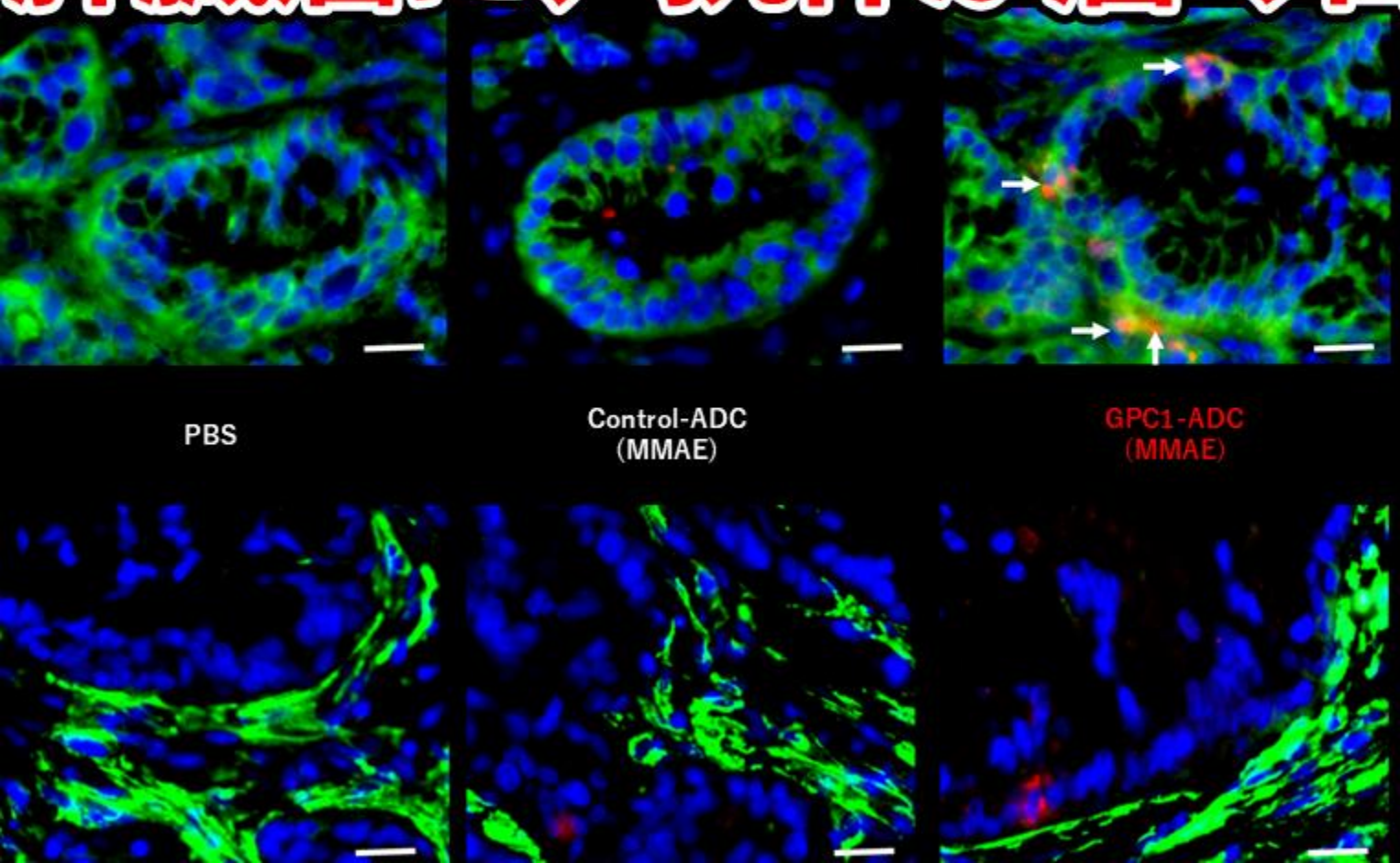
# 抗体

# GPC1-ADCによる膵臓癌治療

## 間質の壁を超える。
## 膵臓癌に、抗体が届く日へ




岩手医科大学　仲 哲治

B-062

# Development of innovative antibody-drug conjugate targeting cancer and stroma against pancreatic cancer

- Development of novel cancer therapy that overcomes the stromal barrier using GPC1-ADC -

# 癌と間質を標的とした抗体薬物複合体による膵臓癌の革新的治療法の創出を目指した研究

- GPC1-ADCを用いた間質のバリアを克服する新規癌治療法の開発 -

Iwate Medical University

岩手医科大学

Prof. Tetsuji Naka, M.D., Ph.D.

教授　仲　哲治

# Executive Summary of this Proposal

## ◇ Owned Research Assets（A）

| | Research assets |
|---|---|
| ☑ | ① Insights/findings on therapeutic target or therapeutic strategy for specific disease |
| ☑ | ② Therapeutic target, ligand for therapeutic target, development candidate (small molecule compound, nuclear acid, antibody, cell for treatment, etc.) |
| ☑ | ③ Various research tools available for verification of therapeutic target or therapeutic strategy mentioned above (patient-derived cell, assay system, screening system, technology for detection, small molecule compound, nuclear acid, antibody, etc.) |
| ☑ | ④ Biomarker or diagnosis technology usable for patient classification, etc. |
| ☑ | ⑤ Platform technology to facilitate drug research (e.g., DDS, technology for drug structure modification) |
| ☐ | ⑥ Technology for drug manufacture or process development |
| ☐ | ⑦ Others (In concrete terms: ) |

×

## ◇ Potential Alliance Scheme（B）

| | Alliance scheme |
|---|---|
| ☐ | Technical assistance |
| ☐ | Material transfer (research tool, etc.) |
| ☑ | Collaborative research |
| ☐ | Contract research |
| ☐ | Patent license |
| ☐ | Start-up establishment |

# Background to this Drug Discovery Project and/or Biotechnology (1)

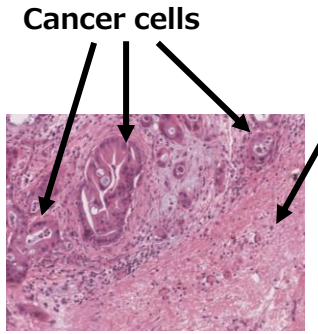
Pancreatic Cancer: An Intractable Malignancy Requiring More Effective Therapies

## ① Challenges in developing therapies for intractable cancers such as pancreatic cancer

・In pancreatic cancer, the stroma constitutes nearly 80% of the tumor tissue, which hinders the penetration of anticancer drugs into cancer cells.

(Changes in the tumor microenvironment)

One of the Major Causes of Anticancer Drug Resistance




**Fibroblasts**
**Activated pancreatic stellate cells**
**Immune cells**
**Inflammatory cells**
**extracellular matrix, etc.**

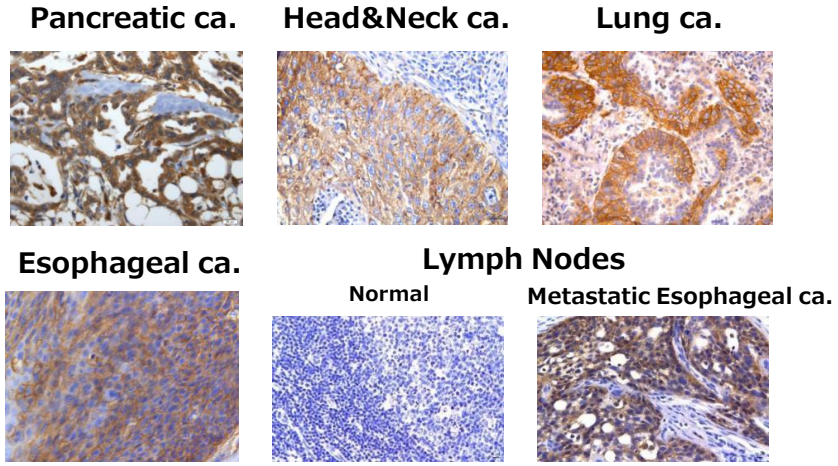
## ② Challenges in antibody–drug conjugate (ADC) development

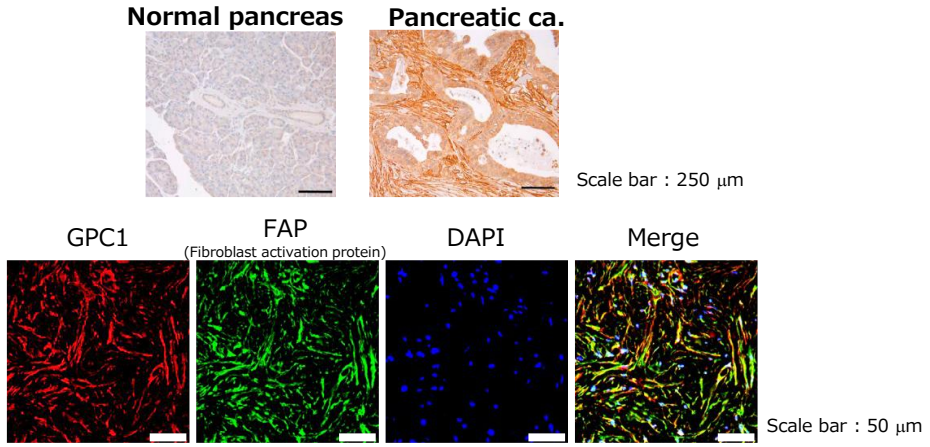
・Currently, ADCs targeting solid tumors are limited to a few antigens such as HER2. In addition, ADCs have not been developed for pancreatic cancer and are mainly applied to cancers such as breast cancer, which have a relatively small amount of stroma within the tumor.

To address these two issues, we aimed to develop an innovative antibody–drug conjugate (ADC) therapy targeting a novel cancer antigen capable of overcoming the stromal barrier in intractable cancers, including pancreatic cancer.

# Background to this Drug Discovery Project and/or Biotechnology (2)

## Glypican-1 (GPC1) as a Novel Cancer Antigen




More than 80% of pancreatic cancer tissue consists of stroma. Cancer-associated fibroblasts (CAFs) within the stromal region express GPC1, whereas GPC1 expression is not observed in normal fibroblasts.

- Through proteomic analyses, we identified Glypican-1 (GPC1) as a novel cancer antigen that is highly expressed in various intractable cancers, including pancreatic, esophageal squamous, head and neck, and lung cancers. (Hara, Naka et al., BrJC. 2016, PCT/JP2014/006455)
- High GPC1 expression is associated with poor prognosis in esophageal squamous cell carcinoma, pancreatic cancer, cholangiocarcinoma, and glioblastoma. (Hara, Naka *et al.*, BrJC. 2016, Nishigaki, Naka *et al.*, BrJC. 2020, Yokota, Naka *et al.*, MCT. 2021, Saito *et al.*, World Neurosurg. 2017)
- GPC1 is expressed in both cancer cells and cancer-associated fibroblasts (CAFs) in pancreatic cancer. (Tsujii, Naka *et al.*, MCT. 2021)
- GPC1 acts as a co-receptor for heparin-binding growth factors. (Matsuda *et al.*, Cancer Res. 2001, Harada, Naka et al., Oncotarget. 2017)
- Expression of GPC1 is induced by mutations in the KRAS gene and other oncogenic alterations. (Tanaka *et al.*, Oncotarget. 2017)

# Background to this Drug Discovery Project and/or Biotechnology (3)

Expression of GPC1 in Pancreatic Cancer and Esophageal Squamous Cell Carcinoma

***Pancreatic cancer***

- GPC1 is highly expressed in 33 (44%) of 75 cases.
  - The immunohistochemical score is determined by multiplying the staining intensity by the proportion of the positive area, resulting in scores ranging from 0 to 4. Cases with scores of 2 or higher are classified as the high-expression group.
  - Only two cases are negative for GPC1 expression.

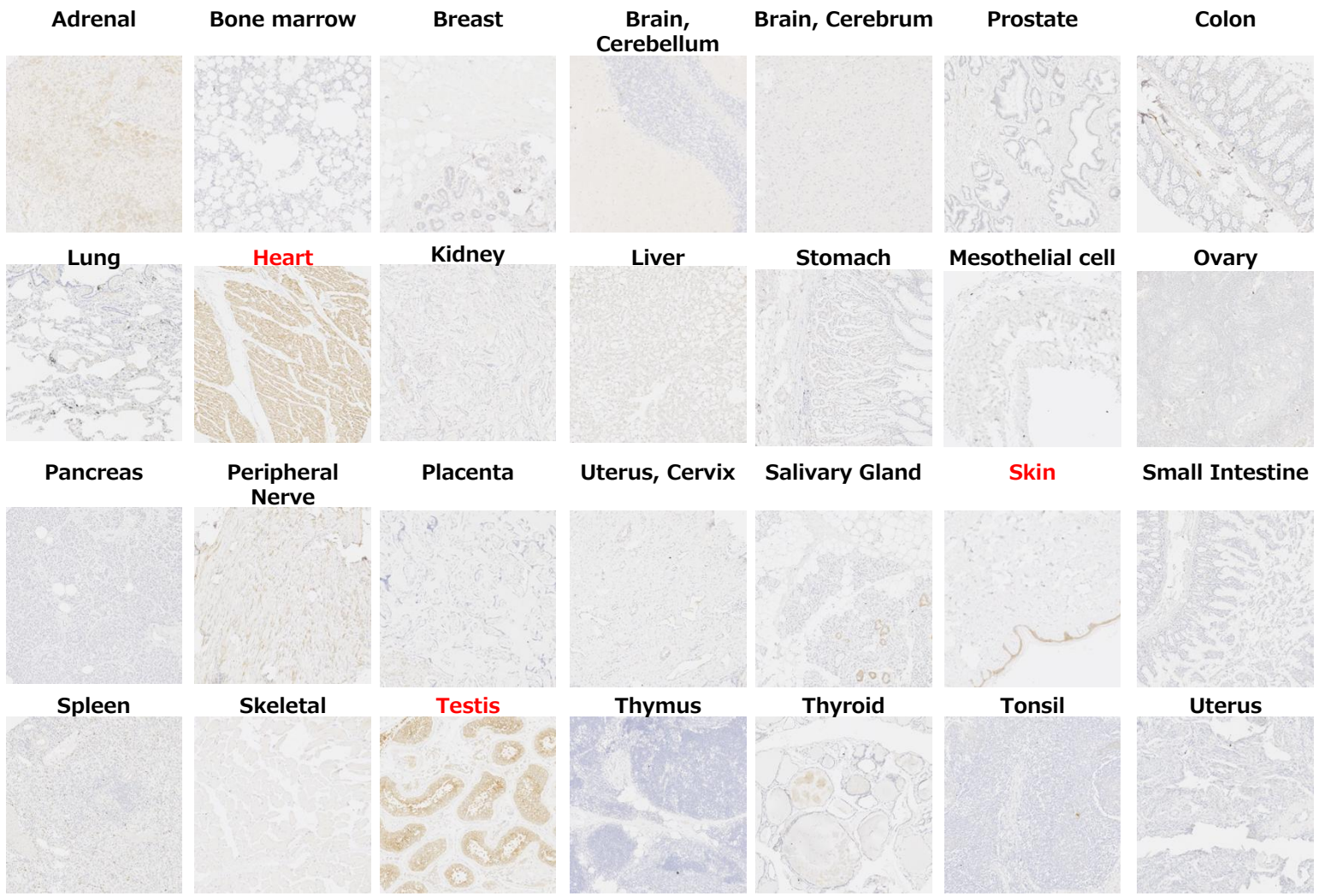
***Esophageal squamous cell carcinoma***

- GPC1 is highly expressed in 76 (43.4%) of 175 patients.
  - The immunohistochemical score is determined by multiplying the staining intensity by the positive area, yielding scores ranging from 0 to 6. Cases with scores of 4 or higher are classified as the high-expression group.
  - Of the 175 cases, only two are negative for GPC1 expression, while the remaining 173 show weakly or strongly positive staining.

Interestingly, the rate of high GPC1 expression in esophageal cancer and pancreatic cancer is approximately 44%, but the expression intensity tends to be higher in esophageal cancer than in pancreatic cancer.

