

Glycoforum 2023年2月1日

# 外科医と研究者で挑む膵癌研究



つくば膵胆腫瘍センター  
Tsukuba Pancreatic-Biliary Tumor Center



筑波大学医学医療系  
消化器外科  
Dept. GI & HBP Surgery, University of Tsukuba



筑波大学附属病院  
University of Tsukuba Hospital

筑波大学医学医療系 消化器外科 下村 治

# COI Disclosure

下村 治

Osamu Shimomura

---

講演内容に関連し、演者について開示すべきCOI関係にある企業等はありません。

The speaker have no financial conflicts of interest to disclose concerning the presentation.

# 本セミナー内容

➤膵癌について 現状と課題

➤膵癌の糖鎖解析

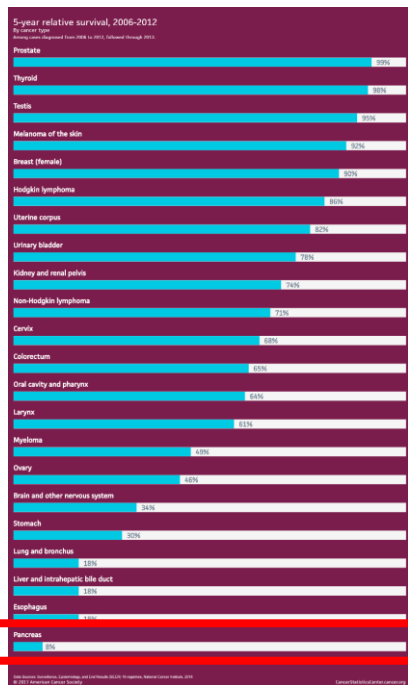
➤レクチンを用いた糖鎖標的治療研究 現状と課題



# 膵癌は最も「予後不良な癌」

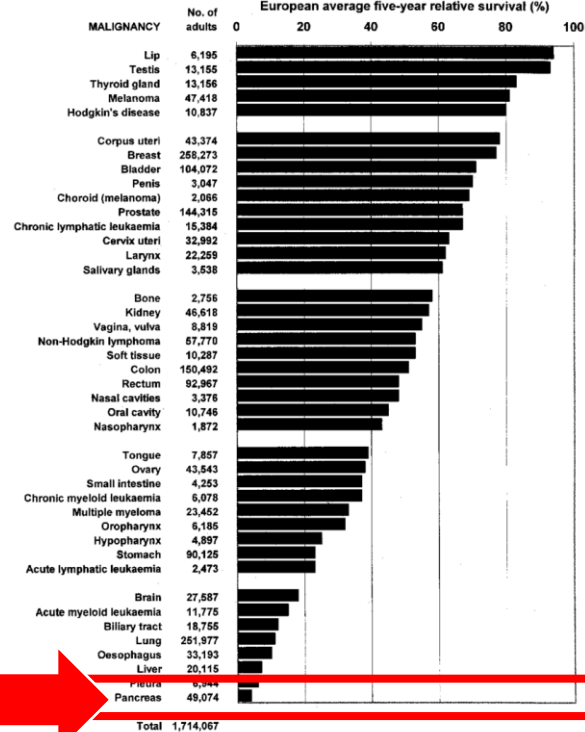
## 癌の種類別 5 年生存率

アメリカ



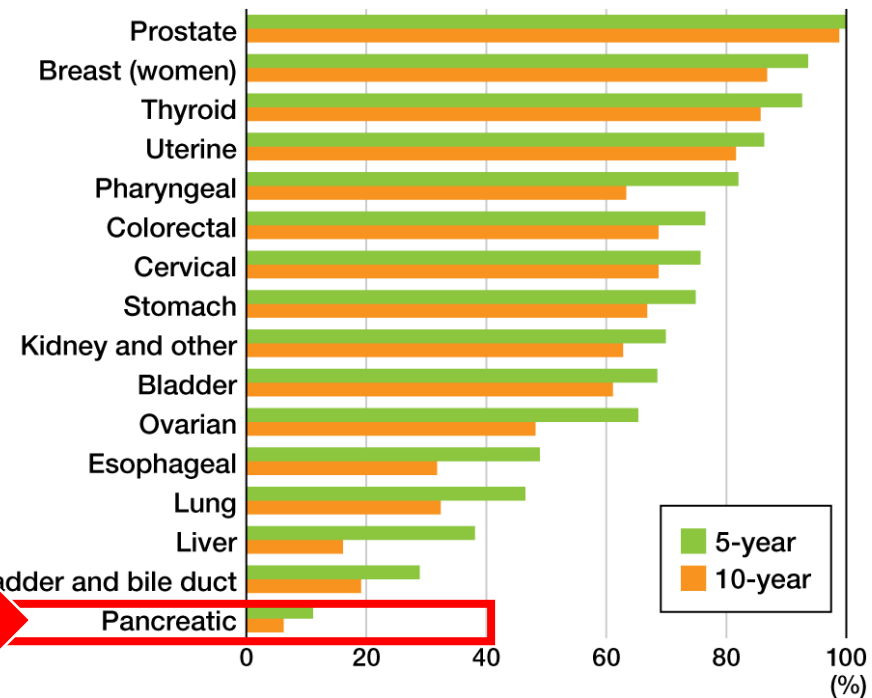
膵癌

ヨーロッパ



膵癌

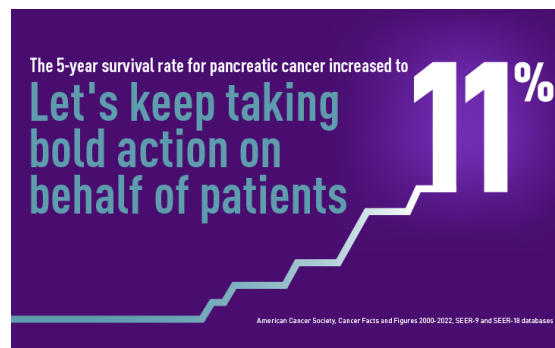
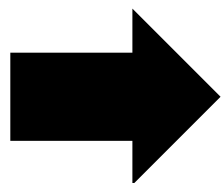
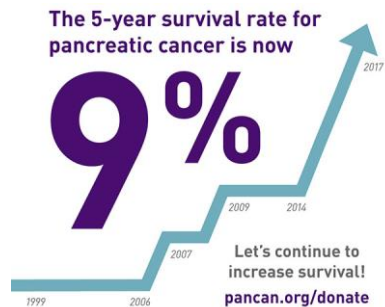
日本



