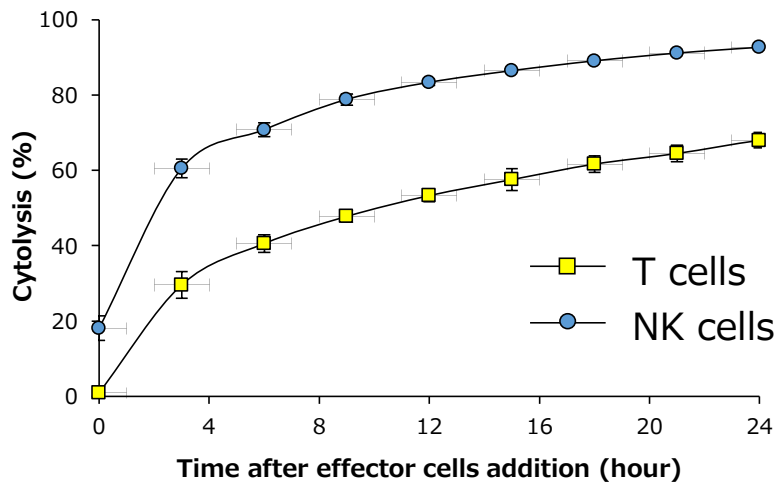
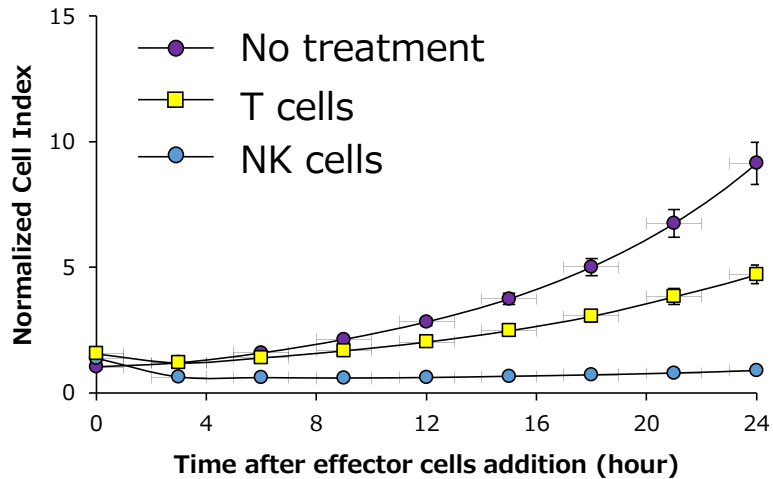
( $1 \times 10^5$  cells or  $2 \times 10^5$  cells/well)

Cell Index measurement  
Cytolysis calculation

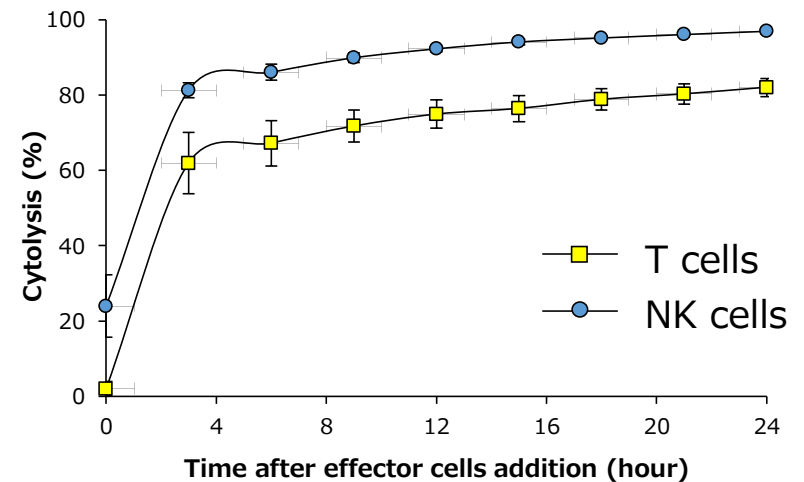
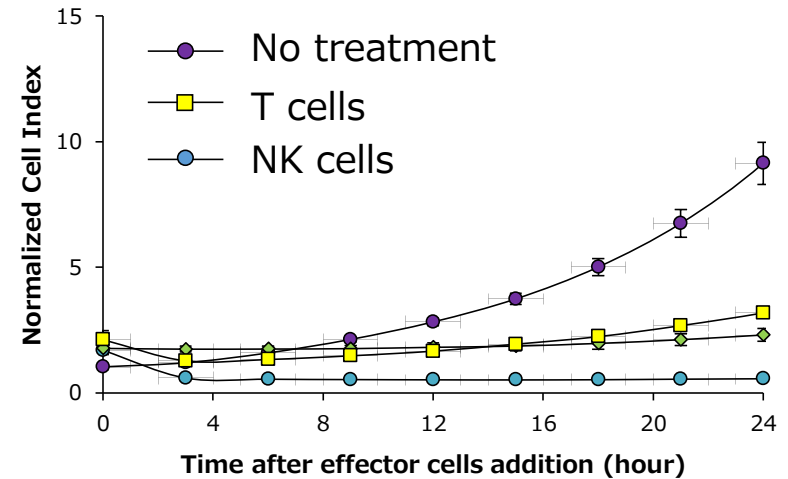
# Cytotoxicity assay by immune cells using F-PDO

## Lung F-PDO: RLUN007, $2 \times 10^4$ cells/well

F-PDO : effector cells = 1 : 5



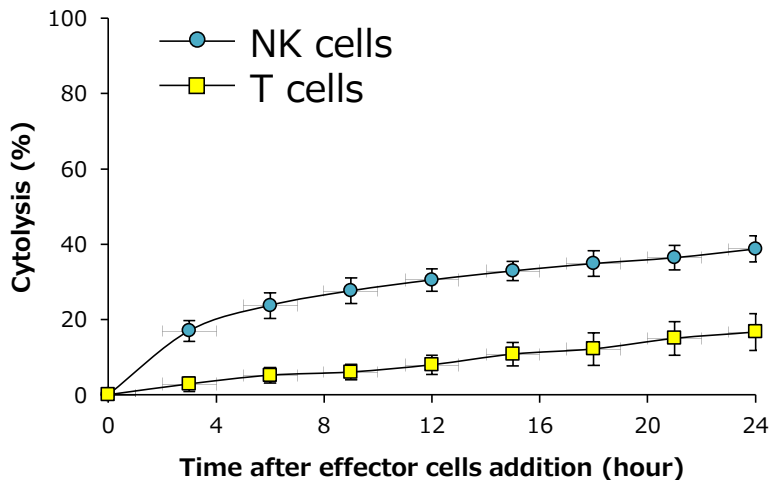
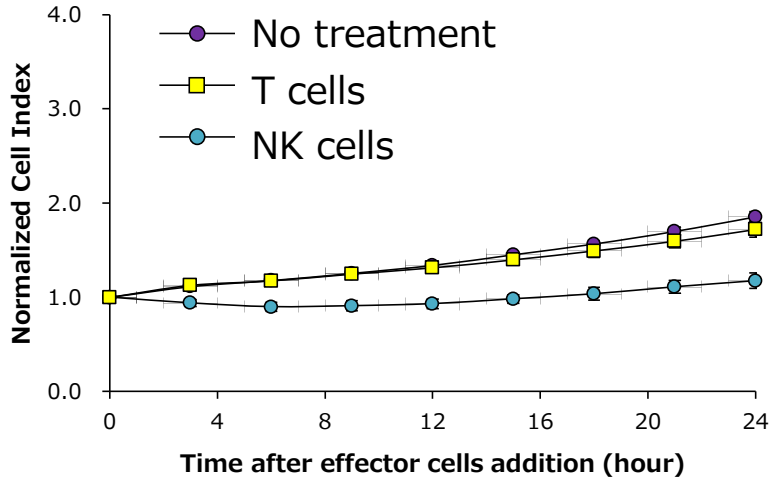
F-PDO : effector cells = 1 : 10