# Background to this Drug Discovery Project and/or Biotechnology (4)

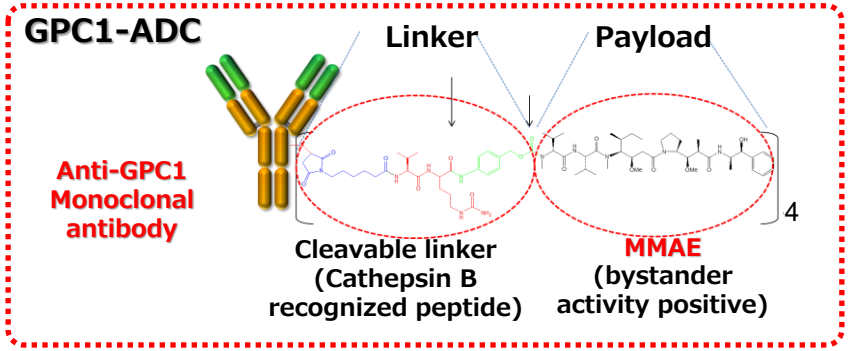
Limited Expression of GPC1 in the Testis, Skin, and Heart



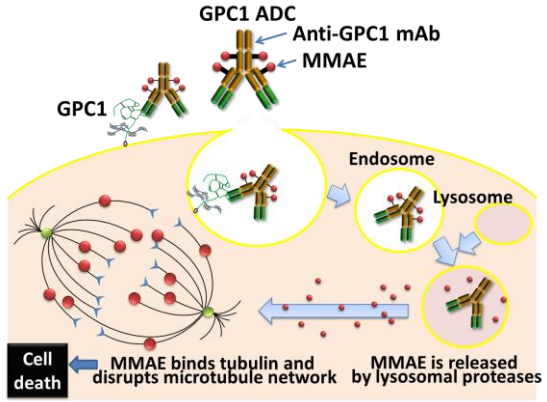

**Normal human tissue microarray (FFPE)** FDA Standard Tissue Array, Biochain, catalog no：T8234701-1, Lot C806075
1st Ab: mouse anti-human GPC1 monoclonal Ab clone PPY7462

# Background to this Drug Discovery Project and/or Biotechnology (5)

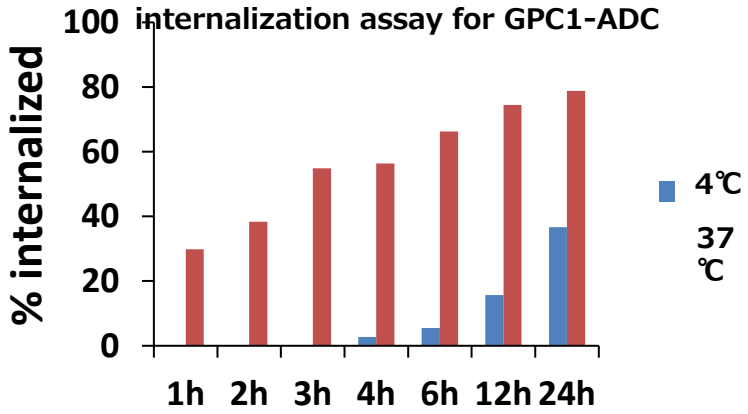
## GPC1 as a Suitable ADC Target Due to Rapid Internalization into Lysosomes



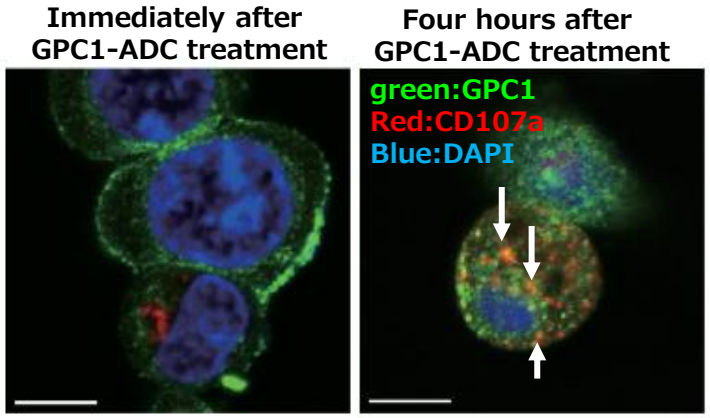

MMAE was selected as the payload because it selectively inhibits proliferating cells, such as cancer cells, but not resting cells, including normal tissues.




Using ADC technology, a chemotherapeutic agent can be selectively delivered to GPC1-positive cancer cells.



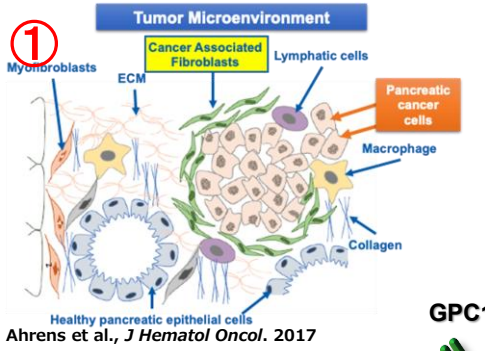

GPC1-ADC is rapidly internalized into GPC1-positive cells.



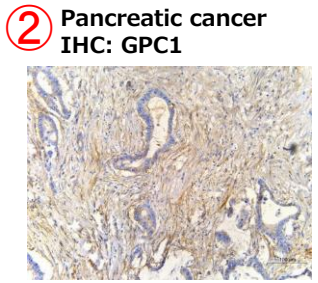

Internalized GPC1-ADC localizes to lysosomes.

# Background to this Drug Discovery Project and/or Biotechnology (6)

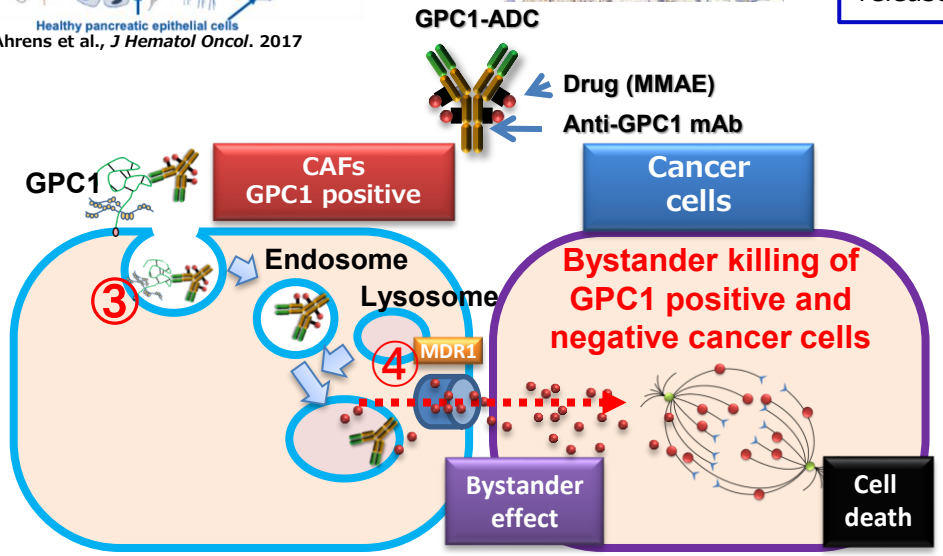
Targeting GPC1-Expressing CAFs with ADCs:
A Promising Strategy for Treating Stroma-Rich Solid Tumors

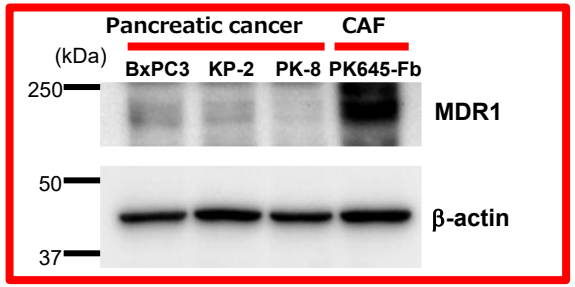


Ahrens et al., *J Hematol Oncol*. 2017




(1) CAFs surround pancreatic cancer cells.
(2) GPC1 is highly expressed in CAFs, which are abundantly present in the tumor stroma.
(3) GPC1-ADC is expected to be internalized into CAFs.
(4) MDR1 is highly expressed in CAFs, enabling the effective release of MMAE from CAFs without causing their damage. The released MMAE is thought to kill adjacent cancer cells.




MMAE is recognized as a substrate for MDR1.

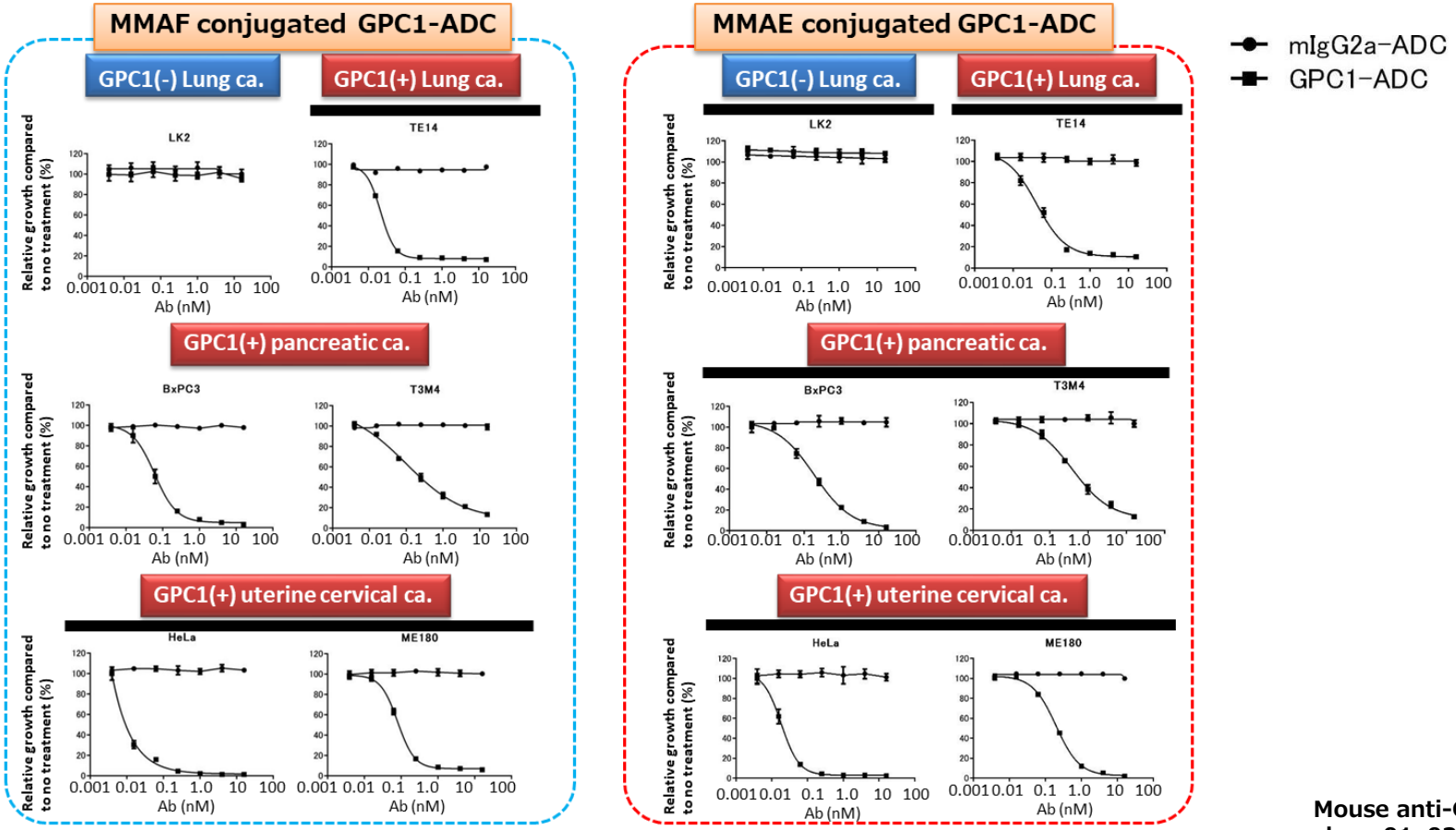
◎ The aim of this study is to address the following points:
- To develop a novel ADC targeting GPC1 for solid tumors that can overcome the stromal barrier.
- To demonstrate the preclinical efficacy of GPC1-ADC.
- To explore the clinical applicability of GPC1-ADC for cancer therapy.

# Summary of this Drug Discovery Project and/or Biotechnology (1)

## GPC1-ADCs Show Potent Cytotoxicity in GPC1⁺ Cancer Cells

**Payload characteristics**

- MMAF is known to have low membrane permeability, whereas MMAE has high membrane permeability.
- It has been reported that, compared to MMAF, MMAE exhibits bystander killing activity.




Two types of GPC1-ADCs were generated, and both effectively inhibited cell growth *in vitro*.

**Mouse anti-GPC1 monoclonal antibody clone 01a033 was used for ADC.**

Matsuzaki, **Naka**. *et al.*, ***IJC*** 2018
Nishigaki, **Naka**. *et al.*, ***BrJC***, 2020

# Summary of this Drug Discovery Project and/or Biotechnology (2)

## GPC1-ADC (MMAF) Shows a Potent Antitumor Effect *In Vivo*

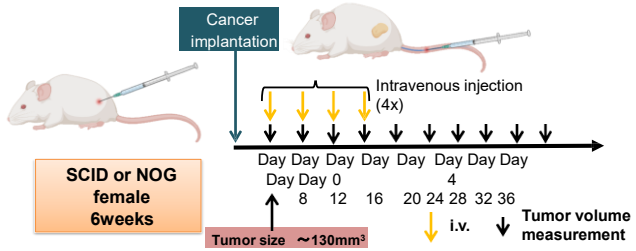
**The following cancer cell lines or tissues were subcutaneously implanted in mice to compare the antitumor effects of GPC1-ADC (MMAF) with those of control-ADC or PBS:**

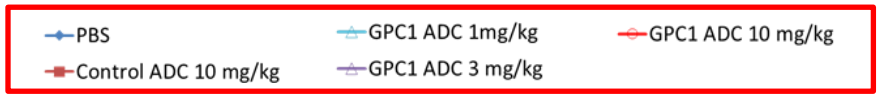
**(i) BxPC3 pancreatic cancer cells**

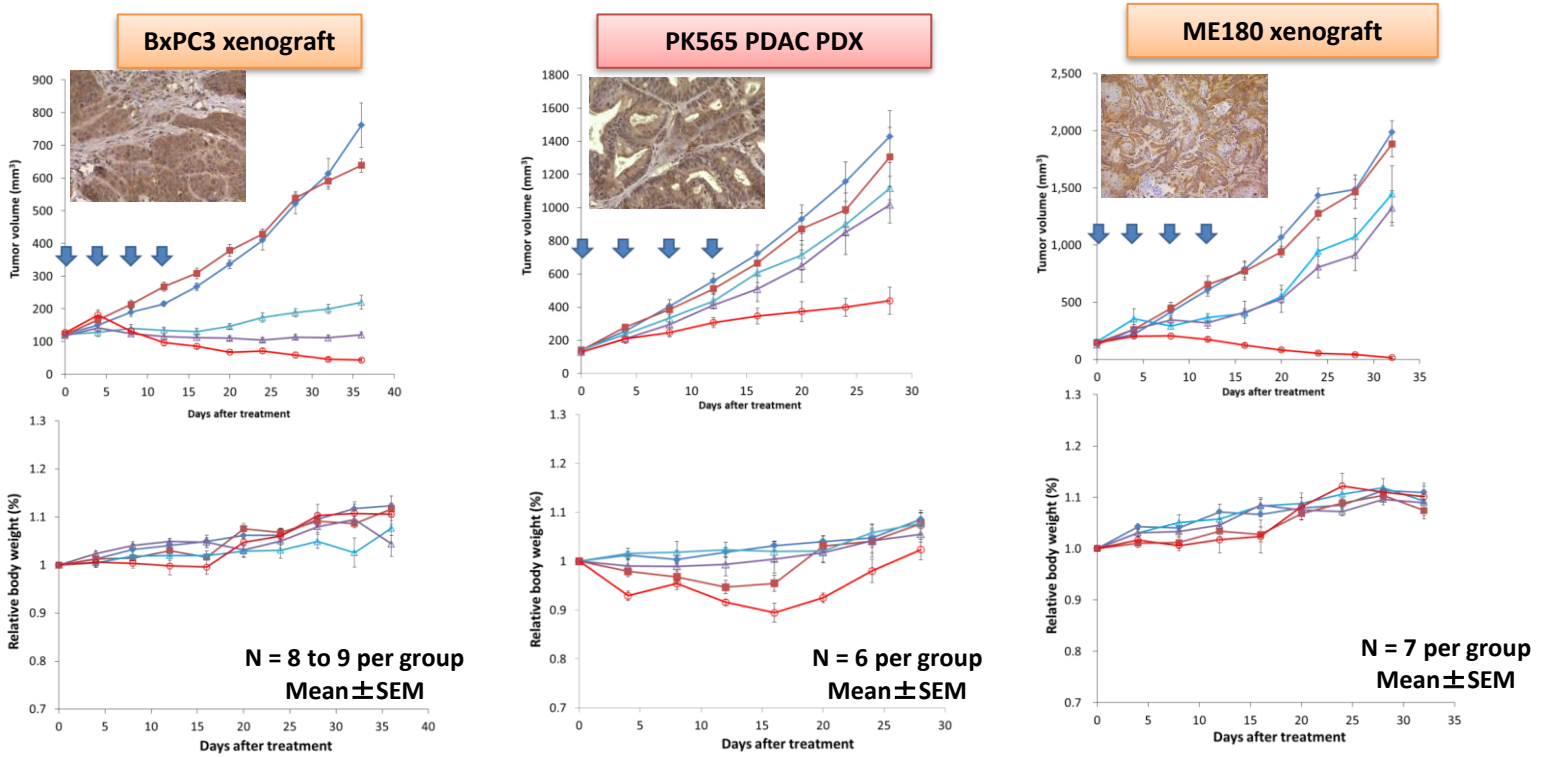
**(ii)Resected tumor tissue from patients with pancreatic cancer**