膵癌

From American Cancer Society

From 国立がん研究センター



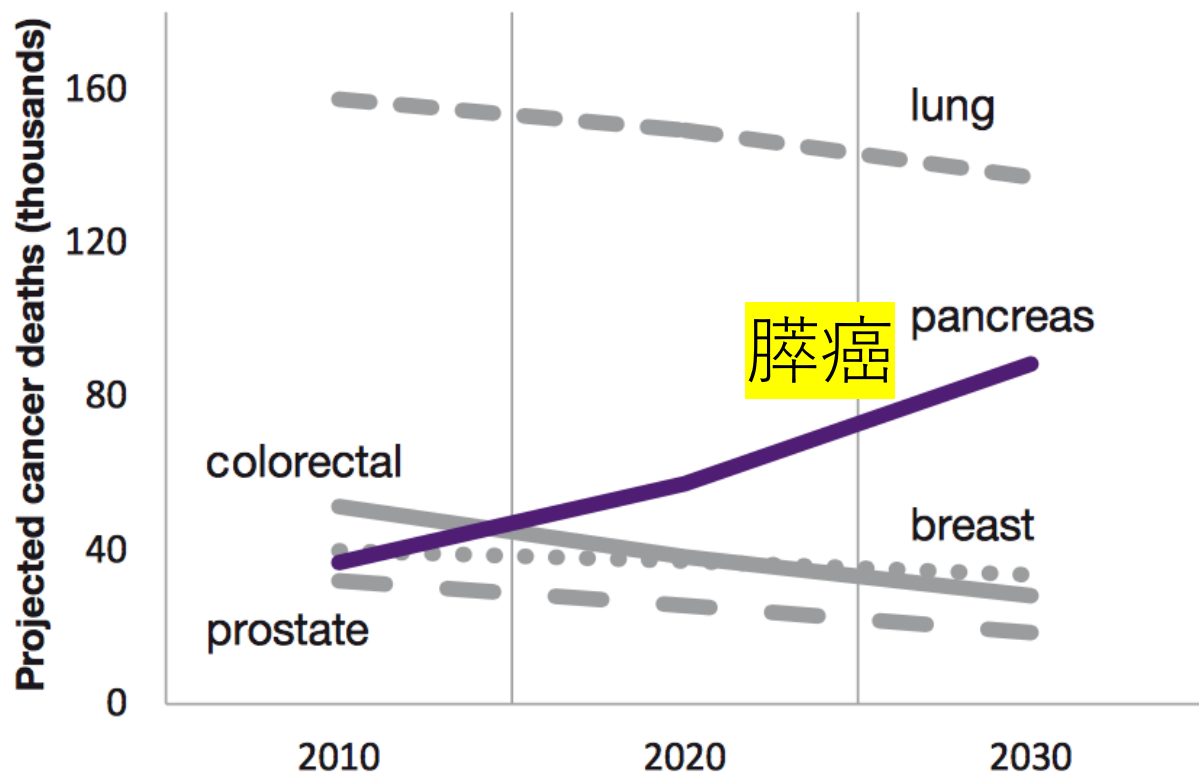
ここ 50 年で漸く 10% 台に



# 膵癌の患者は年々増加している

## アメリカの予想

### PROJECTED CANCER DEATHS



2014 AACR News Releases, Lynn Matrisianより

## 日本の実データ

膵癌で亡くなる方

2014年 男性: 5位 16,411名  
女性: 4位 15,395名  
合計 = 31,800名

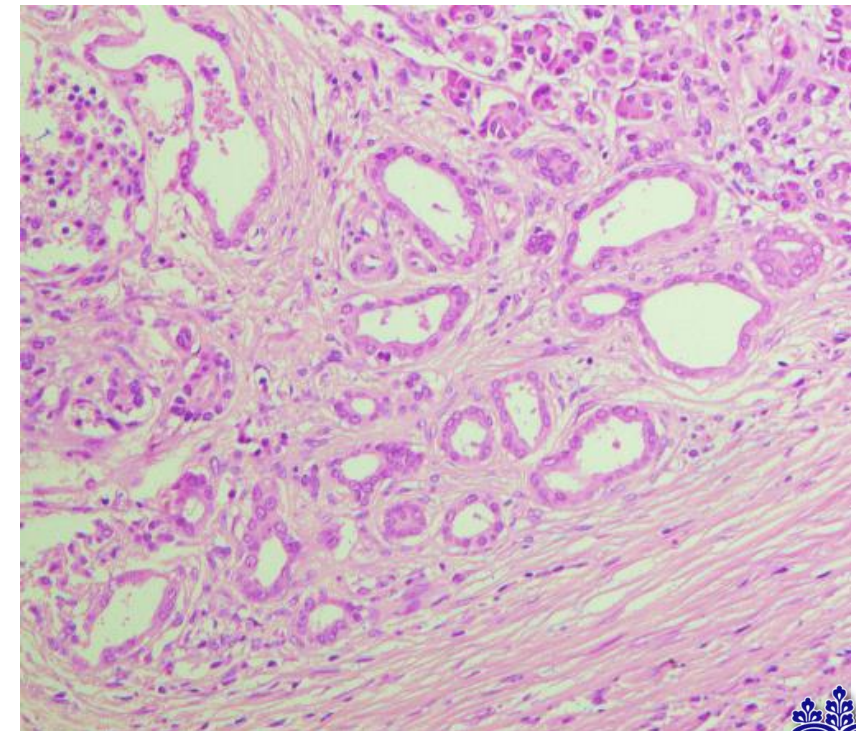
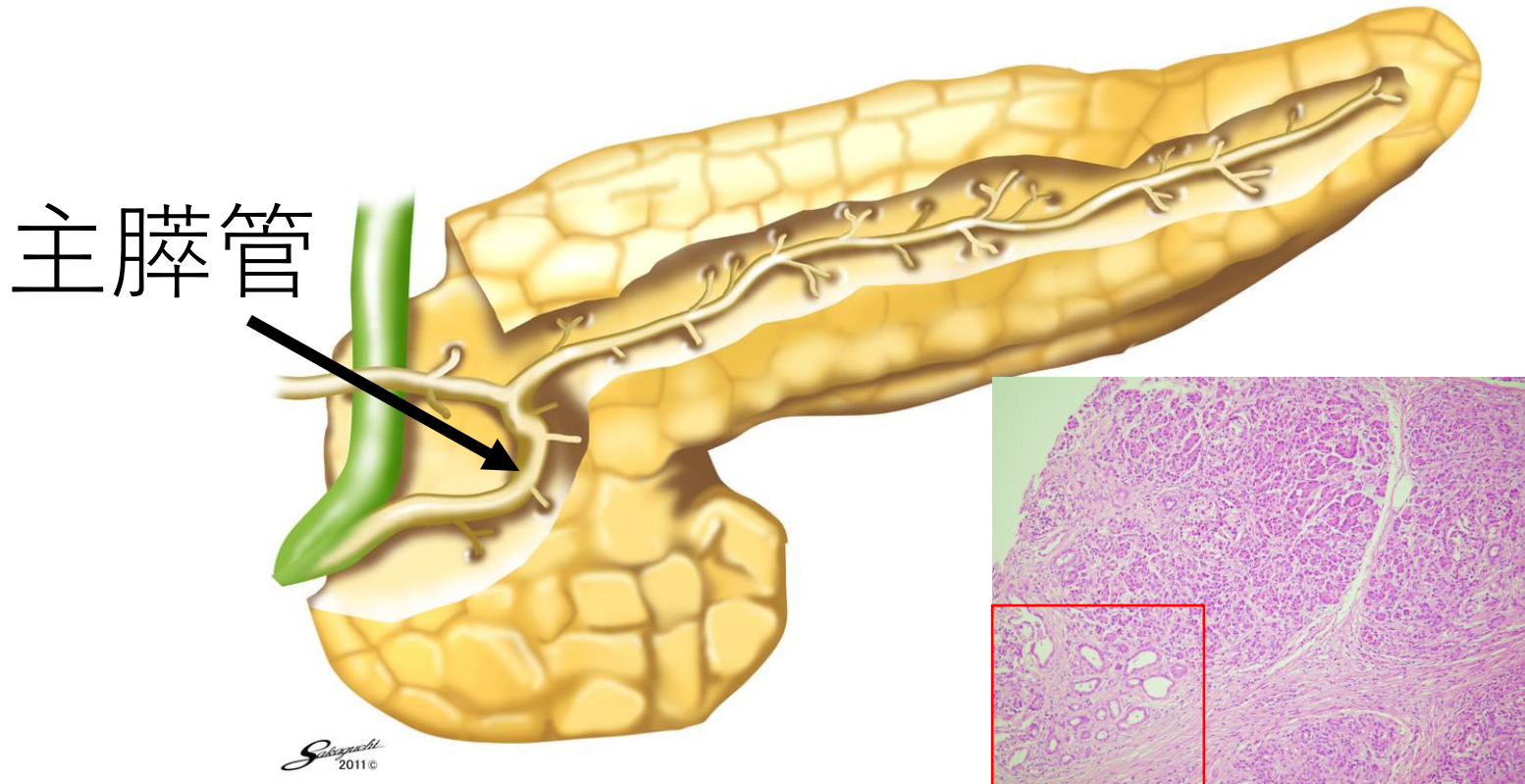