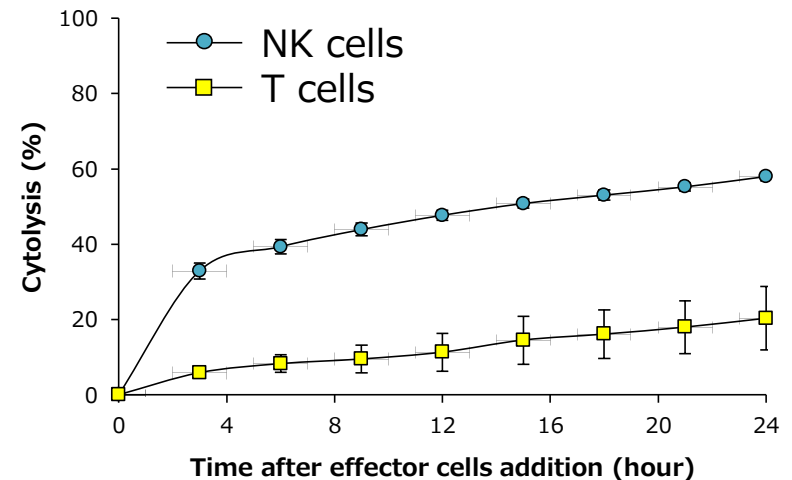
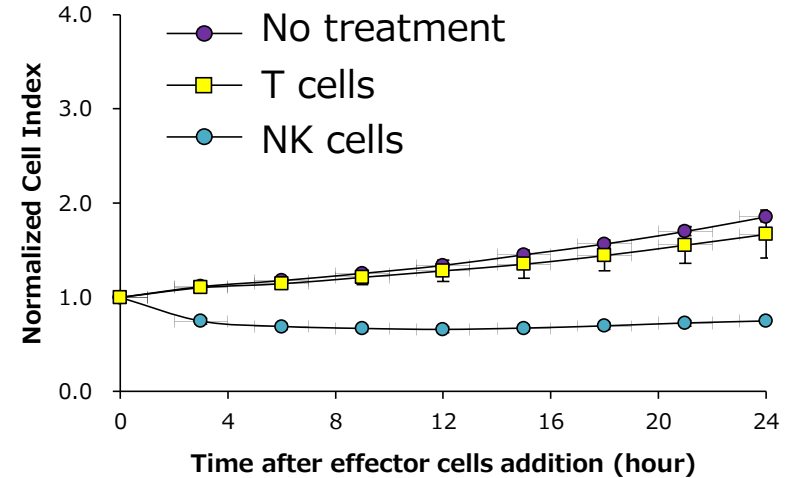
# Cytotoxicity assay by immune cells using F-PDO