**(iii) ME180 cervical cancer cells**

| Treatment: | PBS | Control ADC (10mg/kg) | GPC1- ADC (1mg/kg) | GPC1 -ADC (3mg/kg) | GPC1- ADC (10mg/kg) |
|---|---|---|---|---|---|



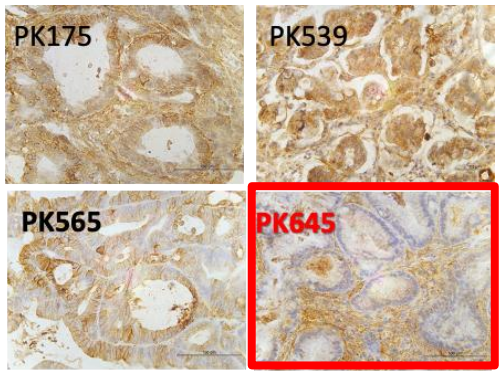

**Mouse anti-GPC1 monoclonal antibody clone 01a033 was used for ADC.**

Matsuzaki, **Naka**. *et al.*, ***IJC*** 2018
Nishigaki, **Naka**. *et al.*, ***BrJC***, 2020

# Summary of this Drug Discovery Project and/or Biotechnology (3)

GPC1-ADC (MMAE), but not GPC1-ADC (MMAF), Exhibits Antitumor Activity Against PK645 Tumors *In Vivo*

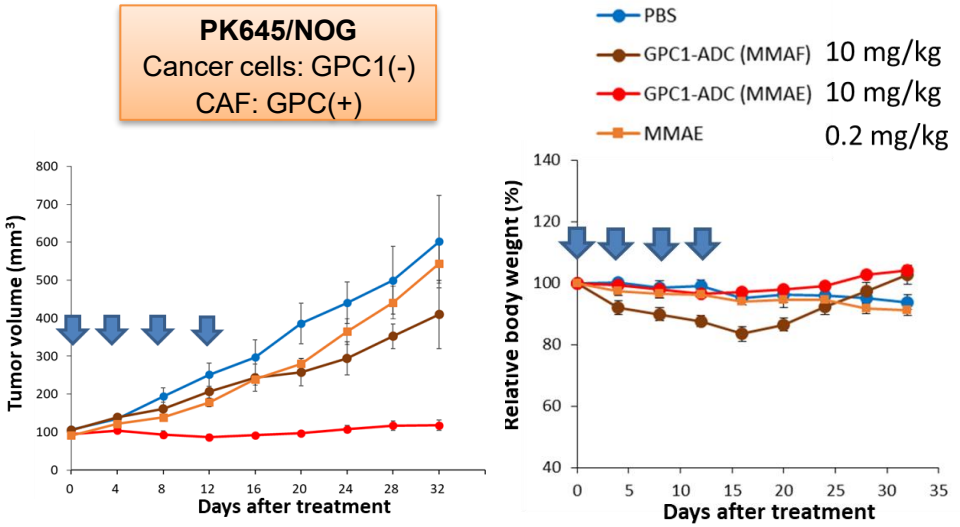
**Screening for GPC1(-) cancer cells, GPC1(+) CAFs PDX tissues.**




| PDX | GPC1 in tumor | GPC1 in CAF |
|---|---|---|
| PK175 | + | ++ |
| PK539 | + | ++ |
| PK565 | + (heterogenous) | Poor Stroma |
| PK645 | − | ++ |
| KPK1 | + | Poor stroma |

Patient-derived xenograft (PDX) models are more suitable than conventional xenograft models for evaluating CAF-mediated bystander effects of GPC1-ADC, since tumor tissues in PDX mice contain both cancer cells and stromal cells derived from human cancer patients. PK645 PDAC PDX was selected and used to demonstrate the importance of GPC1-positive CAFs in the mode of action of GPC1-ADC (MMAE).

**PK645/NOG**
Cancer cells: GPC1(-)
CAF: GPC(+)




・Only GPC1-ADC (MMAE), which possesses bystander effects, showed antitumor efficacy on PK645 tumor tissues.

・The data strongly suggest that GPC1-ADC (MMAE) treatment holds significant potential, as the MMAE released from GPC1-positive CAFs exerts potent cytotoxic effects on cancer cells adjacent to the stroma.

・ GPC1-ADC (MMAE) did not affect mouse body weight.

・MMAE alone, at a dose equivalent to that potentially released by unintended complete cleavage of GPC1-ADC in the blood, did not affect tumor growth, highlighting the importance of CAF-mediated cleavage of GPC1-ADC.

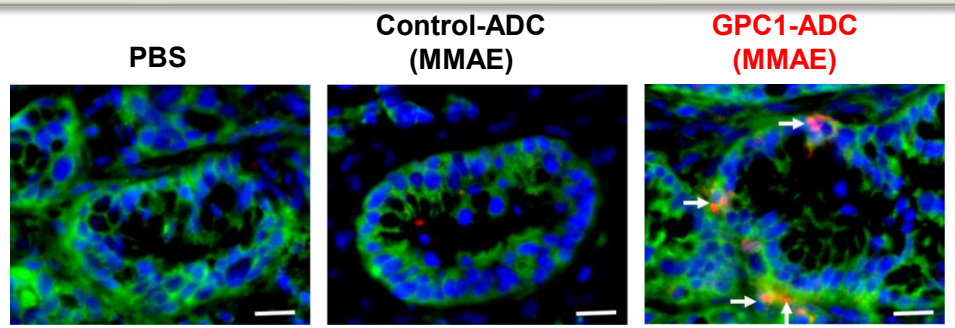
**Mouse anti-GPC1 monoclonal antibody clone 01a033 was used for ADC.**

# Summary of this Drug Discovery Project and/or Biotechnology (4)

## GPC1-ADC (MMAE) Induces Apoptosis in GPC1-Negative Pancreatic Cancer Cells *via* GPC1-Positive CAFs

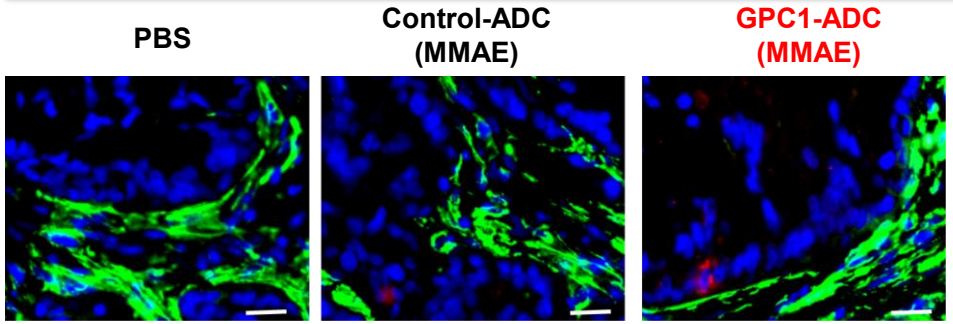
**PK645/NOG**
Cancer cells: GPC1(-)
CAFs: GPC(+)

**GPC1-ADC (MMAE) effectively induces apoptosis in pancreatic cancer cells**




Green: EpCAM, Red: cleaved caspase-3, Blue: DAPI

**GPC1-ADC(MMAE) does not induce apoptosis in CAFs**




Green: α-SMA, Red: cleaved caspase-3, Blue: DAPI

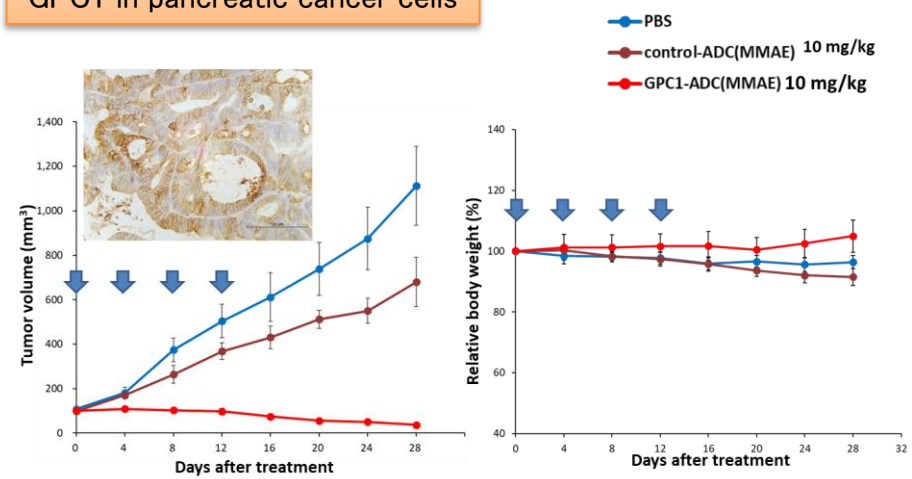
**Mouse anti-GPC1 monoclonal antibody clone 01a033 was used for ADC.**

Apoptosis was detected in GPC1-negative pancreatic cancer cells, but not in CAFs, following treatment with GPC1-ADC (MMAE) in PK645 tumors *in vivo*, suggesting that GPC1-positive CAFs play an important role in the mode of action of GPC1-ADC (MMAE).

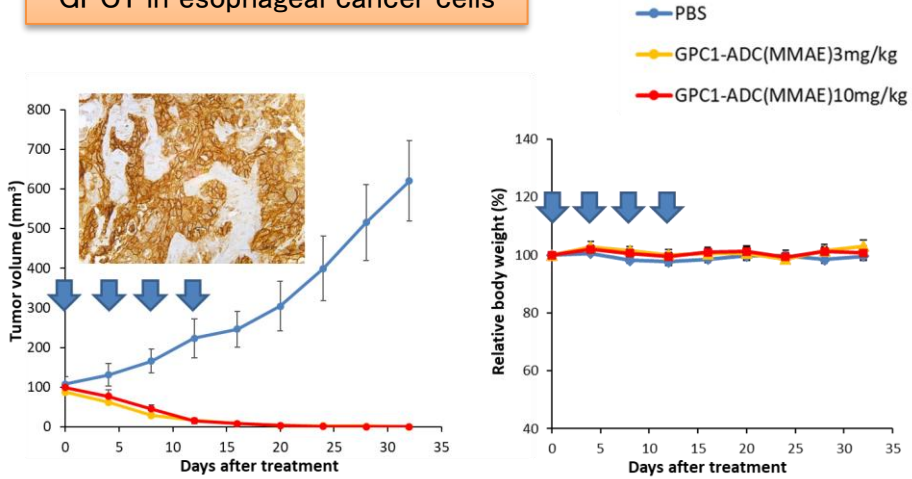
# Summary of this Drug Discovery Project and/or Biotechnology (5)

## GPC1-ADC (MMAE) Exhibits Strong Antitumor Activity In Vivo

**PK565/NOG**
Heterogenous expression of GPC1 in pancreatic cancer cells




**ESCC14/NOG**
Strong, homogenous expression of GPC1 in esophageal cancer cells




・MMAE was selected as the payload for GPC1-ADC.
・GPC1-ADC (MMAE) exhibits potent direct antitumor activity against GPC1-positive cancer cells.
・GPC1-ADC (MMAE) also exerts indirect antitumor activity through MMAE released from GPC1-positive CAFs, as demonstrated by PK645 PDX data.

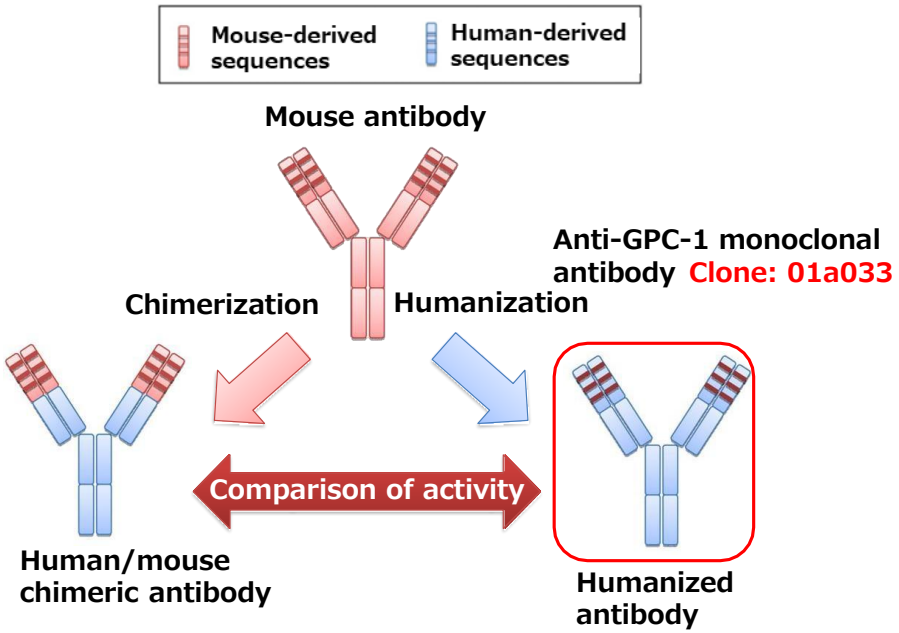
This mode of action of GPC1-ADC (MMAE) represents a promising therapeutic approach for stroma-rich solid tumors.

**Mouse anti-GPC1 monoclonal antibody clone 01a033 was used for ADC.**

Tsujii & **Naka**., et al ***Mol Cancer Ther*** 2021

# Summary of this Drug Discovery Project and/or Biotechnology (6)

## Humanization of a Mouse Anti-Human GPC1 Antibody




http://www.nihs.go.jp/dbcb/mabs.html

### Surface plasmon resonance (SPR) analysis of humanized anti-GPC1 antibodies

| Ligand | ka (1/Ms) | kd (1/s) | Rmax (RU) | KD | Chi² |
|---|---|---|---|---|---|
| Logic-chimeric-mouse Ab | 3.02E+05 | 3.09E-03 | 74.6 | 1.02E-08 | 4.89E-01 |
| Logic-T1 | 2.38E+05 | 1.34E-03 | 58.9 | **5.62E-09** | 2.57E-01 |
| Logic-T2 | 2.40E+05 | 1.25E-03 | 60.3 | **5.20E-09** | 3.34E-01 |
| Logic-T3 | 2.29E+05 | 7.13E-04 | 62.9 | **3.11E-09** | 3.10E-01 |
| Logic-T7 | 3.43E+05 | 4.11E-03 | 64.5 | 1.20E-08 | 5.89E-01 |
| Logic-T8 | 2.34E+05 | 1.18E-03 | 62 | **5.07E-09** | 3.31E-01 |
| Logic-T10 | 1.99E+05 | 1.50E-03 | 56.7 | **7.55E-09** | 2.78E-01 |
| Logic-T11 | 1.86E+05 | 5.06E-02 | 79.6 | 2.72E-07 | 5.46E-01 |
| Logic-T22 | 2.32E+05 | 5.06E-02 | 73.4 | 2.18E-07 | 9.04E-02 |
| Logic-T26 | 1.78E+03 | 1.30E-01 | 13606.9 | 7.28E-05 | 3.98E-01 |
| Logic-T28 | 3.13E+05 | 6.79E-03 | 59 | 2.17E-08 | 4.44E-01 |
| Logic-T29 | 2.05E+05 | 6.64E-02 | 78.4 | 3.24E-07 | 8.95E-02 |
| Logic-T31 | 1.98E+05 | 5.39E-02 | 65.4 | 2.71E-07 | 2.37E-01 |
| Logic-T32 | 2.08E+05 | 6.03E-02 | 81.2 | 2.90E-07 | 6.64E-02 |
| Logic-T33 | 1.60E+05 | 7.38E-02 | 70.4 | 4.62E-07 | 4.14E-02 |
| Logic-T34 | 1.72E+05 | 3.93E-03 | 59.2 | 2.28E-08 | 4.28E-01 |
| Logic-T36 | 2.31E+05 | 2.27E-03 | 60.3 | **9.80E-09** | 1.84E-01 |
| Logic-T43 | 1.69E+05 | 1.06E-02 | 62.4 | 6.24E-08 | 1.99E-01 |
| Logic-T56 | 2.59E+05 | 1.80E-03 | 46.6 | **6.93E-09** | 3.65E-01 |
| Logic-T57 | 1.32E+05 | 1.35E-02 | 97 | 1.02E-07 | 1.36E-01 |
| Logic-T59 | 1.97E+05 | 5.93E-02 | 96.9 | 3.02E-07 | 9.81E-02 |
| Anti-GPC1 mAb 01a033 | 1.23E+05 | 3.88E-023 | 65.7 | 3.16E-08 | 1.15E+00 |

・Problems such as decreased antigen affinity can occur during the process of antibody humanization.