2018年 男性: **4位** 18,124名  
女性: **3位** 18,232名  
合計 = **36,300名**

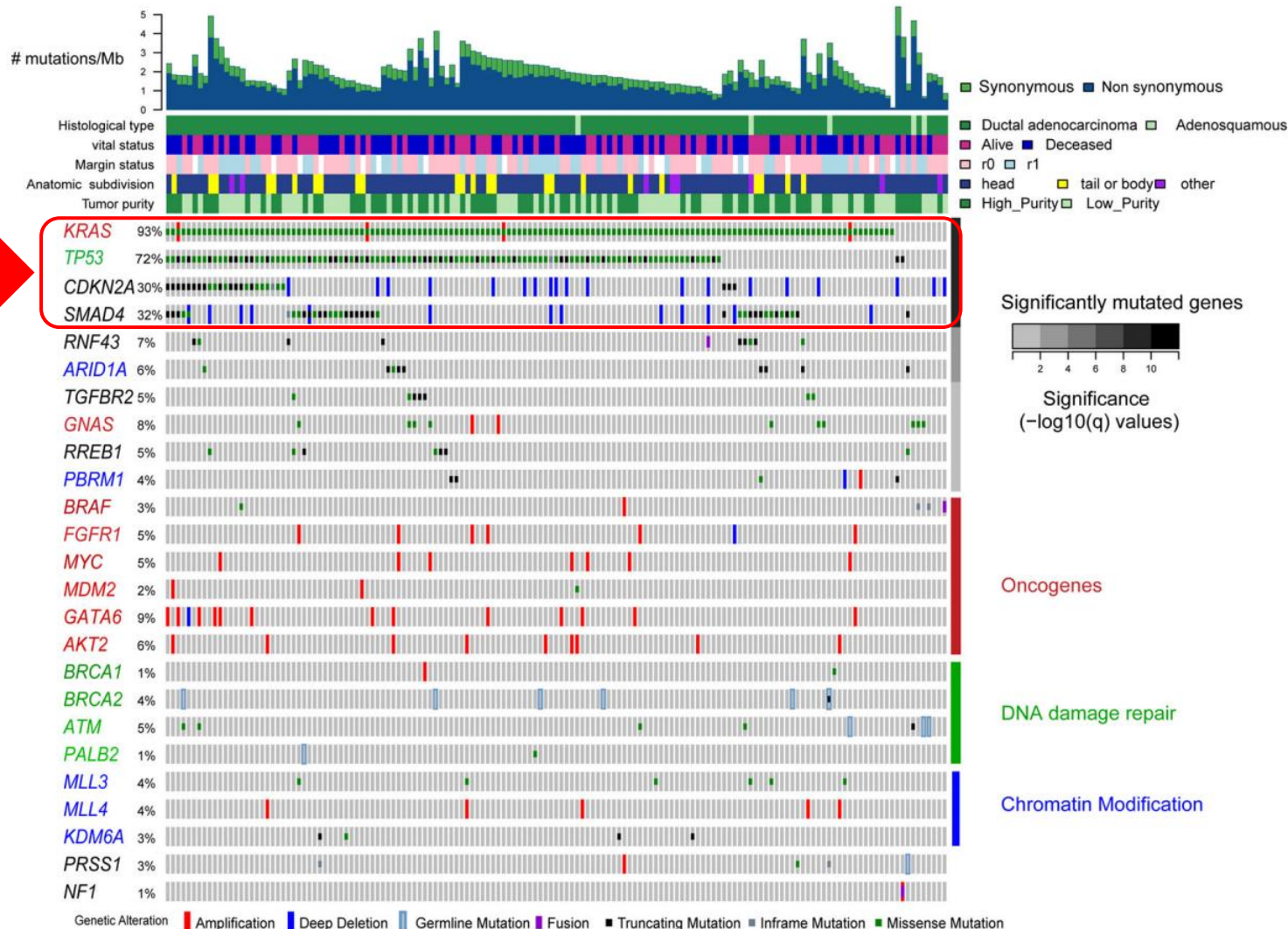


# 膵癌の発生

膵管の上皮（膵液の流れ道）から発生する  
「**浸潤性膵管癌**」が最も代表的



# 明らかになってきた膵がん関連遺伝子



膵癌に関連するBig4遺伝子

KRAS 93%

TP53 72%

CDKN2A 30%

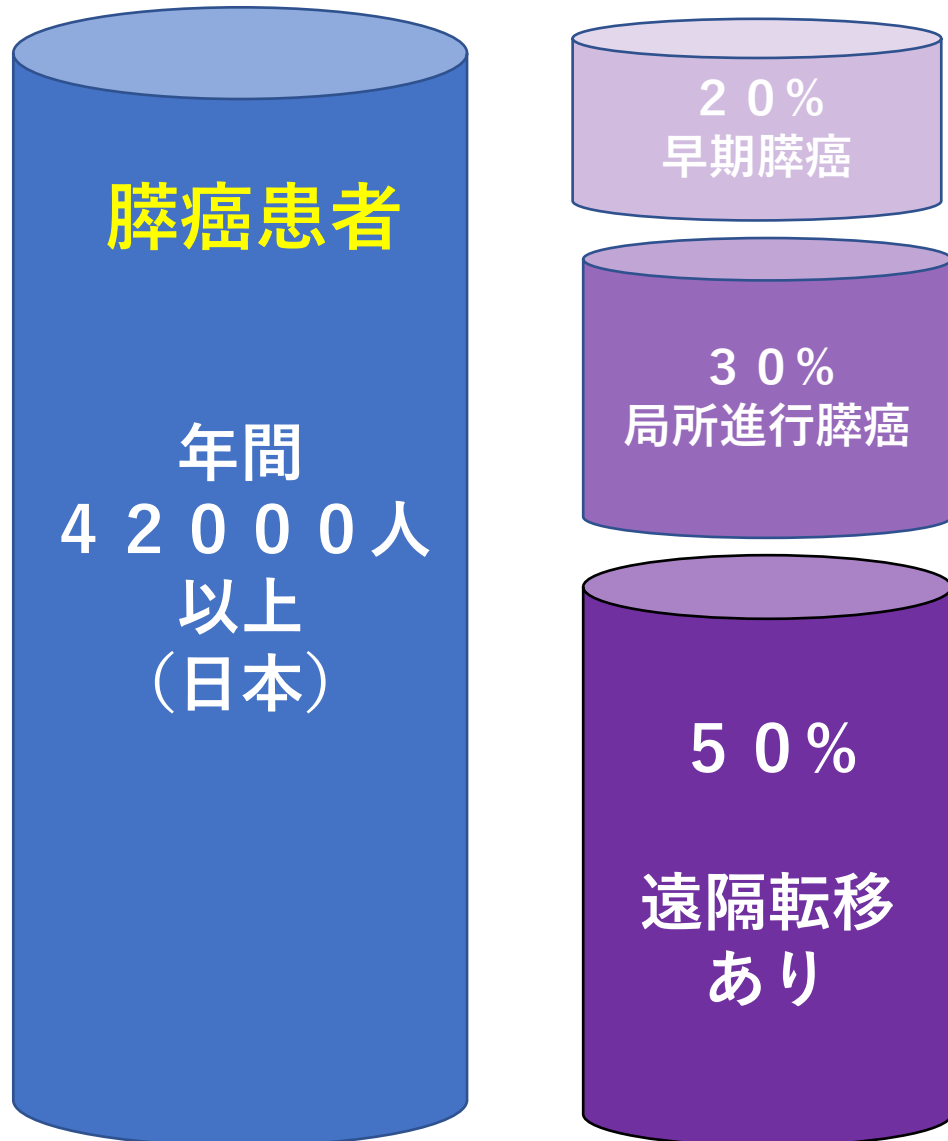
SMAD4 32%

Integrated Genomic Characterization of Pancreatic Ductal Adenocarcinoma. *Cancer Cell* 2017, 32(2):185-203.e113