## Lung F-PDO: RLUN021, $2.5 \times 10^3$ cells/well

F-PDO : effector cells = 1 : 5

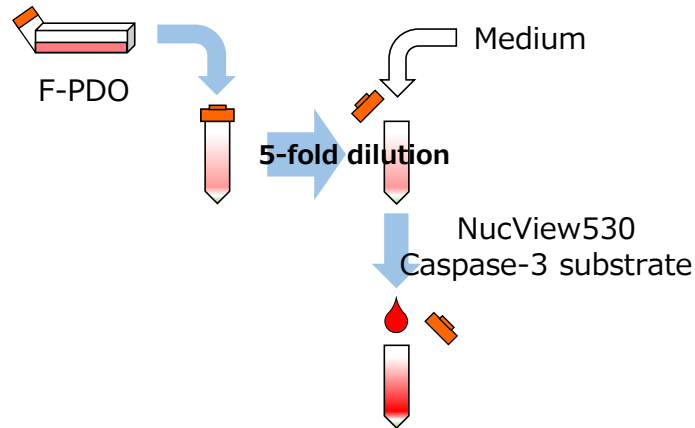


F-PDO : effector cells = 1 : 10

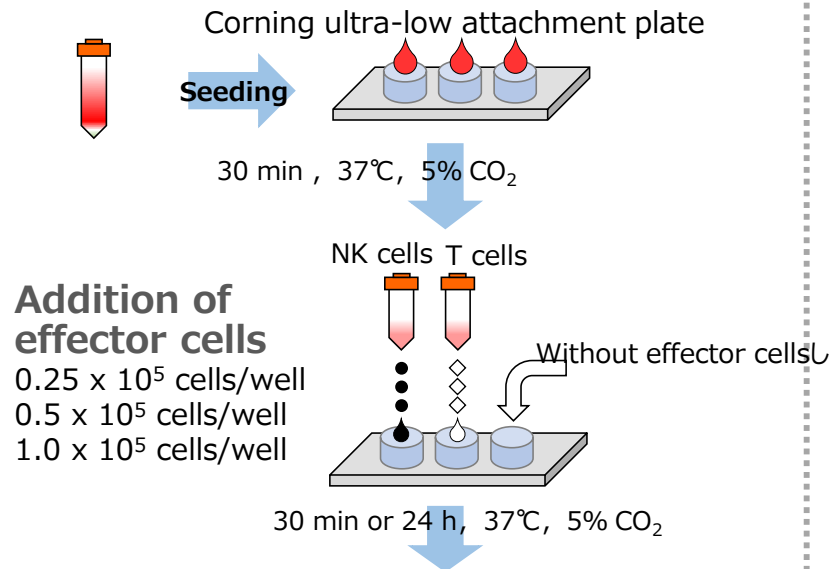


# Cytotoxicity assay by immune cells using NoviSight

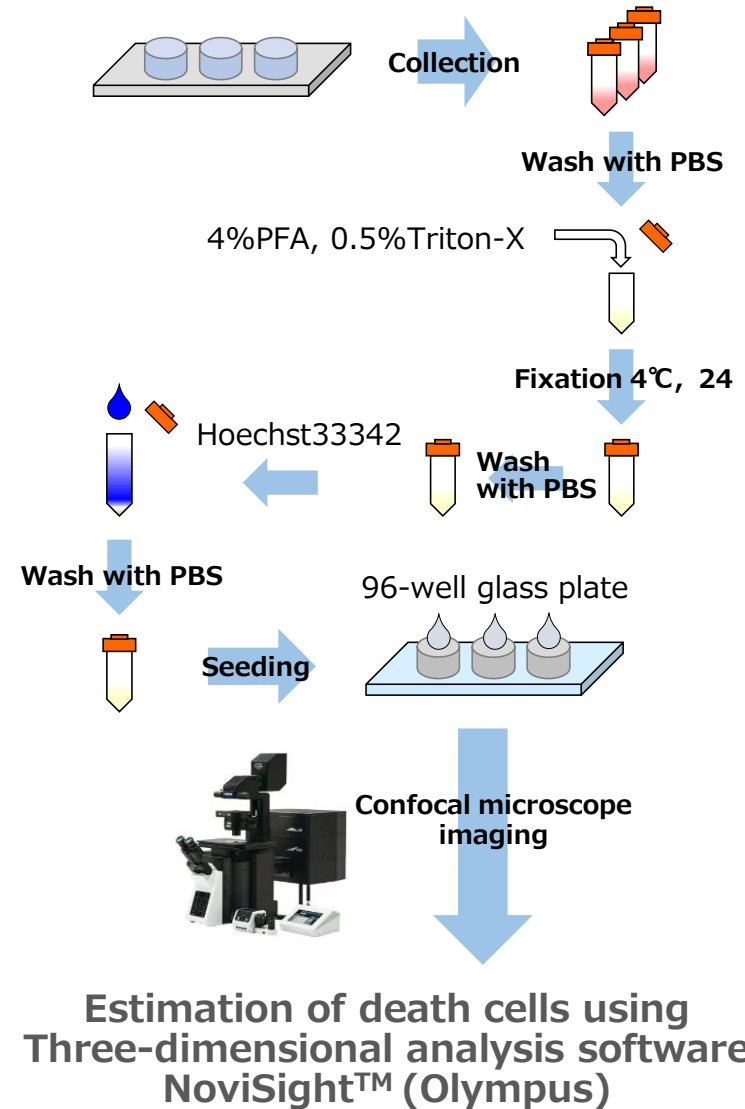
## Preparation of F-PDOs



## Seeding



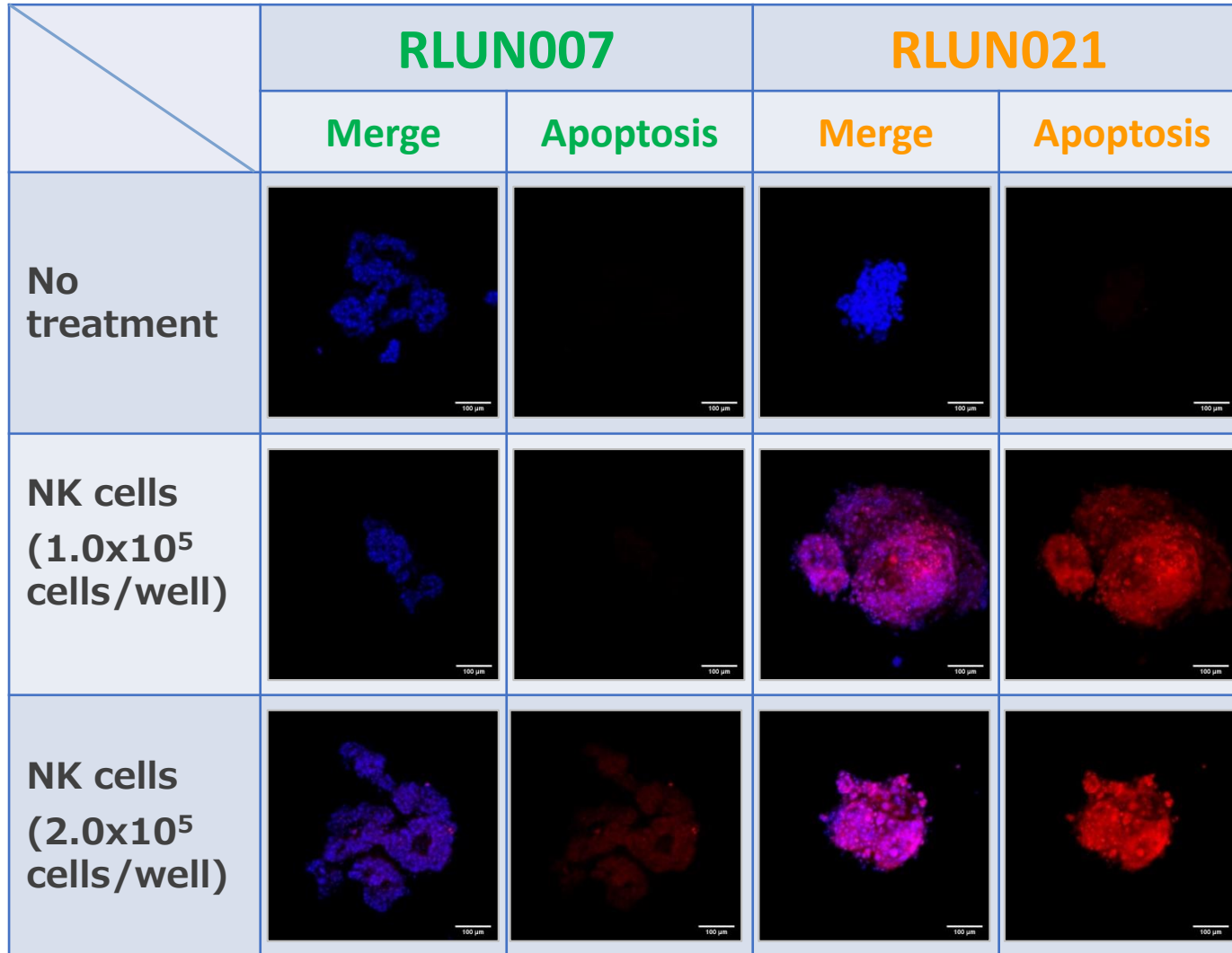
## Fixation, Imaging and analysis



These data were obtained through collaboration with Olympus Corporation.