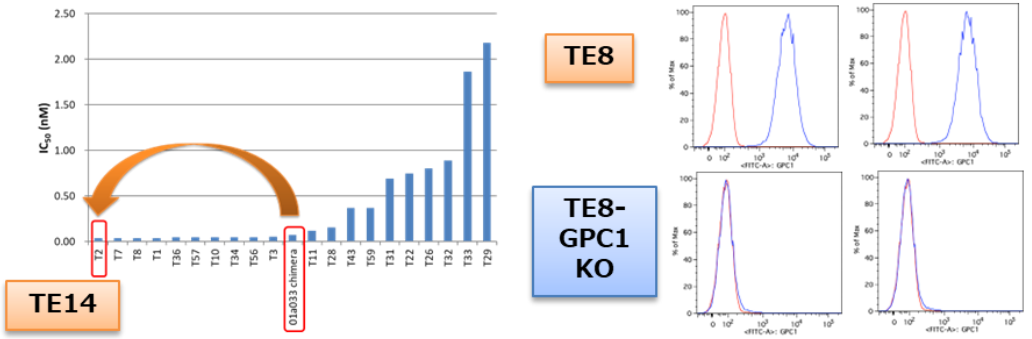
・For 20 humanized antibody candidate clones that were generated, their activity was compared with that of a human–mouse chimeric antibody as a control, and a humanized antibody clone with superior performance was selected.

Munekage, **Naka**. *et al.*, *Neoplasia*. 2021

# Summary of this Drug Discovery Project and/or Biotechnology (7)

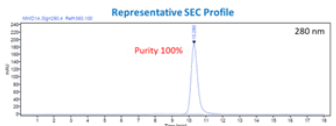
## Generation of Humanized GPC1-ADC through Antibody Humanization and Optimized Linker Conjugation

**Evaluation of antibody internalization activity based on intracellular uptake of an antibody–drug conjugate.**

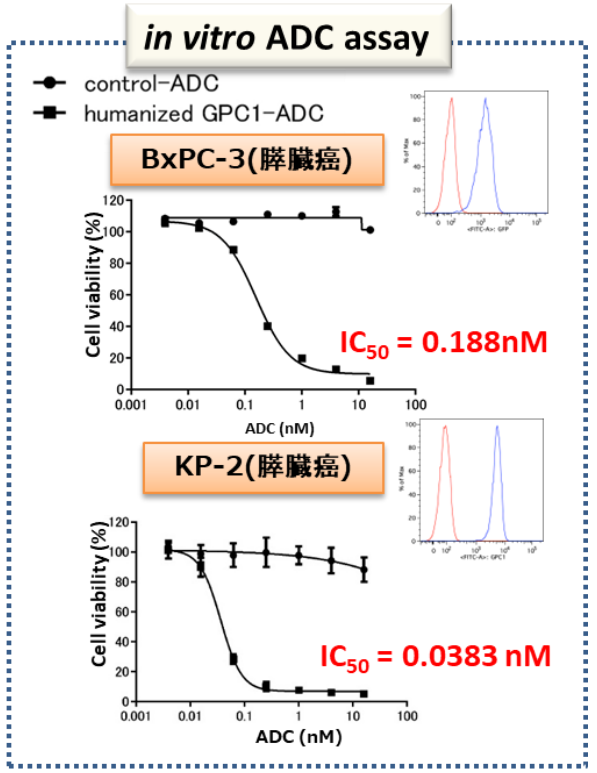



**The intramolecular disulfide bonds of the humanized anti-GPC1 antibody were reduced and conjugated with mc-vc-PAB-MMAE. The optimal manufacturing conditions for the ADC were established, yielding an average drug-to-antibody ratio (DAR) of 4.**


| Conjugation Condition | DAR | Drug Distribution | | | | | Purity by SEC | | |
|---|---|---|---|---|---|---|---|---|---|
| | | D0 (%) | D2 (%) | D4 (%) | D6 (%) | D8 (%) | HMWS (%) | Monomer (%) | LMWS (%) |
| **Protocol 1** | **4.08** | **5.58** | **16.04** | **61.21** | **3.37** | **13.81** | **ND** | **100** | **ND** |
| Protocol 2 | 2.90 | 19.61 | 24.95 | 49.65 | 2.50 | 3.29 | ND | 100 | ND |
| Protocol 3 | 3.86 | 14.05 | 19.64 | 44.66 | 2.68 | 18.98 | ND | 100 | ND |
| Protocol 4 | 4.56 | 8.21 | 14.30 | 46.64 | 3.05 | 27.80 | ND | 100 | ND |
| mAb | NA | NA | NA | NA | NA | NA | ND | 100 | ND |


**A high ratio of DAR4 with excellent efficacy and blood stability** **High quality because no aggregates are observed**



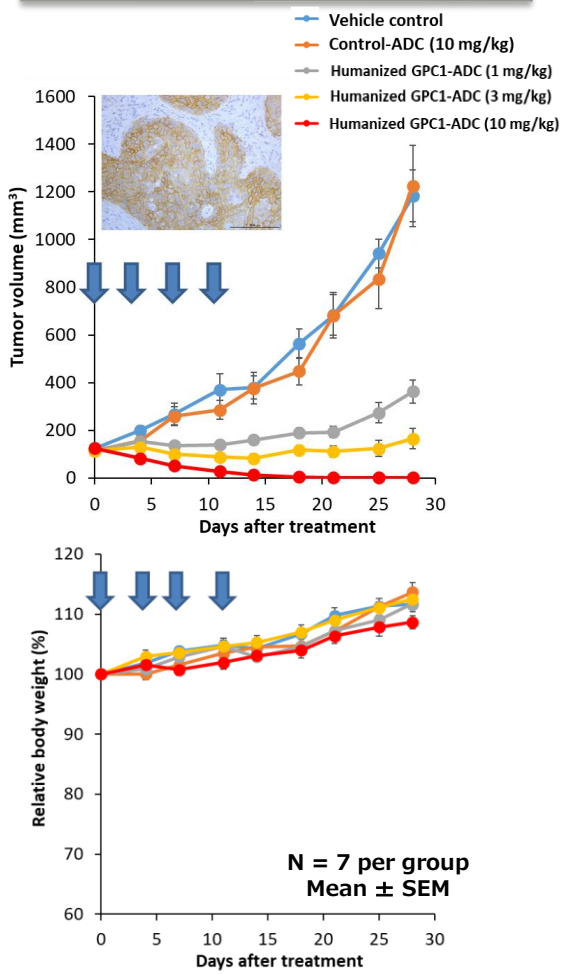

- **Upon humanization of the anti-GPC1 monoclonal antibody, 20 humanized clones were obtained.**
- **Among them, ten humanized anti-GPC1 antibody clones exhibited higher activity than the chimeric (pre-humanized) antibody, with clone T2 showing the highest activity.**
- **Clone T2 demonstrated approximately twofold greater intracellular internalization activity compared to the chimeric antibody and achieved rapid intracellular uptake.**

Munekage, **Naka**. *et al.*, *Neoplasia*. 2021

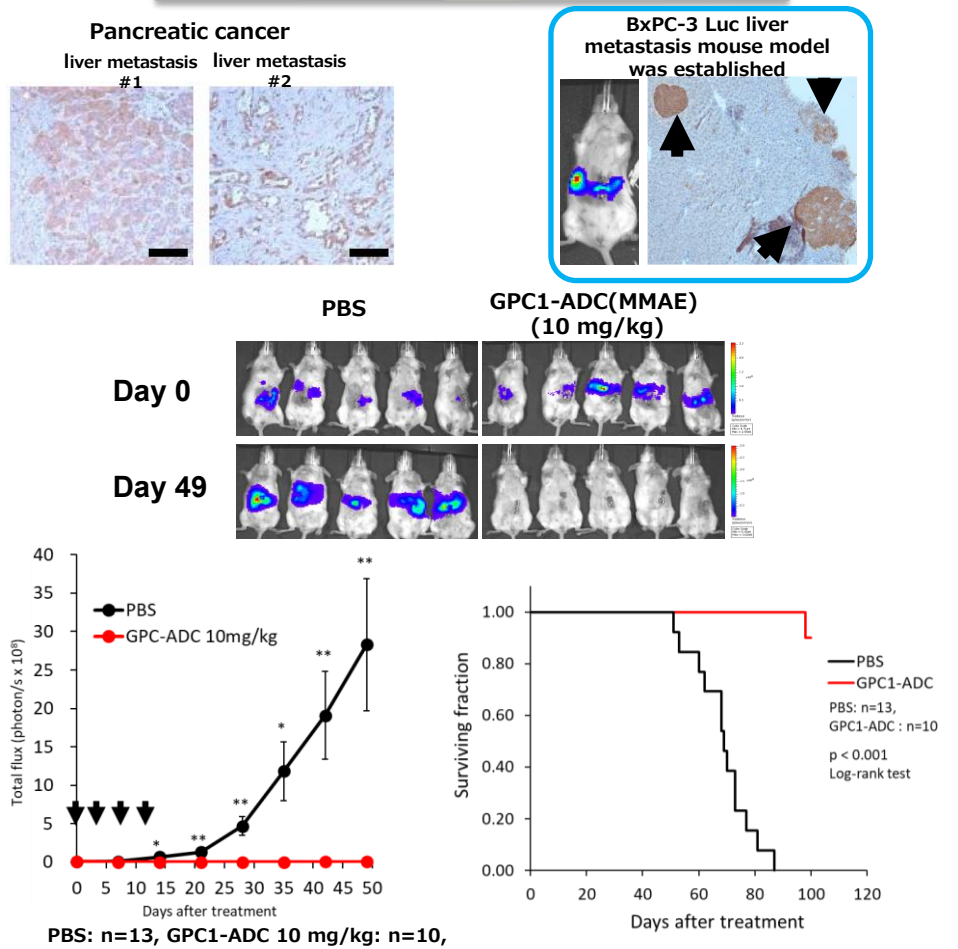
# Summary of this Drug Discovery Project and/or Biotechnology (8)

## Potent Antitumor Activity of Humanized GPC1-ADC (MMAE) in GPC1-Expressing Cancer Cells

### BxPC-3 (pancreatic cancer) subcutaneous implantation model




### BxPC-3 Pancreatic cancer liver metastasis model



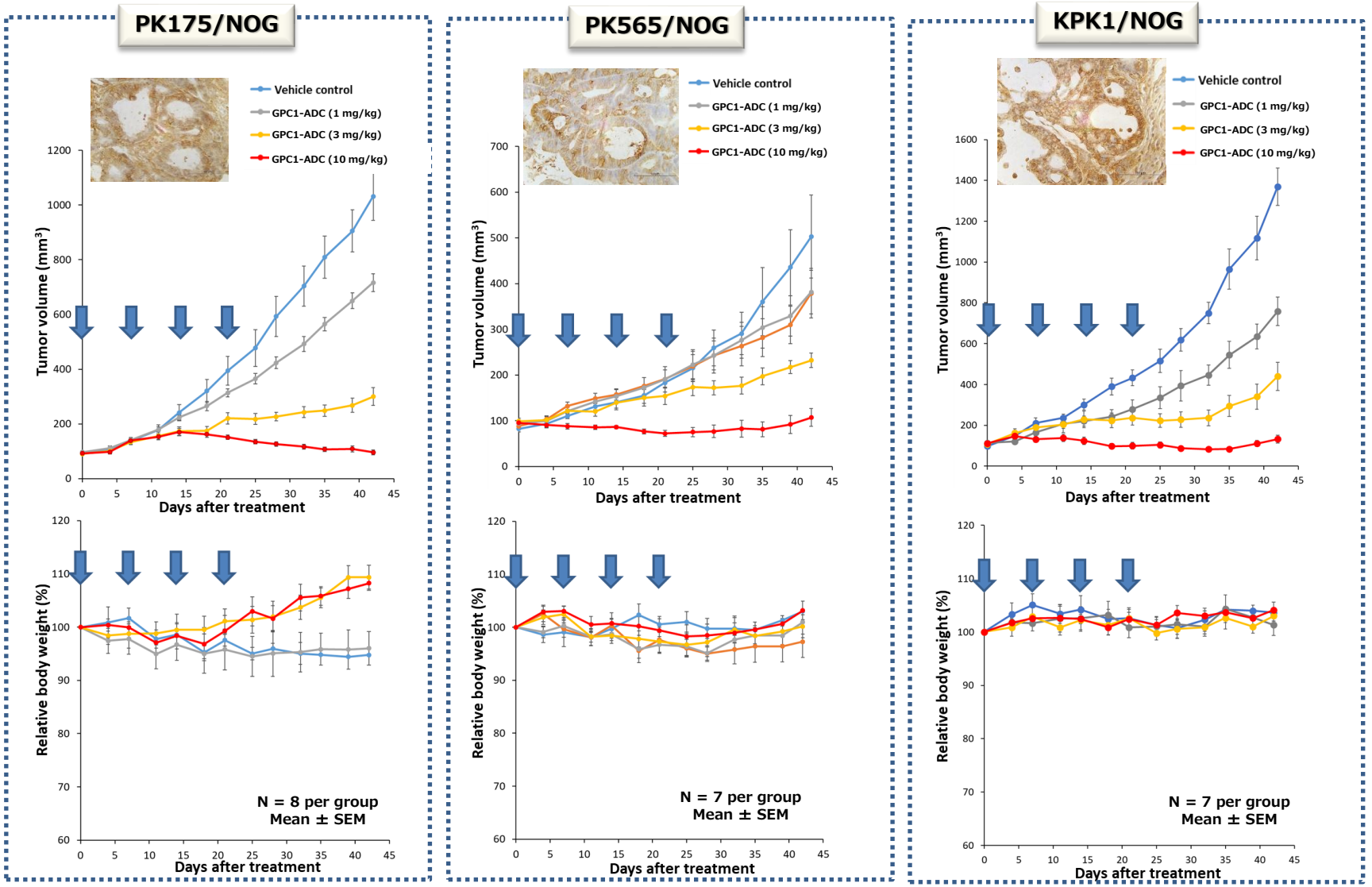

PBS: n=13, GPC1-ADC 10 mg/kg: n=10, Mean ± SEM
*: p<0.05, **: p<0.01

Humanized anti-GPC1 monoclonal antibody clone T2 was used for ADC.