# 膵癌の進行度別の分類

# 切除可能は20%のみ



切除可能膵癌 R Resectable

ステージ1～2相当

切除可能境界型膵癌 BR Borderline Resectable

動脈に浸潤を有する BR-A

門脈のみに浸潤を有する BR-PV

ステージ3相当

切除不能膵癌 UR Unresectable

局所進行切除不能 UR-LA

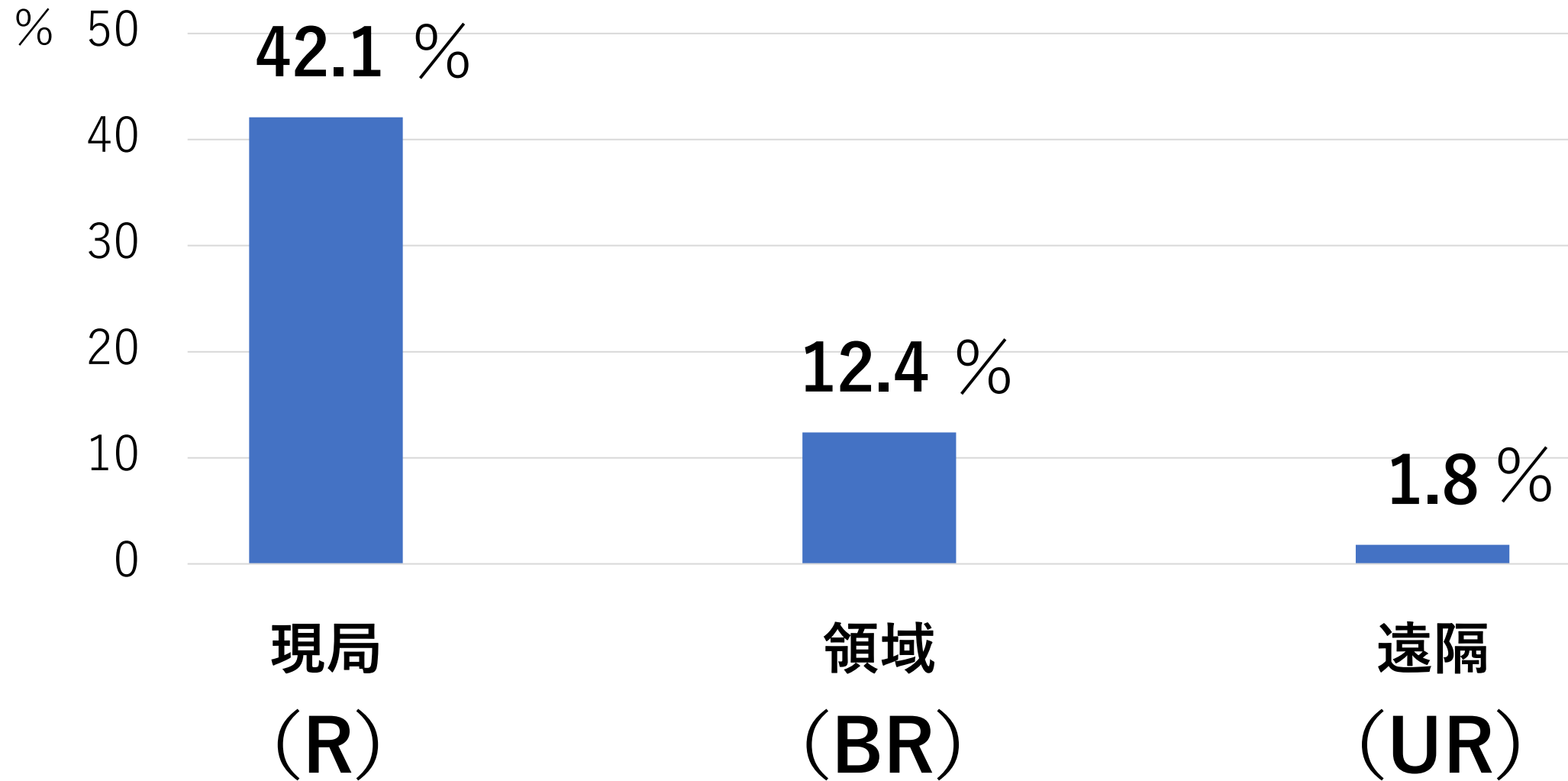
遠隔転移を有する膵癌 UR-M

ステージ4相当