# Cytotoxicity assay by immune cells using F-PDO

## Lung F-PDO

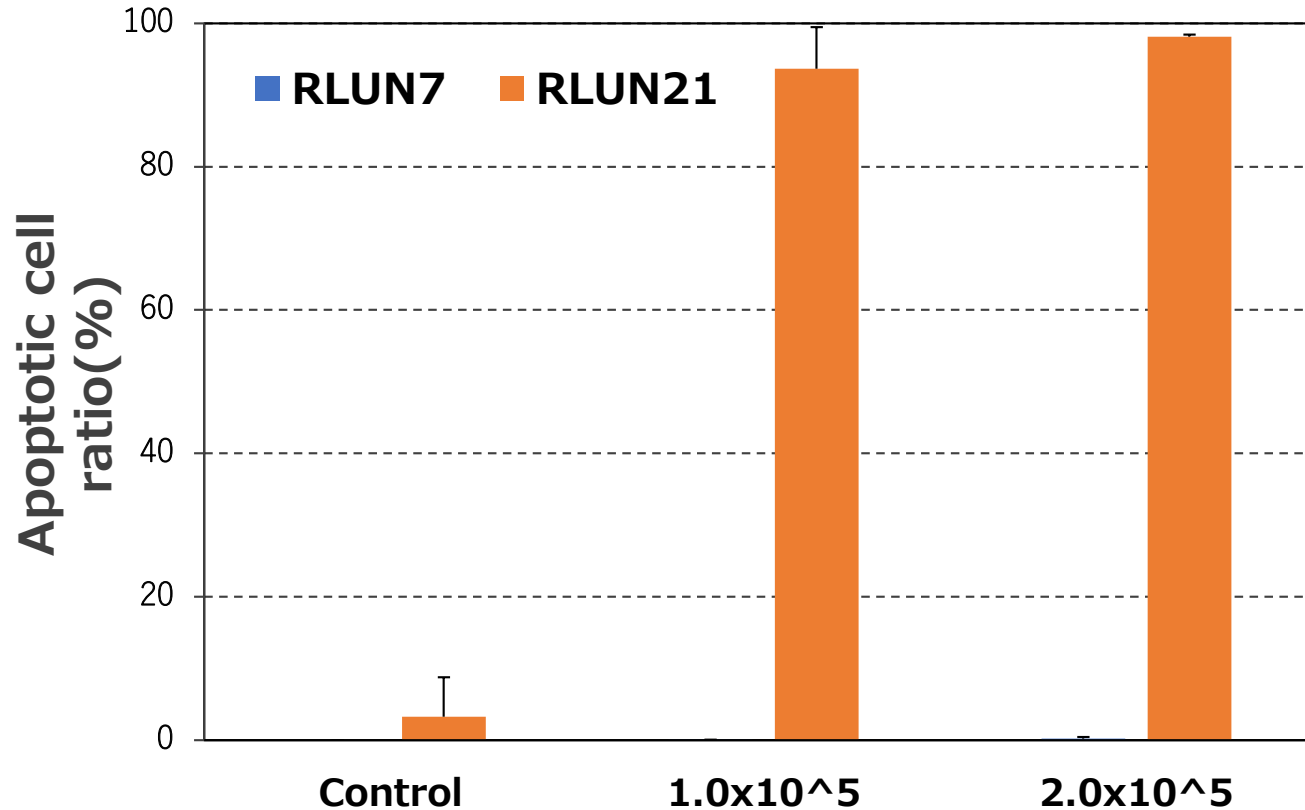


These data were obtained through collaboration with Olympus Corporation.

# Cytotoxicity assay by immune cells using F-PDO

## Lung F-PDO

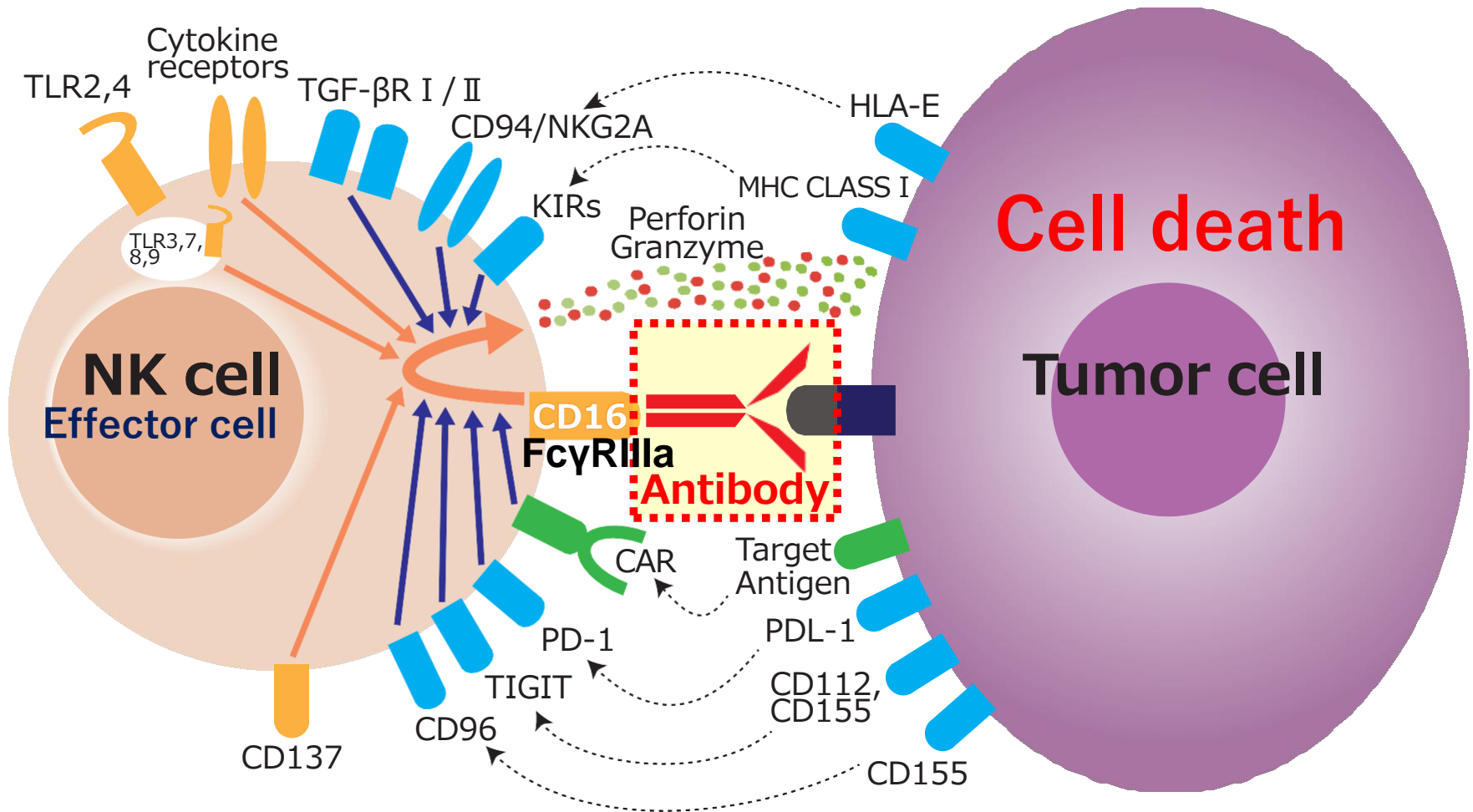
Apoptotic cells ratio in each organoid



Apoptosis induced by NK cells was observed in RLUN021  
Not much was detected in RLUN007.