Munekage, **Naka**. *et al*., *Neoplasia*. 2021

# Summary of this Drug Discovery Project and/or Biotechnology (9)

Potent Antitumor Activity of Humanized GPC1-ADC (MMAE) in **GPC1-Expressing PDAC PDX Models**

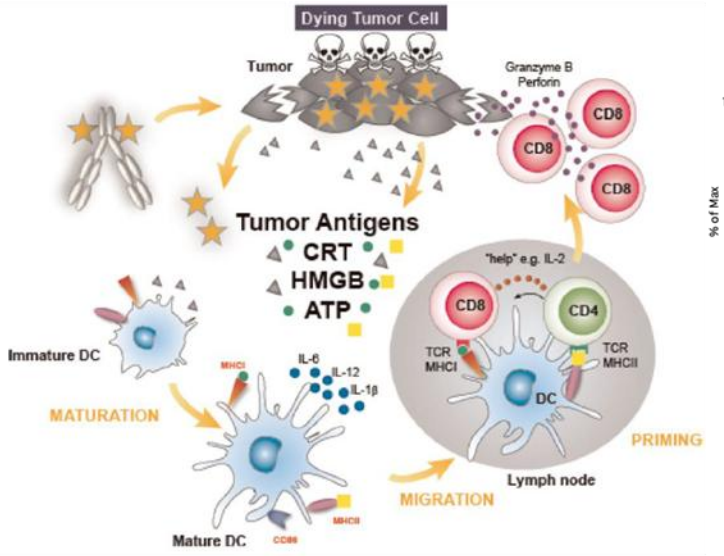



**Humanized anti-GPC1 monoclonal antibody clone T2 was used for ADC.**

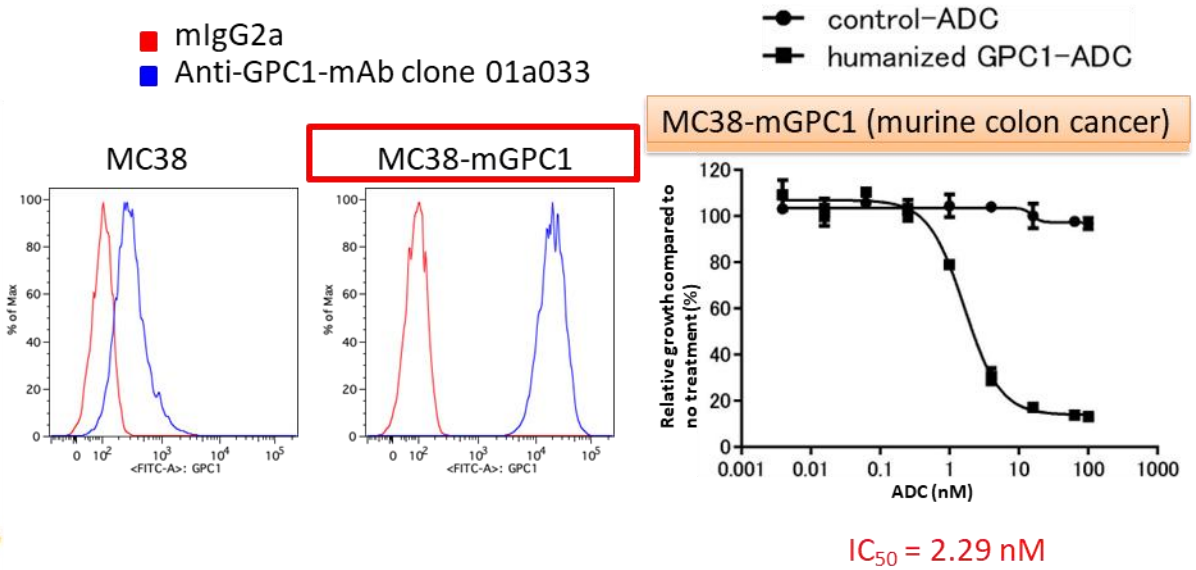
Munekage, **Naka**. *et al.*, *Neoplasia*. 2021

# Summary of this Drug Discovery Project and/or Biotechnology (10)

## Concept of Synergistic Effects Between MMAE-Based ADCs and Immune Checkpoint Inhibitors




Bauzon M et al., ***Oncoimmunology***. 2019.




| | Humanized GPC1-ADC(MMAE) (nM) | MMAE (nM) |
|---|---|---|
| MC38-mGPC1 | 2.29 | 2.16 |

・It has been reported that MMAE induces the release of damage-associated molecular patterns (DAMPs) from cancer cells, leading to the activation of immune cells.

・Therefore, GPC1-ADC employing MMAE is expected to exert additive effects when combined with immune checkpoint inhibitors.

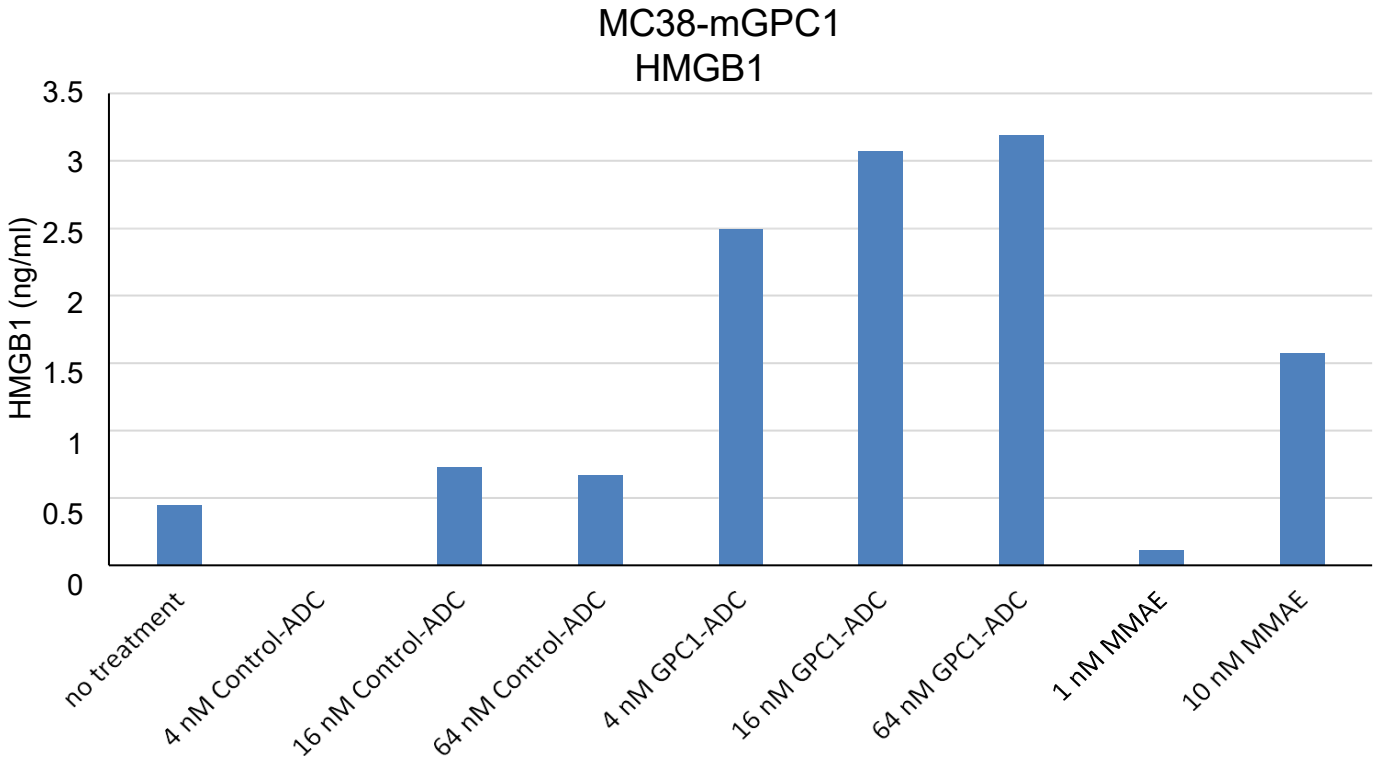
・It is crucial to evaluate the potential synergistic effect between GPC1-ADC (MMAE) and an immune checkpoint inhibitor. To this end, a syngeneic mouse model was established using MC38 cells stably overexpressing murine GPC1, representing a major step forward in our research.

**Humanized anti-GPC1 monoclonal antibody clone T2 was used for ADC.**

**Patent protection: JP7659326 (Japan, Granted); EP4163301 (Europe, Granted)**
**"HUMANIZED ANTI-GPC-1 ANTIBODY"**

# Summary of this Drug Discovery Project and/or Biotechnology (11)

## Humanized GPC1-ADC (MMAE) Induces Extracellular Release of HMGB1




・MC38-mGPC1 cells were treated in vitro with humanized GPC1-ADC (MMAE) or control agents, and the culture supernatant was collected after 48 hours to quantify HMGB1 secretion by ELISA.

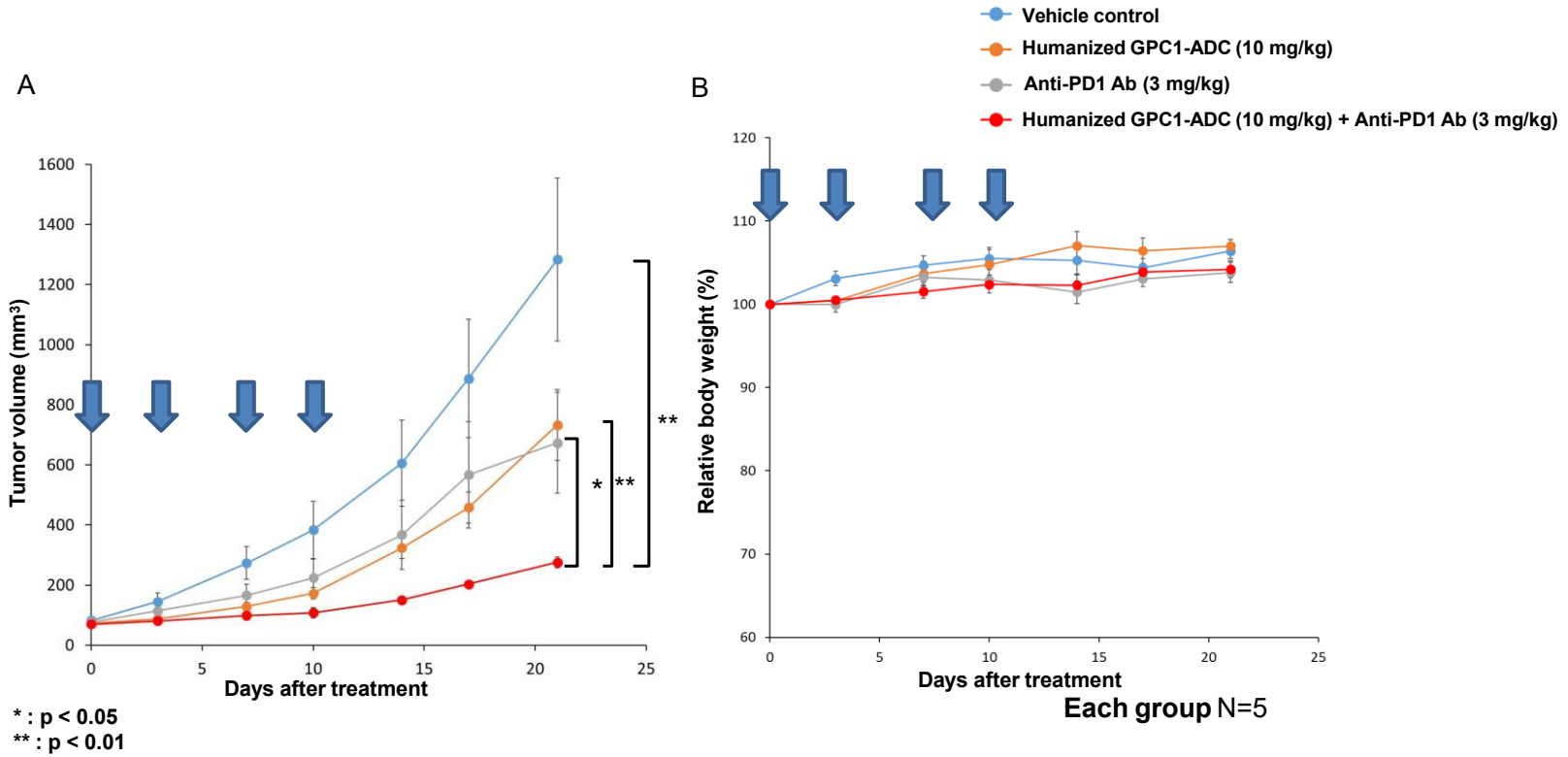
・Treatment with humanized GPC1-ADC (MMAE) resulted in enhanced HMGB1 release from MC38-mGPC1 cells.

* Since the DAR of the ADC is 4, an ADC concentration of 4 nM corresponds to an actual MMAE concentration of 16 nM.

**Patent protection: JP7659326 (Japan, Granted); EP4163301 (Europe, Granted)**
**"HUMANIZED ANTI-GPC-1 ANTIBODY"**

# Summary of this Drug Discovery Project and/or Biotechnology (12)

Combined Administration of Humanized GPC1-ADC (MMAE) and Anti-PD1 Antibody Demonstrates Synergistic Antitumor Effects




・Co-administration of humanized GPC1-ADC (MMAE) and an anti-PD-1 antibody demonstrated synergistic antitumor effects compared with either agent alone in a syngeneic mouse model.

・No significant body weight loss was observed following either the combination or single-agent treatments with humanized GPC1-ADC (MMAE) or the anti-PD-1 antibody.

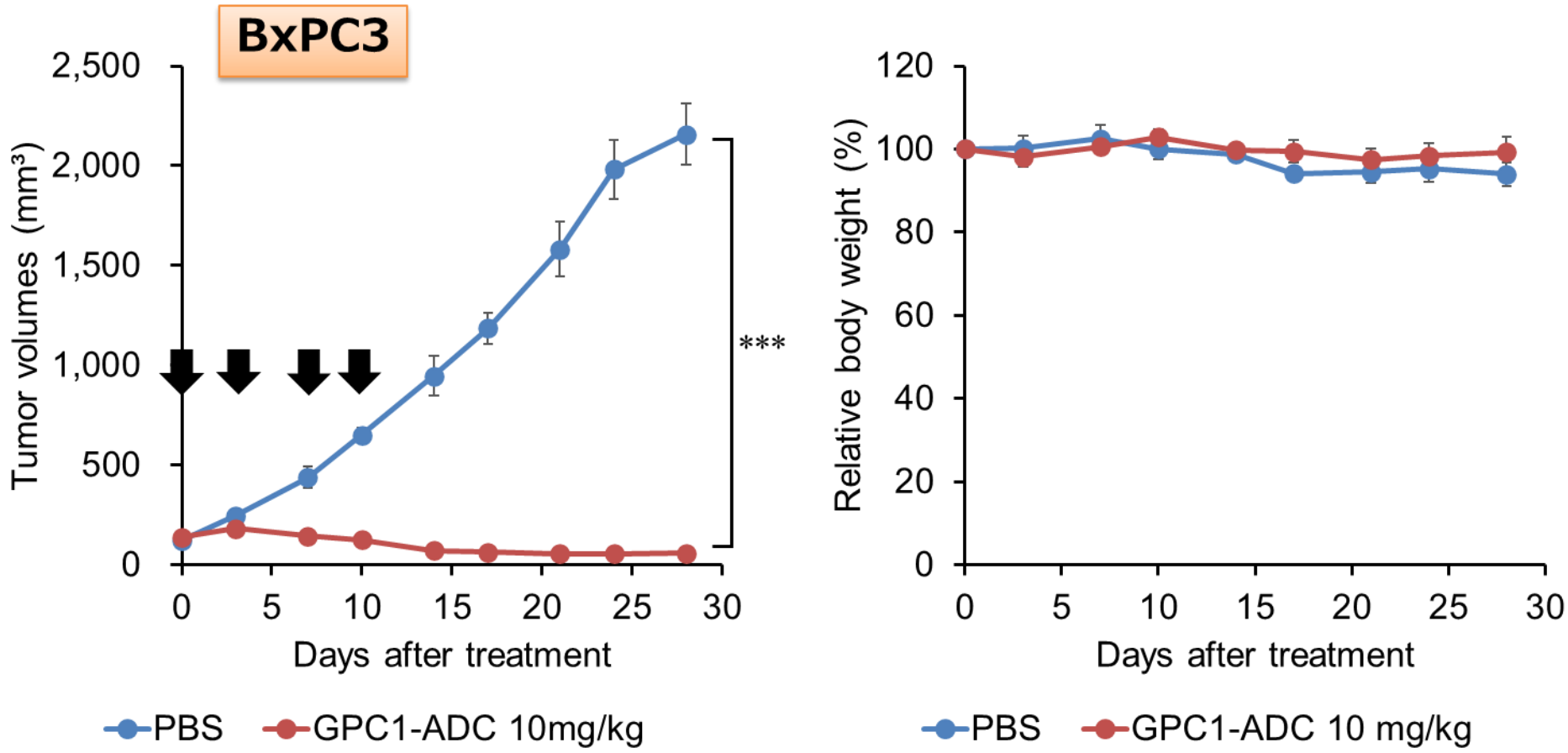
*The lack of complete tumor regression suggests differences in responsiveness depending on the cancer type and/or experimental model conditions.

**Patent protection: JP7659326 (Japan, Granted); EP4163301 (Europe, Granted)**
**"HUMANIZED ANTI-GPC-1 ANTIBODY"**

# Summary of this Drug Discovery Project and/or Biotechnology (13)

## GPC1-ADC Exhibits Antitumor Effects in Humanized Mice

Humanized mice were generated by transferring human PBMCs into NOG MHC double-knockout mice.