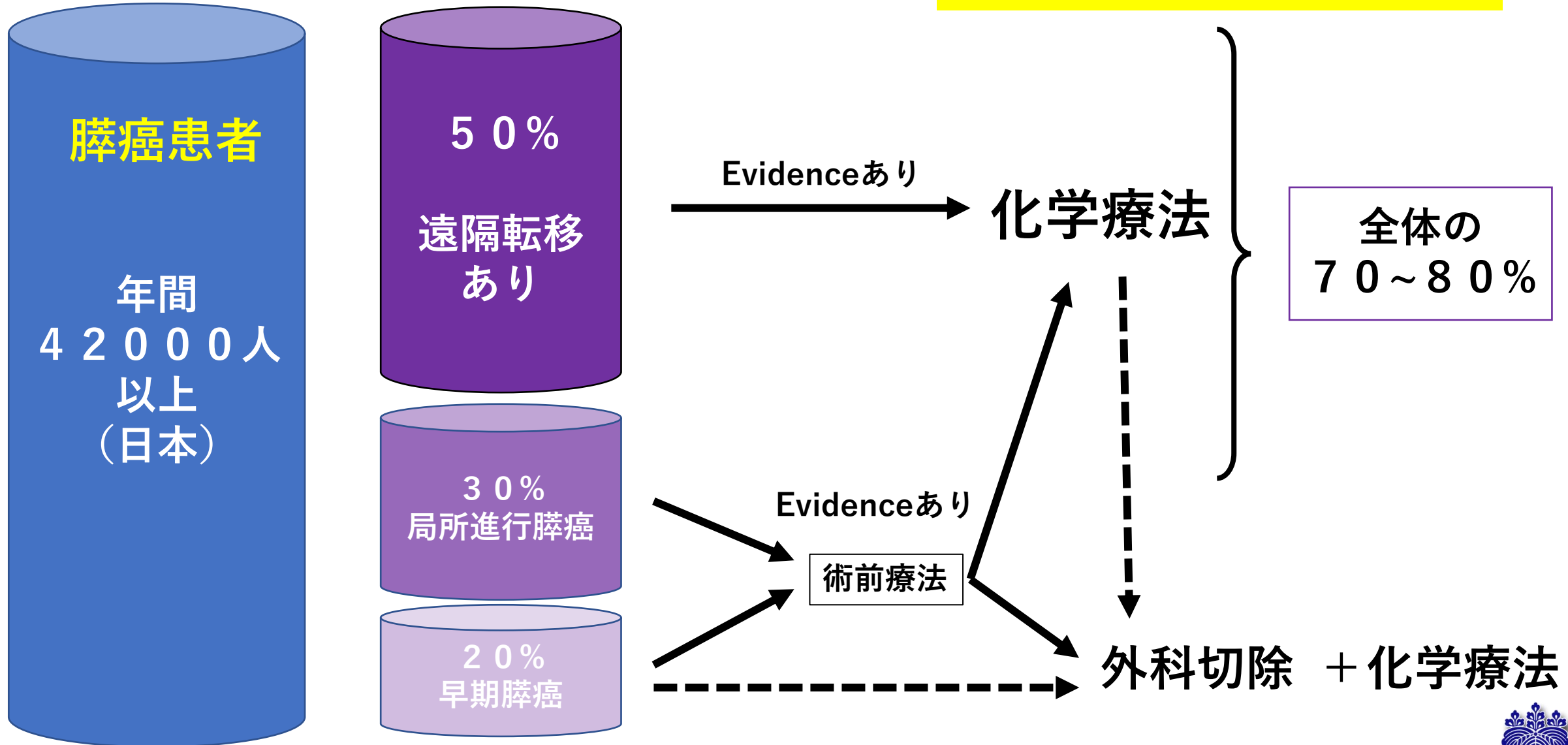


# 膵癌の進行度別の5年生存率



# 通常型膵癌の治療方針

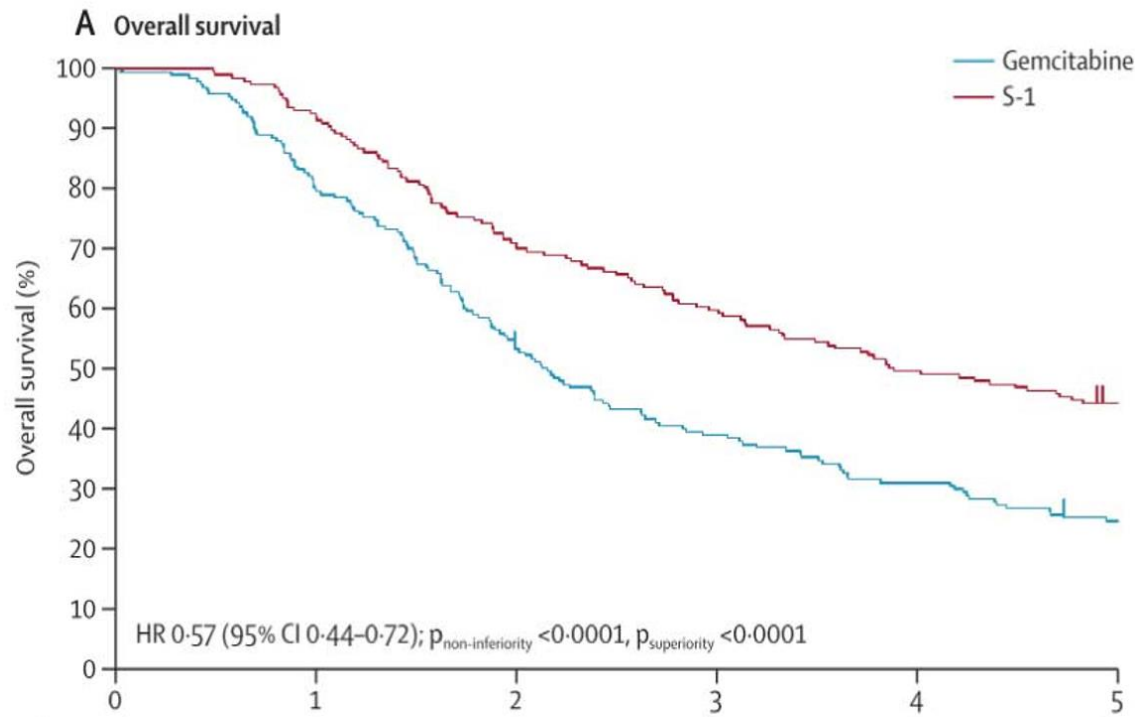
- ✓ 手術のみでは根治不能
- ✓ 全例化学療法が必要



# 切除可能患者に対する切除 + 抗がん剤の効果

20%  
早期膵癌

外科切除 + 化学療法



Number at risk	0	1	2	3	4	5
Gemcitabine	190	151	100	73	58	45
S-1	187	172	130	111	92	80