# **Evaluation of antibody dependent cellular cytotoxicity (ADCC)**

# ADCC



- Activatory mechanism
- Inhibitory mechanism
- Artificial mechanism

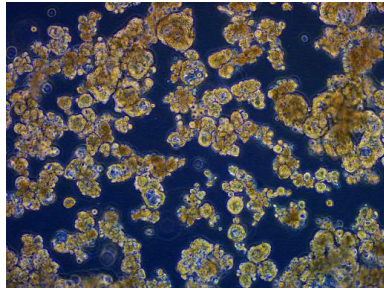
- ADCC-inducing Antibody**
- Surface tumor associated antigen

- Activation
- Inhibition
- Perforin
- Granzyme

# Evaluation of ADCC using lung F-PDO

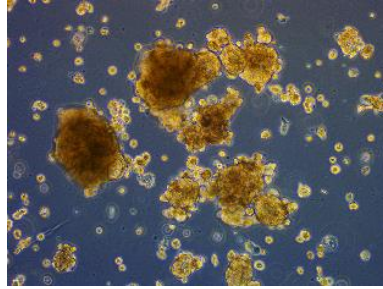
**RLUN007**

EGFR·HER2 expression



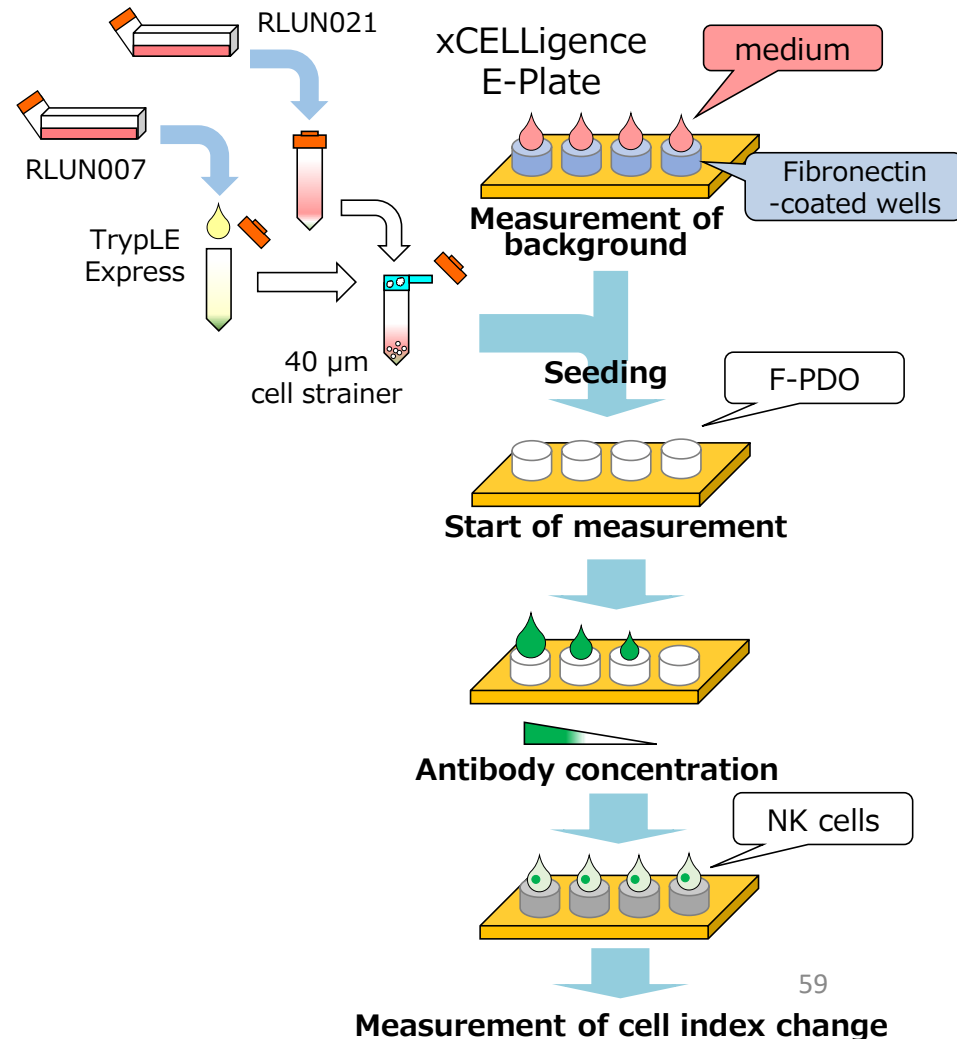
**RLUN021**

EGFR expression



- Cetuximab : anti-EGFR antibody
- Trastuzumab : anti-HER2 antibody

**Treatment concentration(10, 1  $\mu\text{g}/\text{mL}$ )**



**E-Plate 96 coated with fibronectin**

**16 h**

**Addition medium into E-Plate  
Measurement of background**