Student's t test ***<0.001 each group n=6

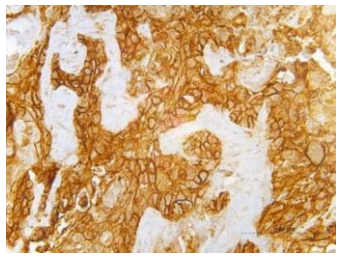
The presence of immune cells had no impact on the antitumor efficacy of GPC1-ADC.

**Humanized anti-GPC1 monoclonal antibody clone T2 was used for ADC.**

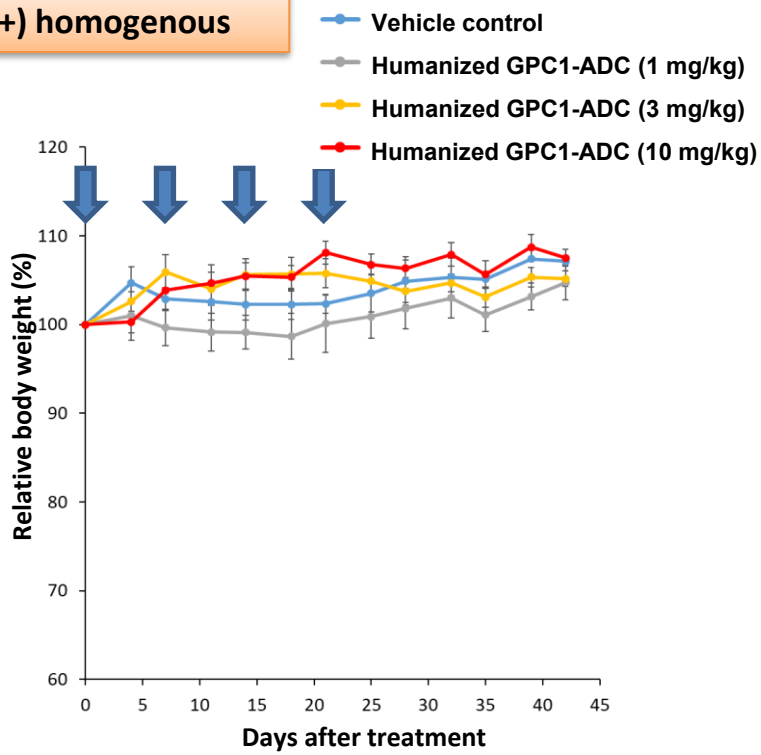
# Summary of this Drug Discovery Project and/or Biotechnology (14)

## Humanized GPC1-ADC (MMAE) Exhibits Potent Antitumor Effects *In Vivo*

**ESCC14（ESCC）/NOG:　GPC1(+) homogenous**






Humanized GPC1-ADC (MMAE) exerted potent antitumor activity in vivo in GPC1-positive esophageal cancer PDX mice.

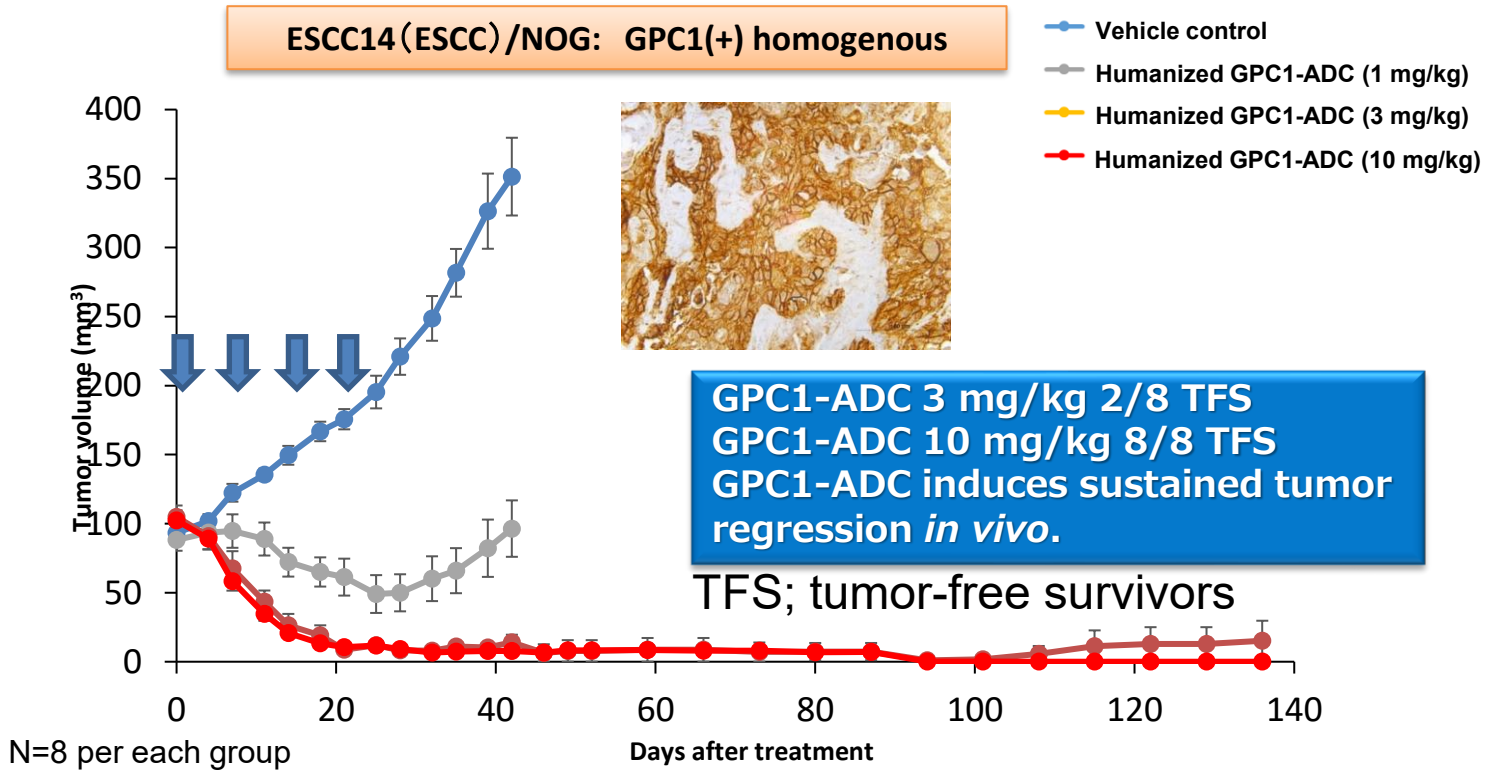
In the ESCC14 PDX model, repeated weekly intravenous administration of humanized GPC1-ADC (MMAE) for four doses resulted in a dose-dependent antitumor efficacy.

No significant reduction in body weight was observed in ESCC14 mice following treatment, indicating acceptable tolerability.

**Munekage, Naka. *et al.*, *Neoplasia*.** 2021

Summary of this Drug Discovery Project and/or Biotechnology (15)

# Humanized GPC1-ADC (MMAE) Exhibits Potent Antitumor Effects *In Vivo*

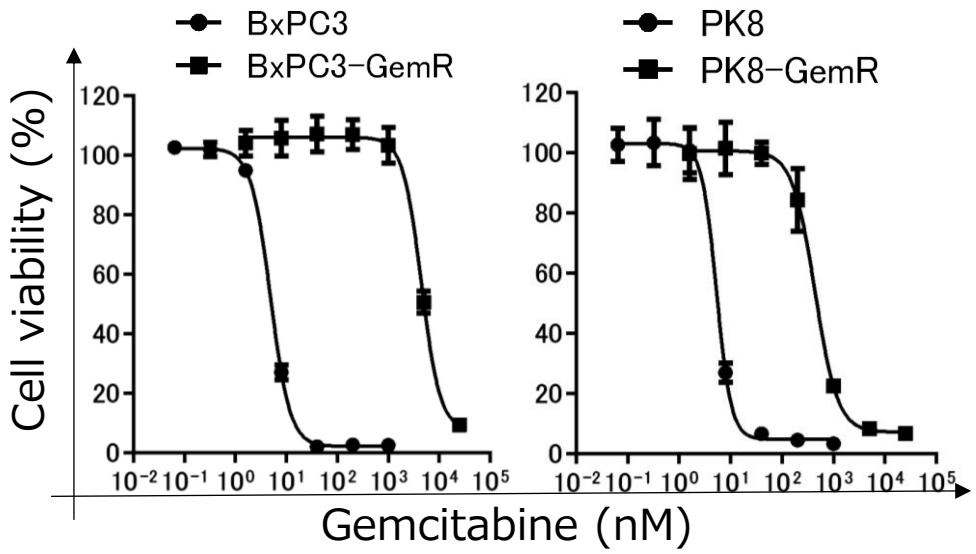


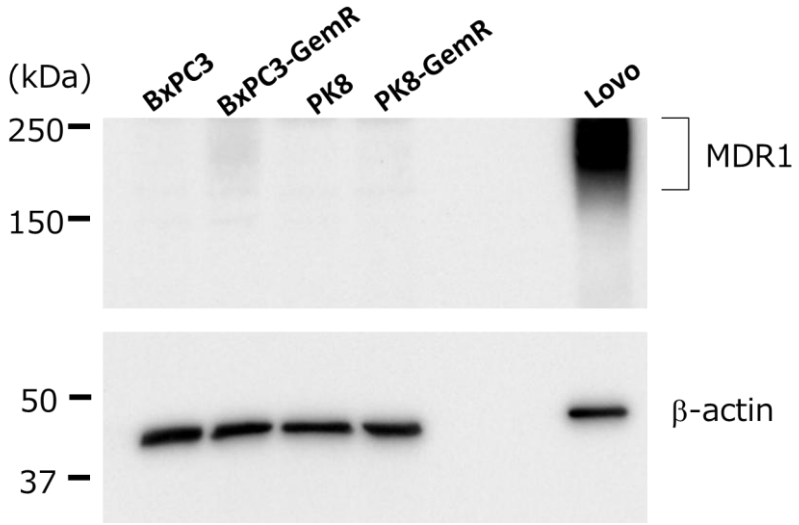

After long-term observation for 136 days, tumor recurrence was observed in 2 of 8 animals in the GPC1-ADC (MMAE) 3 mg/kg group.
In contrast, no tumor recurrence was observed in any of the eight animals treated with 10 mg/kg of GPC1-ADC (MMAE).

Humanized anti-GPC1 monoclonal antibody clone T2 was used for ADC.

# Summary of this Drug Discovery Project and/or Biotechnology (16)

## Establishment of Gemcitabine-Resistant Pancreatic Cancer Cell Lines

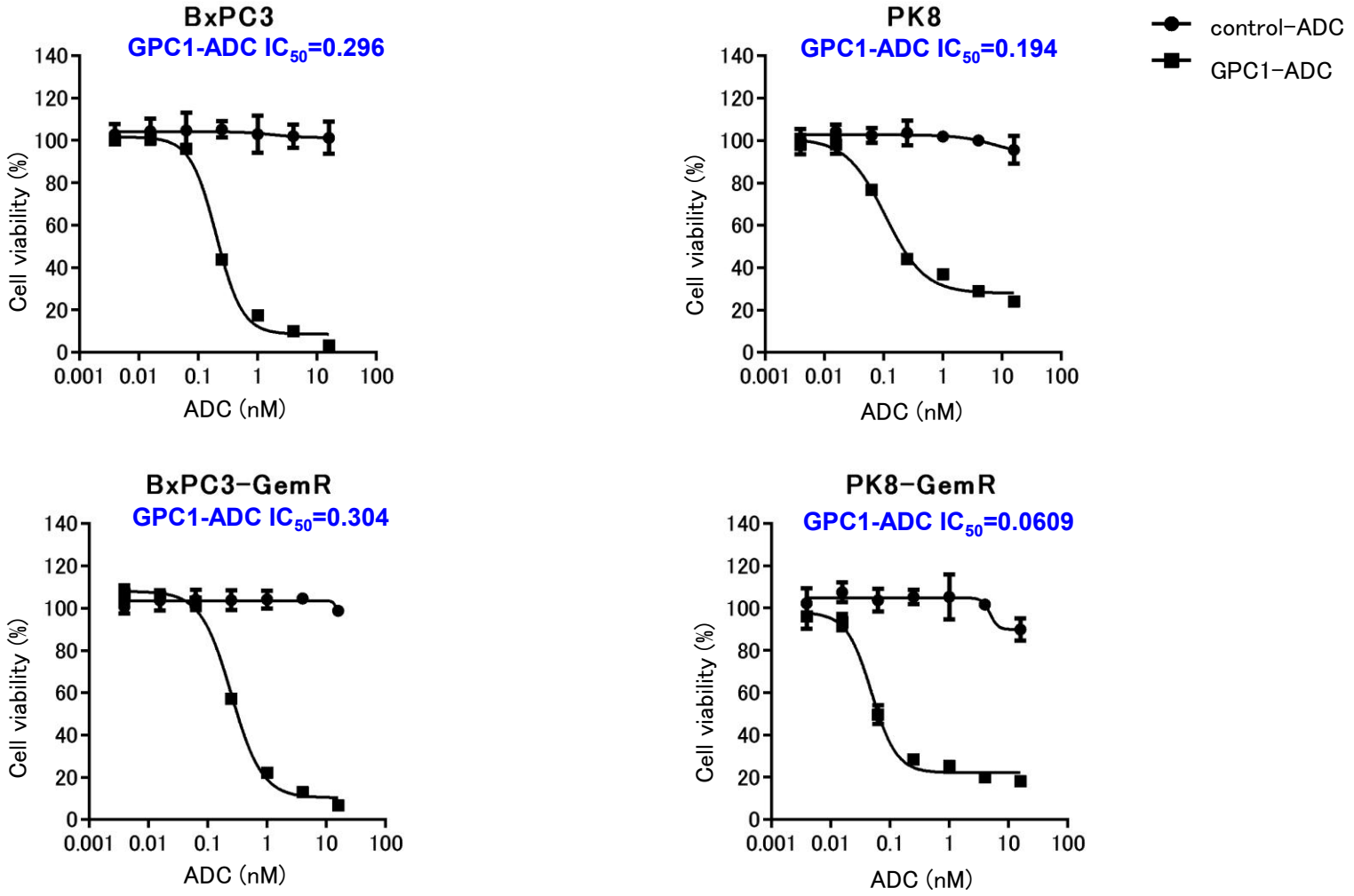



| Cell line/$IC_{50}$ | $IC_{50}$ MMAE (nM) | $IC_{50}$ Gem (nM) | Gem resistance index (Gem-resistant cells / parental cells) |
|---|---|---|---|
| BxPC3 | 0.29 | 4.62 | 1109.74 |
| BxPC3-GemR | 0.29 | 5127.04 | |
| PK8 | 0.22 | 4.84 | 105.19 |
| PK8-GemR | 0.10 | 564.4 | |