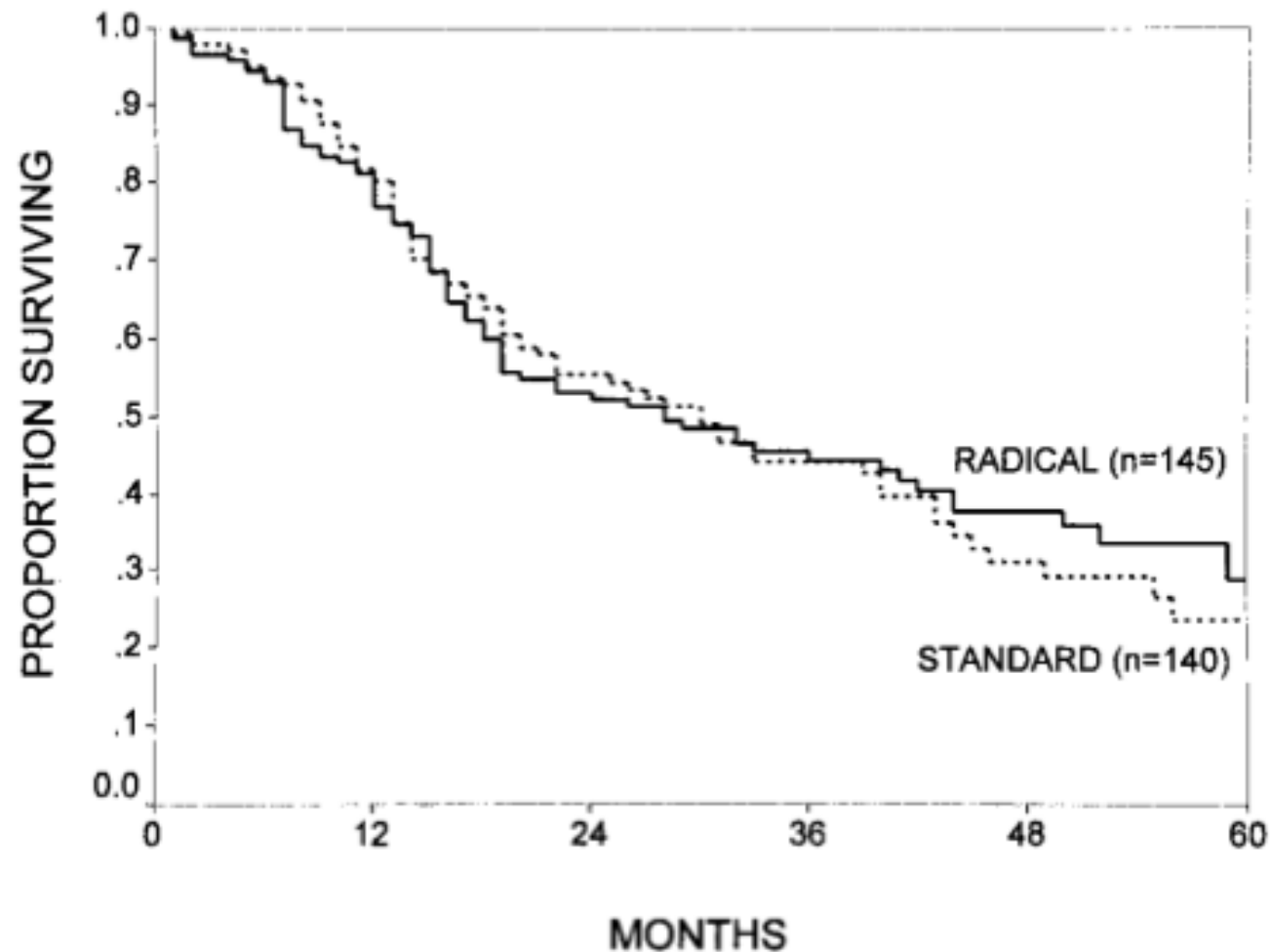
5年生存率: 44.1%  
5年無再発生存: 33.3%

Uesaka K et al., Lancet 2016



# 拡大手術 vs 標準手術 の生存率

(2002 The Johns Hopkins Medical Institutions, Baltimore, USA)



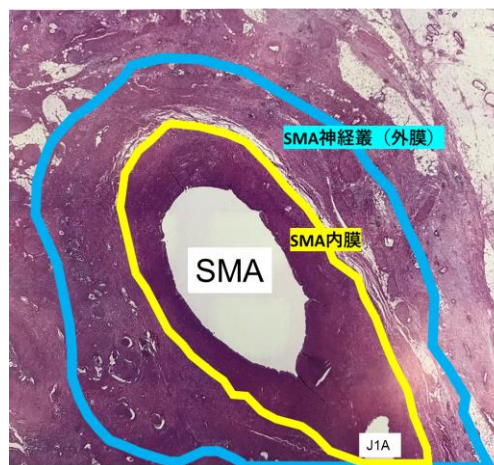
拡大郭清 vs. 標準郭清

**予後に差は無し！**



# 膵癌 拡大vs.標準 郭清 → 拡大リンパ節郭清の意義は否定

	Italy 多施設	USA J Hopkins	USA Mayo	Japan 多施設	Korea 多施設