**Seeding of F-PDO**

**Set on xCELLigence  
Measurement of Cell index**

**24 h**

**Antibody treatment (100, 10, 1  $\mu\text{g}/\text{mL}$ )**

**1 h**

**Seeding of NK cells**

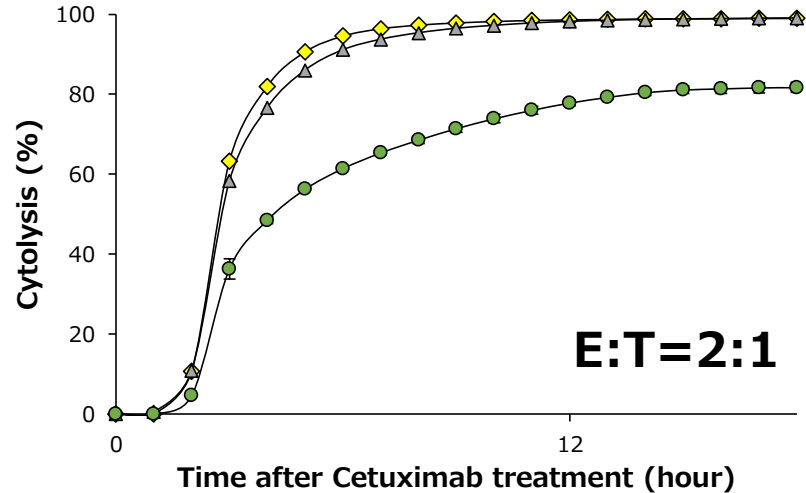
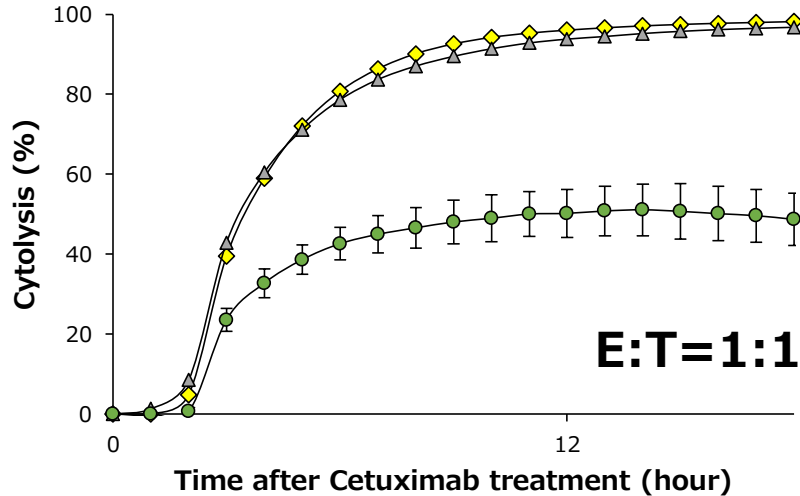
**24 h**

**End of measurement**

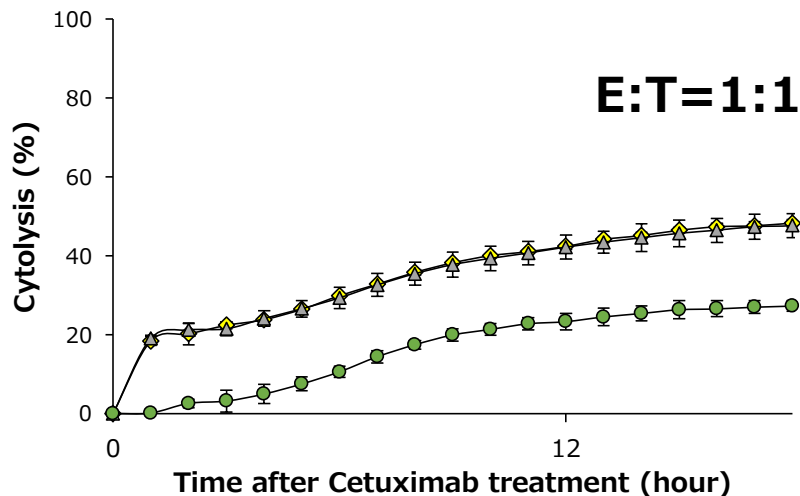
# Evaluation of ADCC using lung F-PDO

## Cetuximab: anti-EGFR antibody

RLUN007: EGFR and HER2 overexpression



## RLUN021: EGFR overexpression



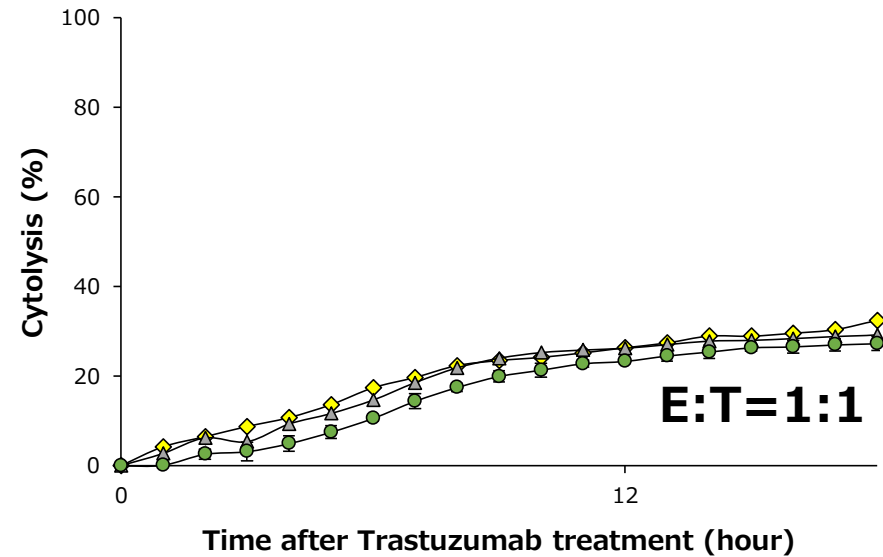
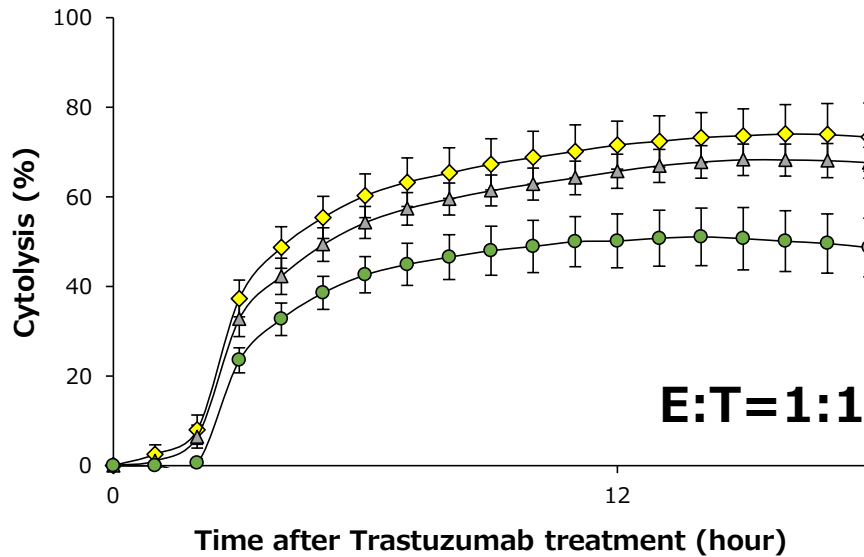
- ◆ 10 µg/mL Cetuximab + NK cells
- ▲ 1 µg/mL Cetuximab + NK cells
- F-PDO + NK cells

# Evaluation of ADCC using lung F-PDO

## Trastuzumab : Anti-HER2 antibody

RLUN007 (high expression of HER2)