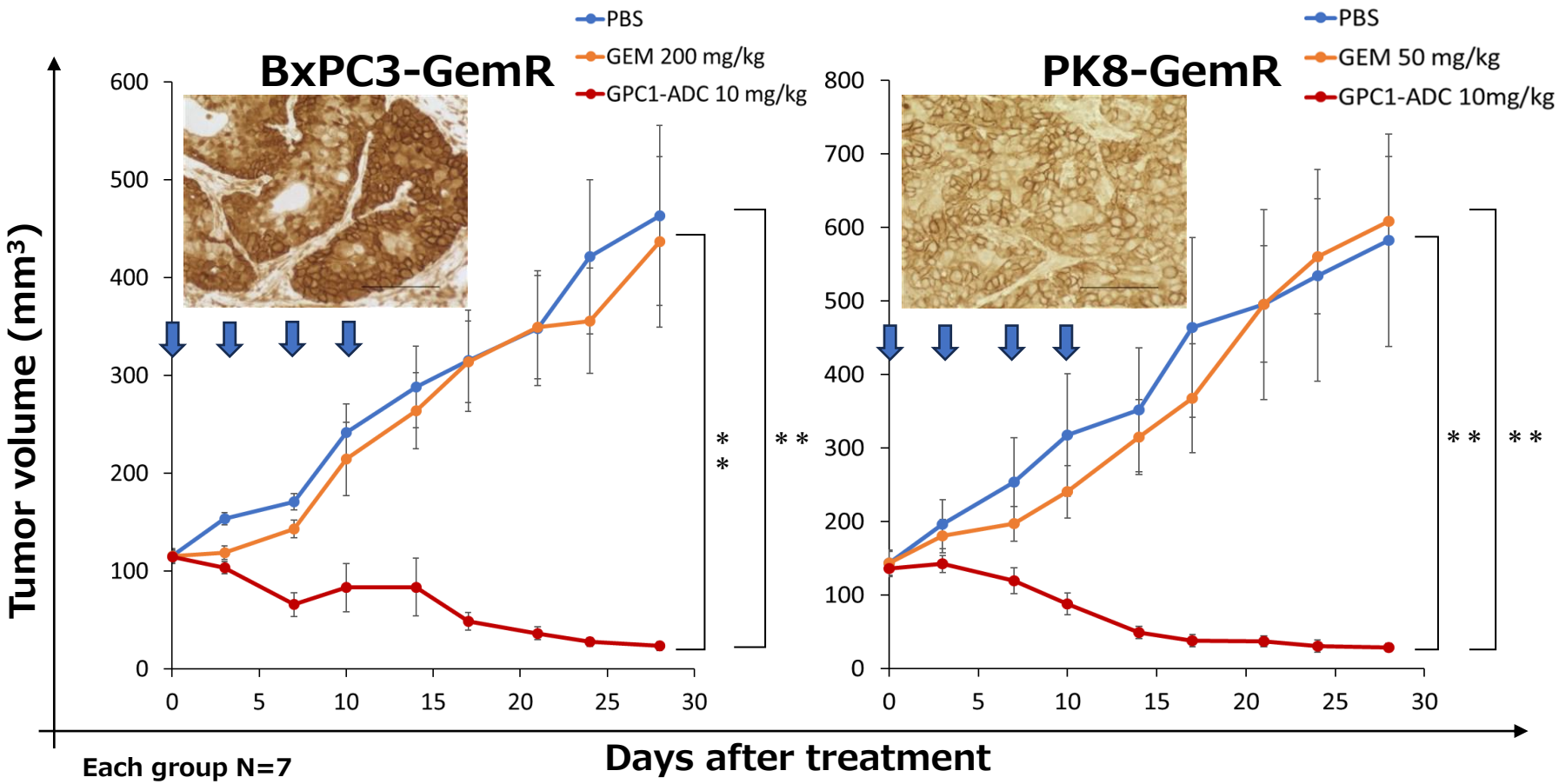
# Summary of this Drug Discovery Project and/or Biotechnology (17)

GPC1-ADC Exhibits In Vitro Growth-Inhibitory Activity Against Gemcitabine-Resistant Pancreatic Cancer Cell Lines



# Summary of this Drug Discovery Project and/or Biotechnology (18)

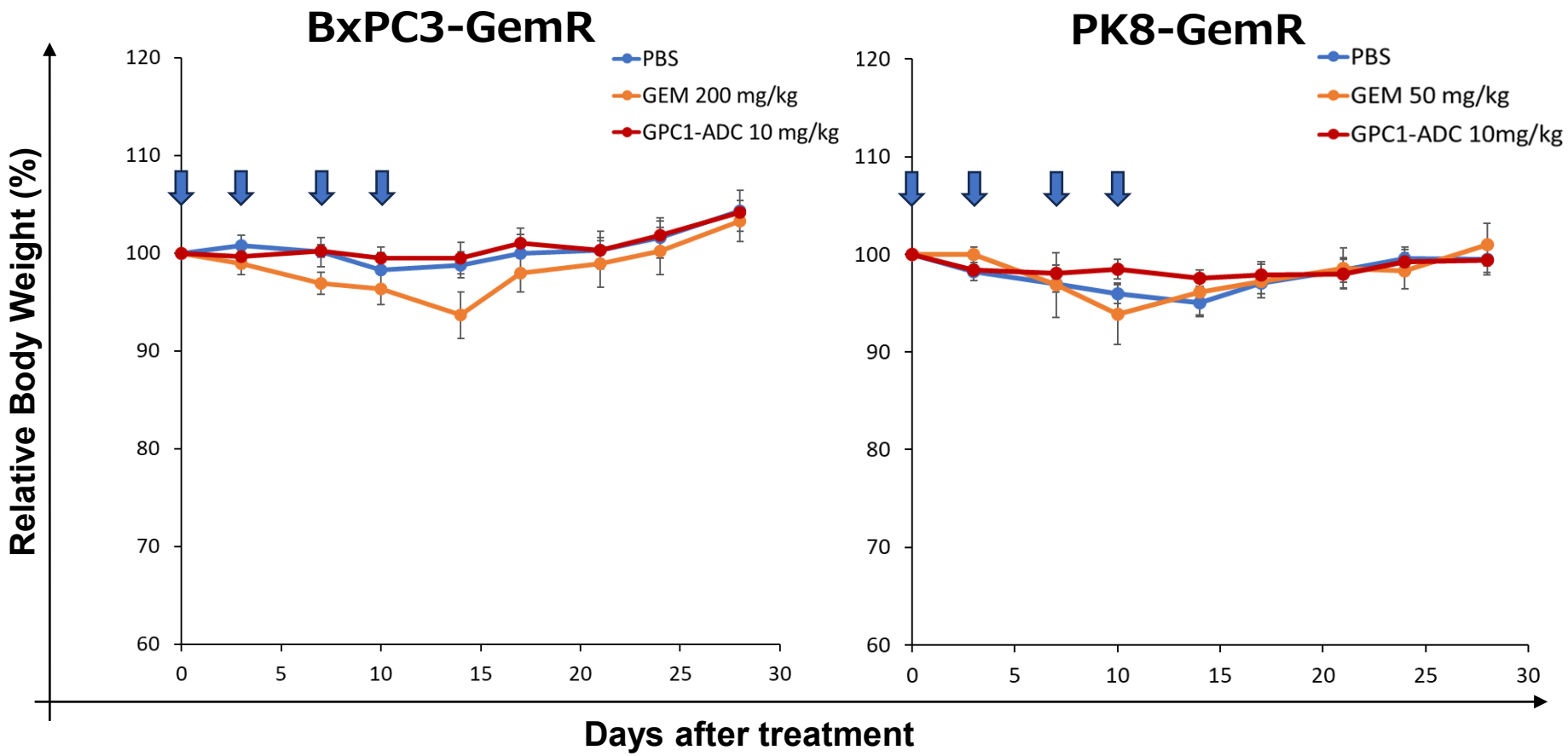
GPC1-ADC Exhibits Antitumor Effects Against Gemcitabine-Resistant Pancreatic Cancer Xenograft Models




one-way ANOVA, followed by Scheffe's post hoc test, * p<0.05, ** p<0.01

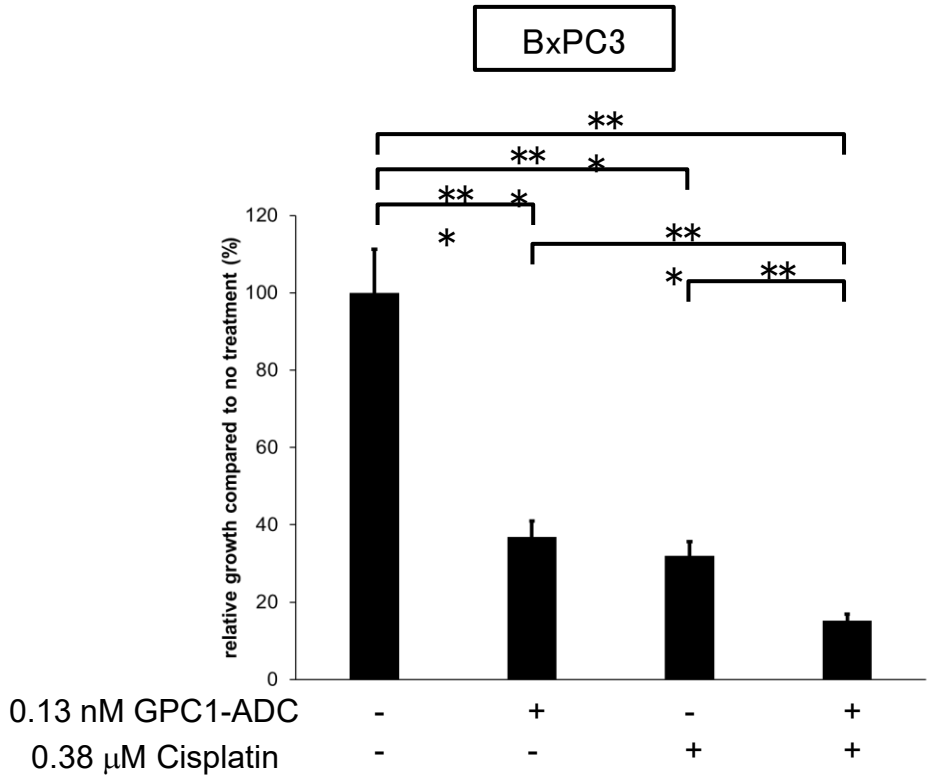
## Summary of this Drug Discovery Project and/or Biotechnology (19)

GPC1-ADC Inhibits Tumor Growth without Affecting Body Weight in Subcutaneous Models of Gemcitabine-Resistant Pancreatic Cancer



# Summary of this Drug Discovery Project and/or Biotechnology (20)

GPC1-ADC Shows Additive Growth-Inhibitory Activity When Combined with Cisplatin




Two-way ANOVA, followed by Bonferroni's *post hoc* test
**p<0.01, ***p<0.001

# Summary of this Drug Discovery Project and/or Biotechnology (21)

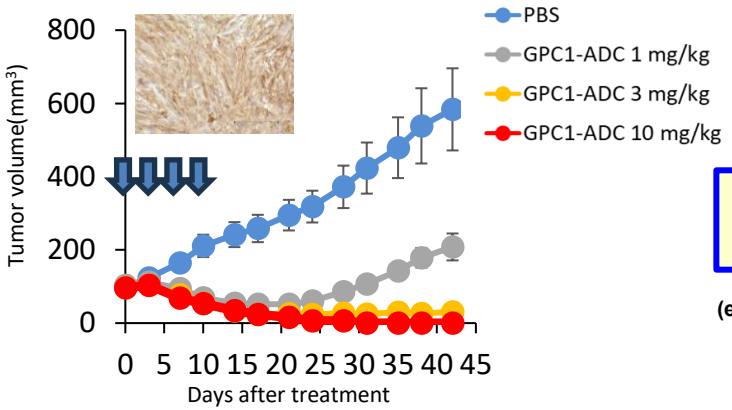
## GPC1-ADC Exhibits Potent Antitumor Effects Against Cancer Cells with High GPC1 Expression

GPC1 expression on cancer cell surface was quantified *in vitro* by flow cytometric analysis using QIFIKIT

**KALS-1 (Glioblastoma)**

**GPC1: 155,353 molecules/cell**

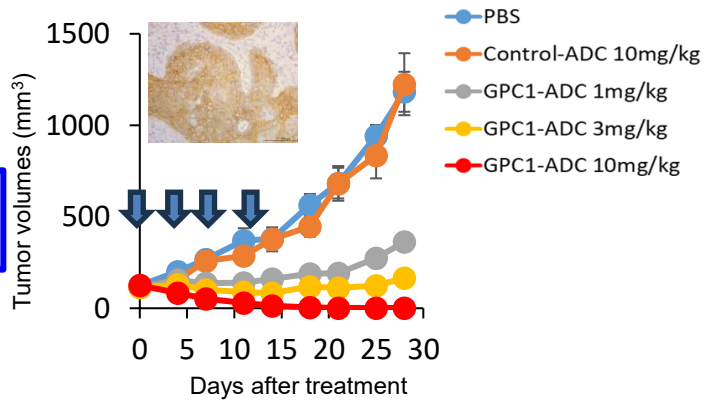
**(each n = 7 per group)**




**BxPC3 (Pancreatic cancer)**

**GPC1: 93,290 molecules/cell**

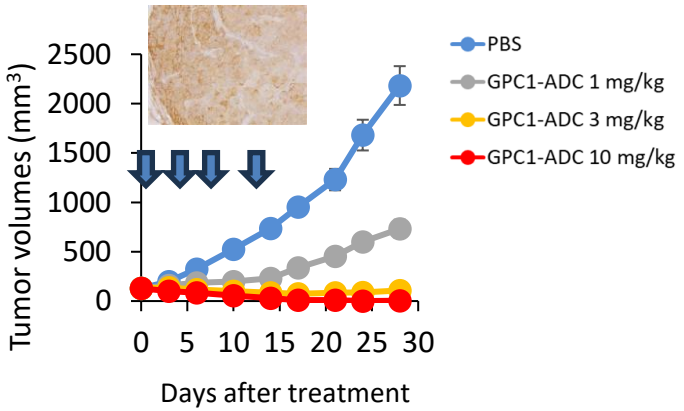
**(each n = 7 per group)**




**HARA (squamous cell lung cancer)**

**GPC1: 71,847 molecules/cell**

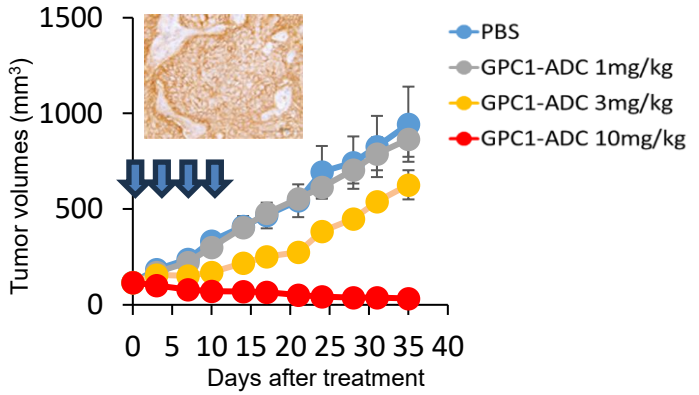
**(each n = 7 per group)**




**H103 (oral squamous cell carcinoma)**

**GPC1: 45,778 molecules/cell**

**(each n = 7 per group)**




GPC1-ADC (10 mg/kg) is expected to exert antitumor efficacy in tumor-bearing mice even when GPC1 expression on cancer cells is as low as 45,778 molecules per cell.

**Munekage, Naka. *et al.*, *Neoplasia*. 2021** **Uchida, Naka. *et al.*, *Neoplasia*. 2024**

## Summary of this Drug Discovery Project and/or Biotechnology (22)

Dose Conversion Based on Human Equivalent Dose (HED)

| HED conversion based on body surface area(1) animal species | divisor |
|---|---|
| Mouse | 12.3 |
| Cynomolgus monkey | 3.1 |

| HED conversion based on body surface area(1) animal species | 10 mg/kg in mice converted to human dose | 6 mg/kg in monkeys converted to human dose |
|---|---|---|
| Human (Assumes 60 kg body weight) | 0.813 mg/kg | 1.94 mg/kg |

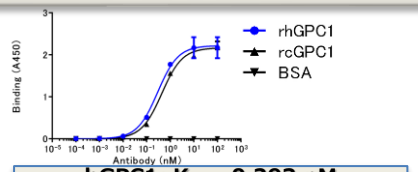
**Approx. 2.4 times**

Safety was confirmed in cynomolgus monkeys at approximately 2.4 times the pharmacologically effective dose of GPC1-ADC observed in mice.

# Summary of this Drug Discovery Project and/or Biotechnology (23)

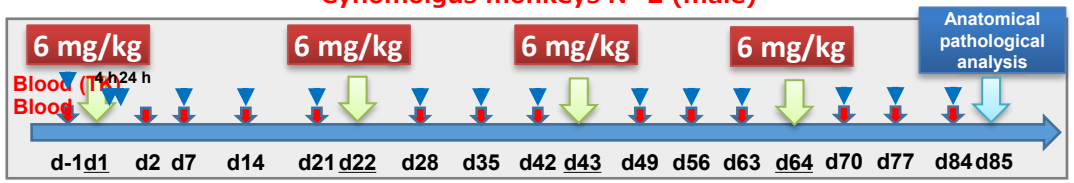
## Preliminary Safety Pharmacology Study Using Cynomolgus Monkeys (Non-GLP)

The humanized anti-GPC1 antibody clone T2 binds to human GPC1 with almost the same affinity as to cynomolgus monkey GPC1, indicating cross-reactivity between the two species.