発表年	1998年	2002年	2005年	2012年	2014年
症例数	40 vs 41	146 vs 148	40 vs 39	51 vs 50	83 vs 86
生存中央値 標準 vs 拡大 Month	11.2 vs 16.7	30 vs 28	26 vs 18.8	19.9 vs 13.8	18.8 vs 16.5
予後	有意差なし	有意差なし	有意差なし	有意差なし	有意差なし



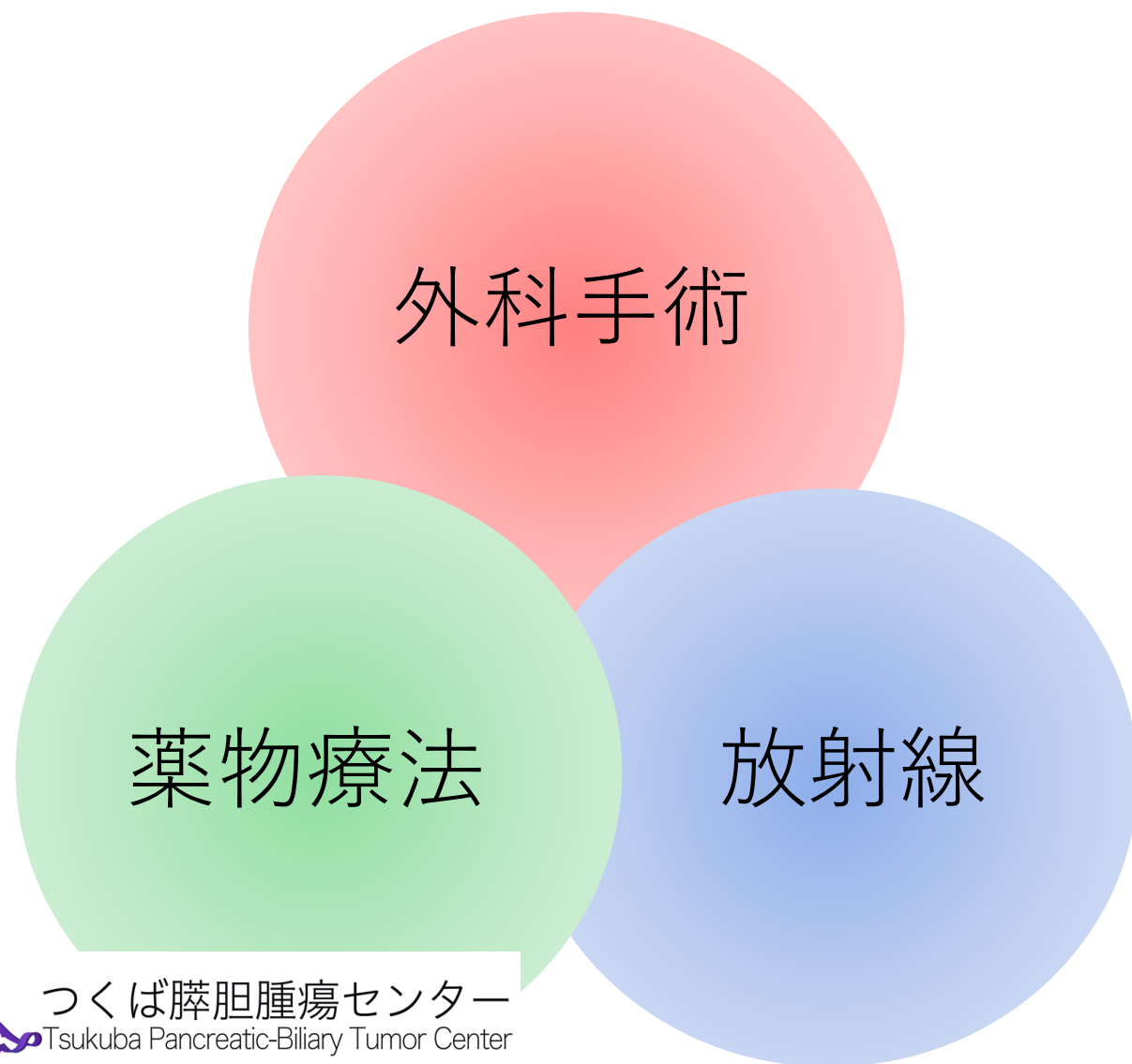
(藤井務、川井学ら 膵癌診療治療のバイブル p174より)