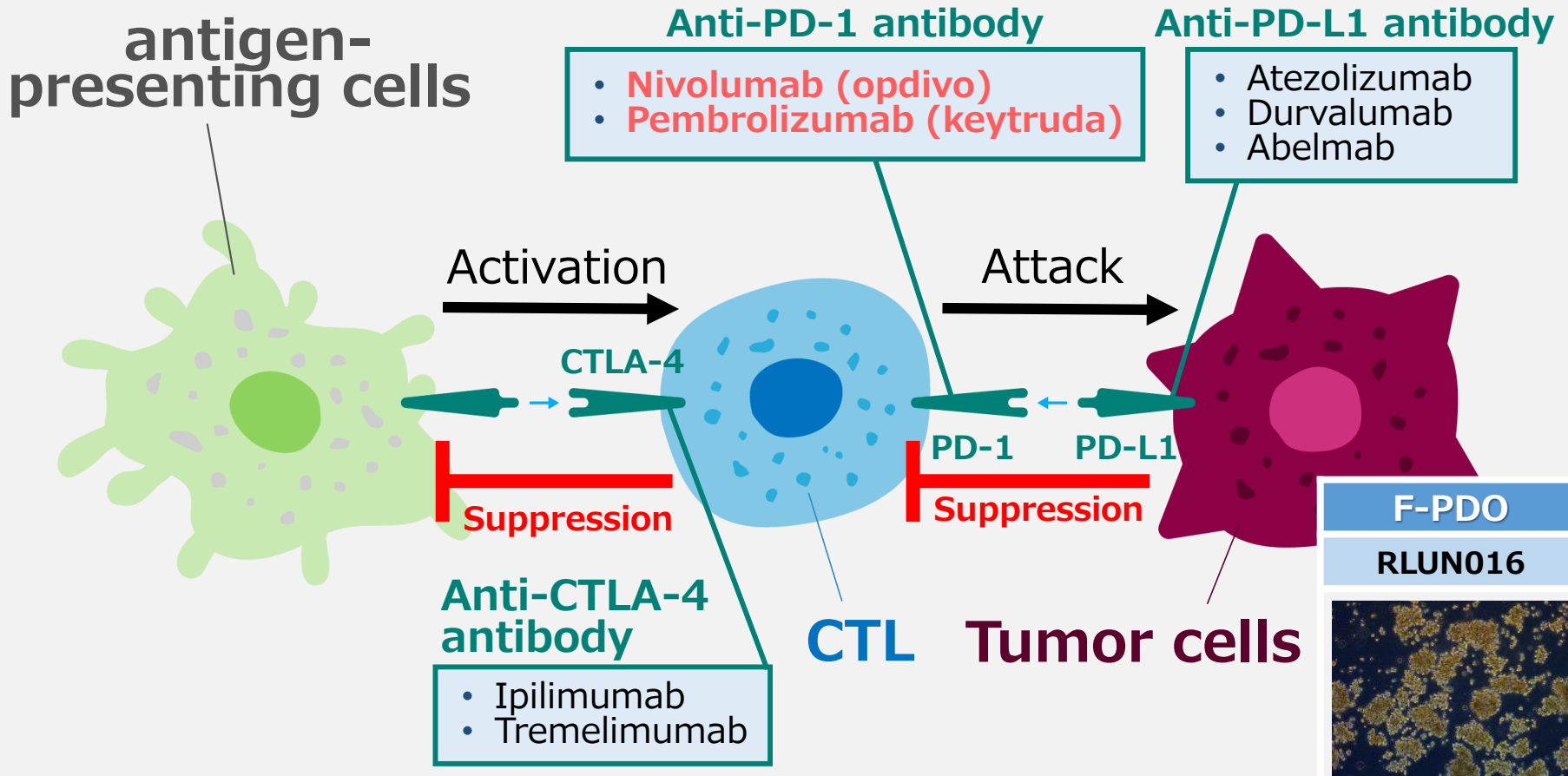
RLUN021



- ◆— 10 µg/mL Trastuzumab + NK cells
- ▲— 1 µg/mL Trastuzumab + NK cells
- F-PDO + NK cells

# Mechanism of action of immune checkpoint inhibitors

— Immune checkpoint inhibitors —

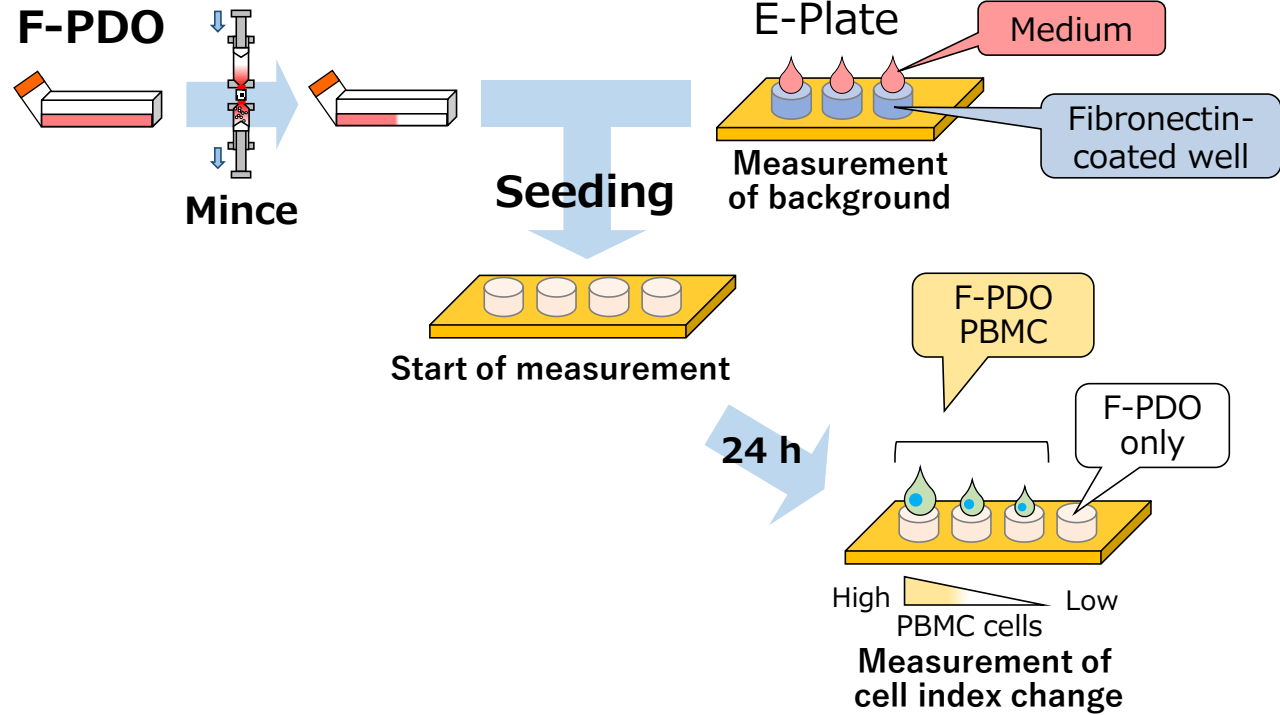
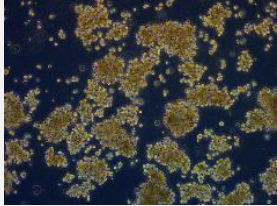