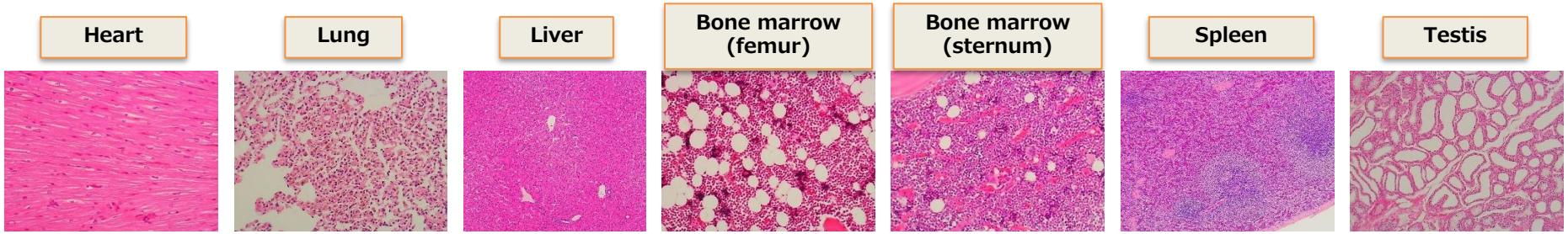

hGPC1: $K_D$ = 0.293 nM
cynoGPC1: $K_D$ = 0.442 nM

**Humanized GPC1-ADC intravenous dosing analysis schedule**
**Cynomolgus monkeys N=2 (male)**



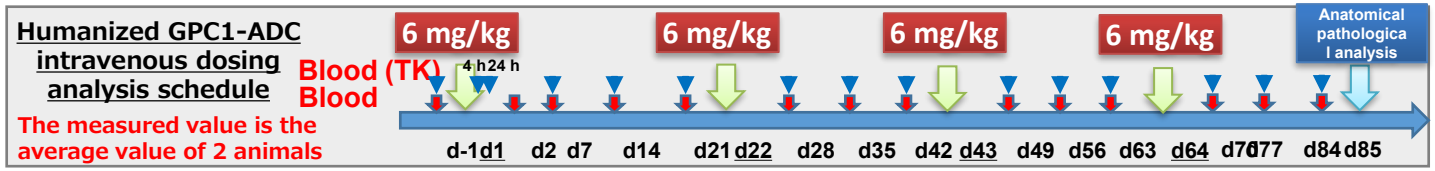

## Summary of test results

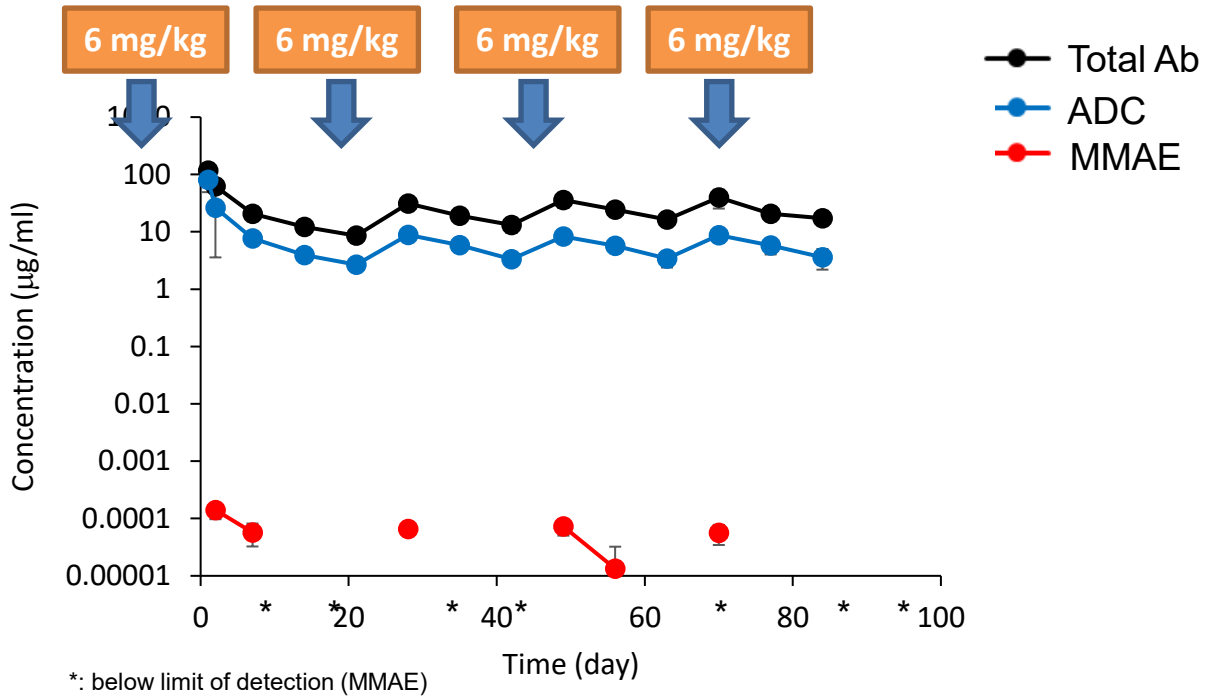
・In the study, no treatment-related changes were observed following four repeated doses of humanized GPC1-ADC (6 mg/kg) administered every 21 days.
・Although a transient decrease in neutrophil counts was noted, both monkeys fully recovered within 20 days after administration, alleviating any safety concerns.
・Notably, no abnormalities were detected across a comprehensive range of parameters, including body weight, electrocardiogram, blood pressure, respiratory rate, ophthalmologic and gross examinations, or organ weights, supporting the safety profile of the drug.
・No abnormalities in liver or renal function were observed in biochemical analyses.
・Histopathological examination revealed no treatment-related abnormalities in any organs.
・The changes described above are consistent with those observed for other anticancer agents and were not considered indicative of severe toxicity.



# Summary of this Drug Discovery Project and/or Biotechnology (24)

## Plasma Concentrations of GPC1-ADC in Cynomolgus Monkeys Are Stable



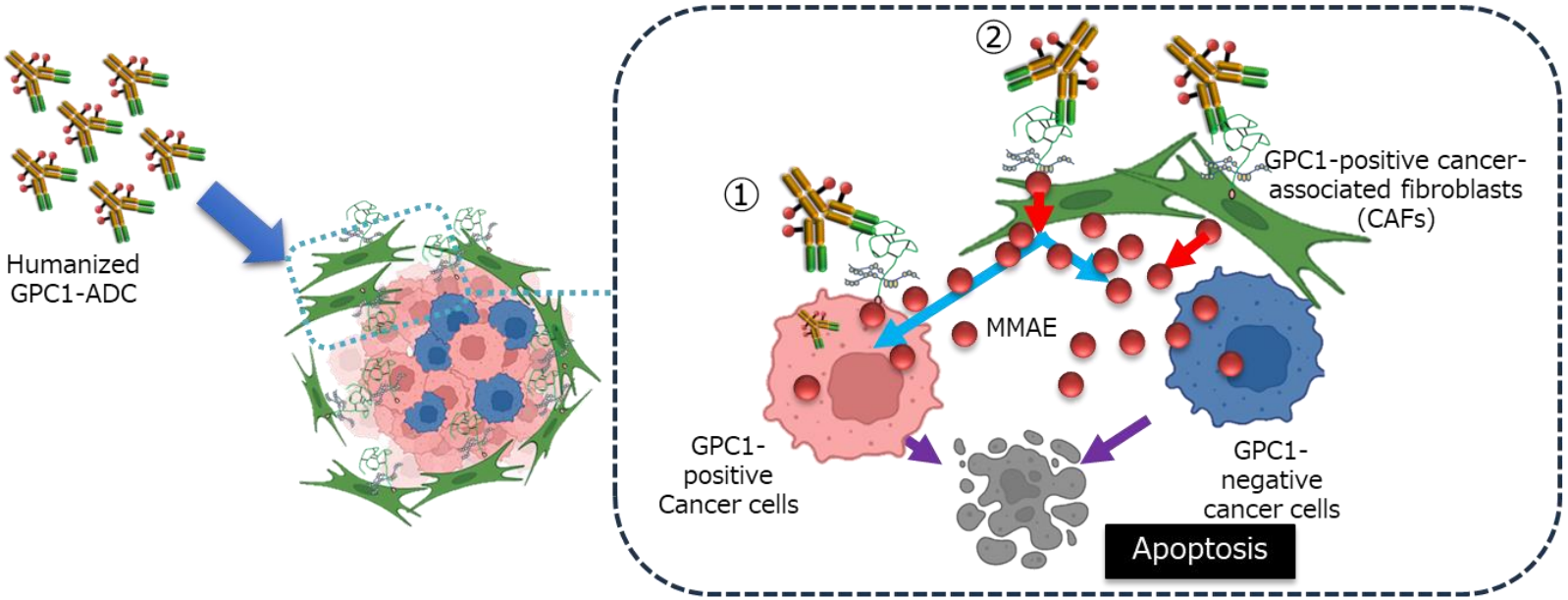




Both total antibody and GPC1-ADC maintained sufficient plasma concentrations throughout the observation period.
Plasma concentrations of free MMAE remained very low.

# Advantage of this Project and/or Biotechnology (1)

**GPC1-ADC is a promising therapeutic candidate for GPC1-positive, stroma-rich solid tumors.**

**Currently, most approved ADCs target cancer cell–specific antigens; however, the treatment of stroma-rich solid tumors, such as pancreatic cancer, remains challenging because the dense stromal barrier hinders efficient ADC delivery to cancer cells.**
**GPC1 is expressed not only in pancreatic cancer cells but also in cancer-associated fibroblasts (CAFs), which are abundantly present in pancreatic tumor tissues.**
**With its unique mechanism of action, GPC1-ADC exerts potent antitumor activity by directly targeting GPC1-positive cancer cells and indirectly suppressing tumor growth through MMAE released from GPC1-positive CAFs, opening new avenues for cancer therapy.**




① Humanized GPC1-ADC binds to GPC1-positive pancreatic cancer cells and is internalized *via* GPC1, resulting in a direct antitumor effect.
② When the humanized GPC1-ADC is internalized into CAFs, the linker is cleaved and MMAE is released from the ADC. The released MMAE is then excreted by CAFs via the P-glycoprotein drug transporter, killing both GPC1-negative and GPC1-positive cancer cells through a bystander effect.

Among various potential payloads, MMAE stands out as particularly suitable for pancreatic cancer treatment, especially in CAF-rich tumors, providing a strong scientific rationale for our research.
- Can be exported from cells via the P-glycoprotein drug transporter (MDR1)
- High membrane permeability enables bystander killing of neighboring cancer cells.

# Advantage of this Project and/or Biotechnology (2)

Limitations of currently approved ADCs

| Name | Generic name | Antigen | Indication | Payload |
|---|---|---|---|---|
| **Kadcyla** | **Trastuzumab emtansine** | **HER2** | HER2-positive unresectable or recurrent **breast cancer** | DM1 |
| **Padcev** | **Enfortumab vedotin** | **Nectin-4** | Adult patients with locally advanced or metastatic **urothelial cancer** (mUC) who:<br>have previously received a programmed death receptor-1 (PD-1) or programmed death-ligand 1 (PD-L1) inhibitor and platinum-containing chemotherapy, or<br>are ineligible for cisplatin-containing chemotherapy and have previously received one or more prior lines of therapy | **MMAE** |
| **Enhertu** | **Trastuzumab deruxtecan** | **HER2** | Unresectable or metastatic HER2-positive (IHC 3+ or ISH positive) **breast cancer** who have received a prior anti-HER2-based regimen either:<br>In the metastatic setting, or<br>In the neoadjuvant or adjuvant setting and have developed disease recurrence during or within six months of completing therapy | DXd |
| **Trodelvy** | **Sacituzumab govitecan** | **Trop-2** | **Triple-negative breast cancer** received more than two lines of treatment | SN38 |
| **Tivdak** | **Tisotumab vedotin** | **Tissue Factor** | Recurrent/metastatic **cervical cancer** | **MMAE** |
| **Elahere** | **Mirvetuximab soravtansine** | **FRα** | **Platinum-resistant ovarian cancer, primary peritoneal carcinoma, or fallopian tube cancer** with a history of 1 to 3 lines of therapy | DM4 |

## Differences between GPC1-ADC and approved ADCs

**GPC1-ADC demonstrates therapeutic efficacy against stroma-rich tumors that are difficult to treat with currently approved ADCs.**

## Plan to maximize the value of this drug discovery Project and/or Biotechnology

### ~~1)~~ Goal and its plan for research and/or development

・The goal of this study is to obtain regulatory approval for the treatment of intractable tumors, including pancreatic cancer.

・Preclinical GLP studies using cynomolgus monkeys are scheduled following the production of non-GMP humanized GPC1-ADC. Cynomolgus monkeys for the GLP study have already been secured.

A Phase I clinical study is planned after the generation of GMP-grade humanized GPC1-ADC.

・Companion diagnostics for humanized GPC1-ADC are being developed in parallel, including IHC assays for detecting GPC1 expression in FFPE tumor tissues.

# Reference and/or Patent(s)

## 1 ) Potential target disease and/or therapeutics area on this proposal

Various GPC1-positive solid tumors could be potential targets for this proposal, including:
gastric cancer, cholangiocarcinoma, cervical cancer, oral squamous cell carcinoma, glioblastoma, lung cancer, head and neck cancer, breast cancer, and prostate cancer.

## 2) Key papers ~~and/or~~


**1.** **Overexpression of glypican-1 implicates poor prognosis and their chemoresistance in oesophageal squamous cell carcinoma.**
Hara H, Takahashi T, Serada S,, Doki Y, **Naka T**. *et al*., ***Br J Cancer*. 2016;115(1):66-75.**

**2.** **Glypican-1 targeted antibody-based therapy induces preclinical antitumor activity against esophageal squamous cell carcinoma.**
Harada E, Serada S, Doki Y, Kaneda Y, **Naka T**. *et al*., ***Oncotarget*. 2017;8(15):24741-24752.**

**3.** **Anti-glypican-1 antibody-drug conjugate exhibits potent preclinical antitumor activity against glypican-1 positive uterine cervical cancer.**
Matsuzaki S, Serada S, Kimura T, **Naka T**. *et al*., ***Int J Cancer*. 2018;142(5):1056-1066.**

**4.** **Anti-glypican-1 antibody-drug conjugate is a potential therapy against pancreatic cancer.**
Nishigaki T, Takahashi T, Serada S, Doki Y, **Naka T**. *et al*., ***Br J Cancer*. 2020;122(9):1333-1341.**

**5.** **Anti-glypican-1 antibody–drug conjugate as potential therapy against tumor cells and tumor vasculature for glypican-1 positive cholangiocarcinoma.**
Yokota K, Serada S, Doki Y, Hanazaki K, **Naka T**. *et al*., ***Mol Cancer Ther*. 2021;20(9):1713-1722.**

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Tsujii S, Serada S, **Naka T**. *et al*., ***Mol Cancer Ther*. 2021;20(12):2495-2505.**

**8.** **Glypican-1-targeted antibody-drug conjugate inhibits the growth of glypican-1-positive glioblastoma.**
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***9.*** **Assessing efficacy of anti-glypican-1 antibody-drug conjugate as potential therapeutic approach for gastric cancer.**
Suzuki Y, Serada S, **Naka T**. *et al*., ***Gasctric Cancer*. 2025 In Press**

# Reference and/or Patent(s)

3) Patent and its status

**(1) Title of Invention: Esophageal cancer marker and its use**

Application number/Patent number: PCT application PCT/JP2014/006455 Transferred to Japan and the US
United States (15/108,251) Japan (Japanese Patent Application 2015-554576, Japanese Patent Application 2017-88418 (2017/4/27, divisional application from Japanese Patent Application 2015-554576))
Filing date: December 25, 2014 (international filing date)
Registration number: Patent No. 6500933 (Patent application 2017 - 88418, registration date: March 29, 2019)
Registration number: Patent No. 10,077,316 (United States, registration date: September 18, 2018)
Applicant: Iwate Medical (application filed in Japan, April 24, 2017)
International Publication WO2015/098112 (PCT), “ESOPHAGEAL CANCER MARKER AND USE THEREOF” published Jul. 2, 2015

**(2) Title of invention: Humanized Anti-GPC-1 Antibody**

Application number: 2020-101856, Application date: June 11, 2020, Applicant: Iwate Medical University
International application number: PCT/JP2021/022085, filing date: June 10, 2021, applicant: Iwate Medical University
Transferred to Japan, US and EU. Japan
(Japanese Patent Application 2022-530617)
United States (18/009,024)
EU (21823133.0)
Registration number: Patent No. 7659326 (Patent application 2022-530617, registration date: March 18, 2025)
Registration number: Patent No. 4163301 (Patent application 21823133.0 - 1111, registration date: October 10, 2025)
International Publication WO 2021/251459 (PCT), “HUMANIZED ANTI-GPC-1 ANTIBODY” published Dec. 16, 2021

## Related Information

R&D system