**Original immunity is restored by blocking the signals of immunosuppressive system  
(Release the brakes on the immune system)**

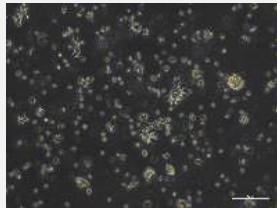
# Evaluation of immune checkpoint inhibitors using F-PDO

Lung F-PDO

RLUN016

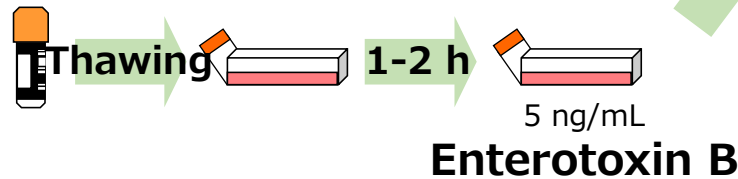


Peripheral Blood Mononuclear Cells (PBMC)



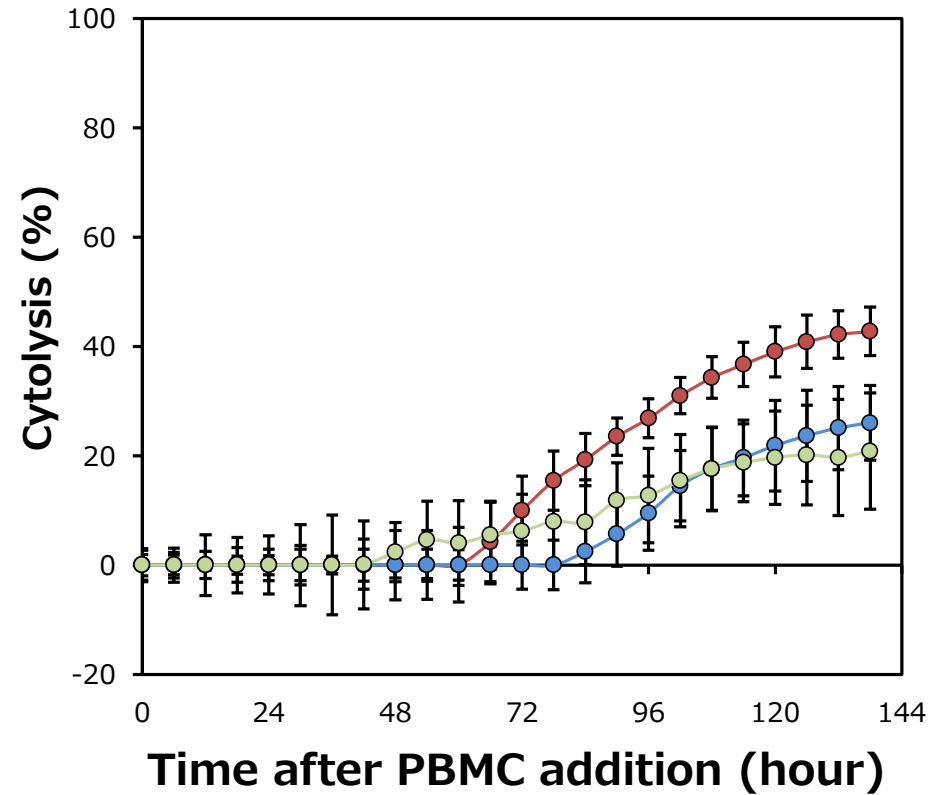
Seeding number of cells:  
1, 2.5, 5 x 10<sup>4</sup> cells/well

**PBMC**



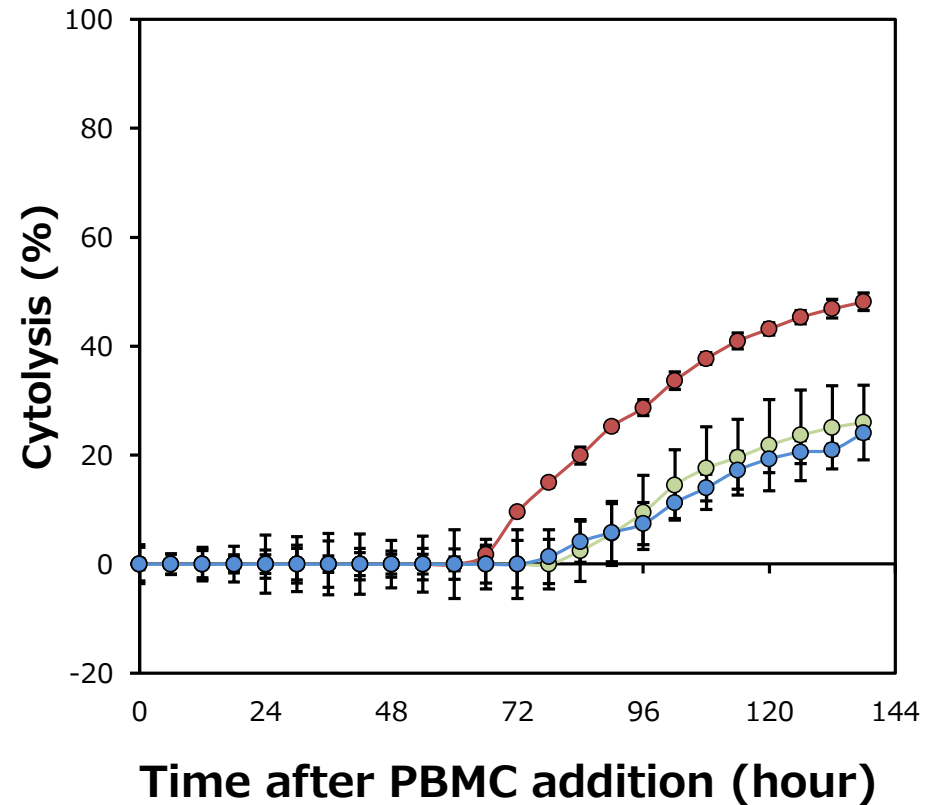
# Evaluation of immune checkpoint inhibitors using F-PDO

## Opdivo



- RLUN016 + 50 µg/mL Opdivo
- RLUN016 + 50 µg/mL Opdivo + PBMC (+SEB)
- RLUN016 + PBMC (+SEB)

## Keytruda



- RLUN016 + 1 µg/mL Keytruda
- RLUN016 + 1 µg/mL Keytruda + PBMC (+SEB)
- RLUN016 + PBMC (+SEB)

# Fukushima Translational Research Project

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