手術のみでは膵癌の予後改善は難しい



# 膵癌は集学的に治療する時代へ



外科手術

+

抗癌剤

+

放射線

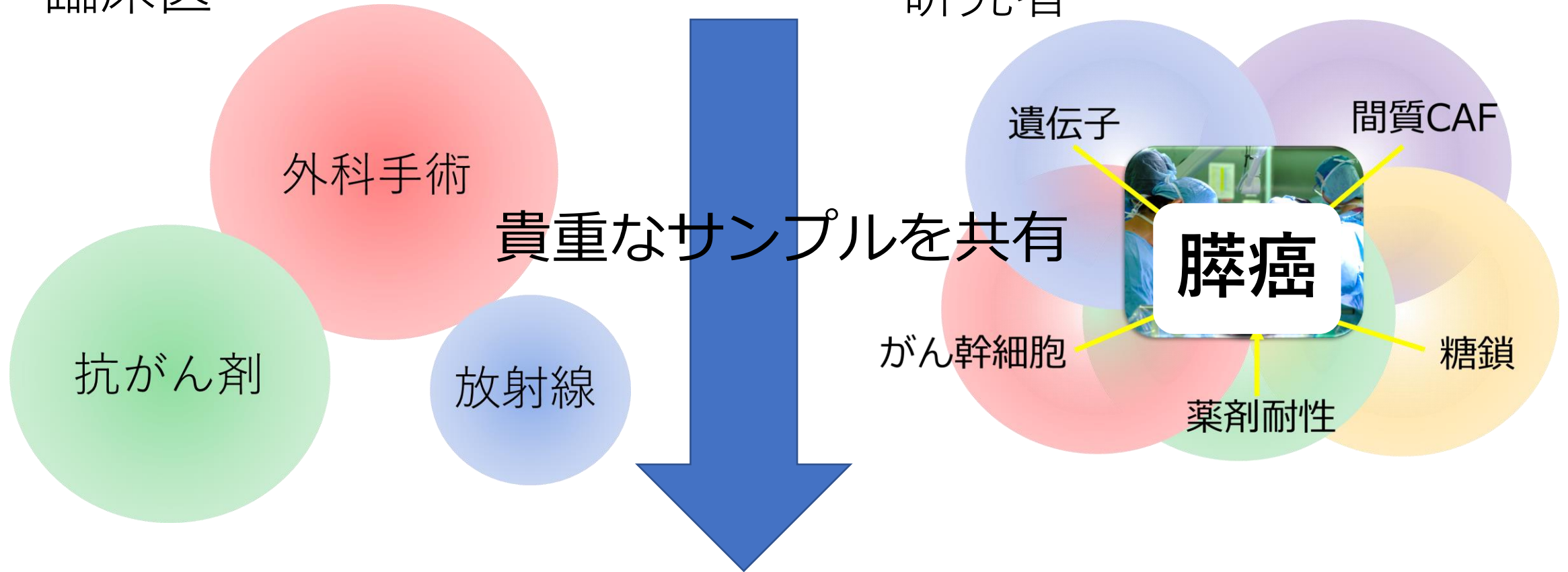
= 根治を目指す



# 最難治悪性腫瘍 膵癌への挑戦

臨床医

研究者



新薬開発、診断法開発 力を合わせることも重要



# 膵癌の予後を改善するには

- 1、 早期診断し、切除症例を増やす
- 2、 革新的な新規薬物治療を開発する
- 3、 病態に応じた真の個別化医療を導入する





# 膵癌の予後を改善するには

- 1、 早期診断し、切除症例を増やす
- 2、 革新的な新規薬物治療を開発する
- 3、 病態に応じた真の個別化医療を導入する



# 本セミナー内容

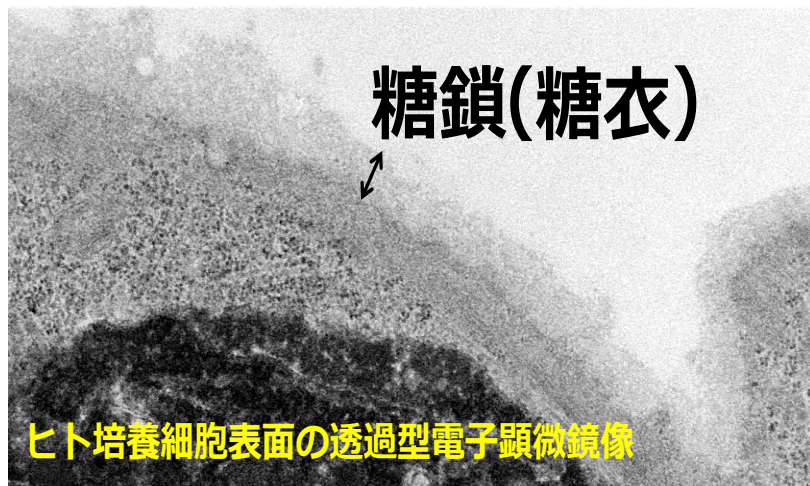
▶膵癌について 現状と課題

▶膵癌の糖鎖解析

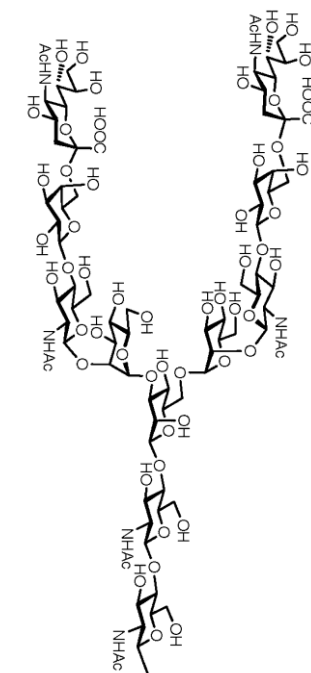
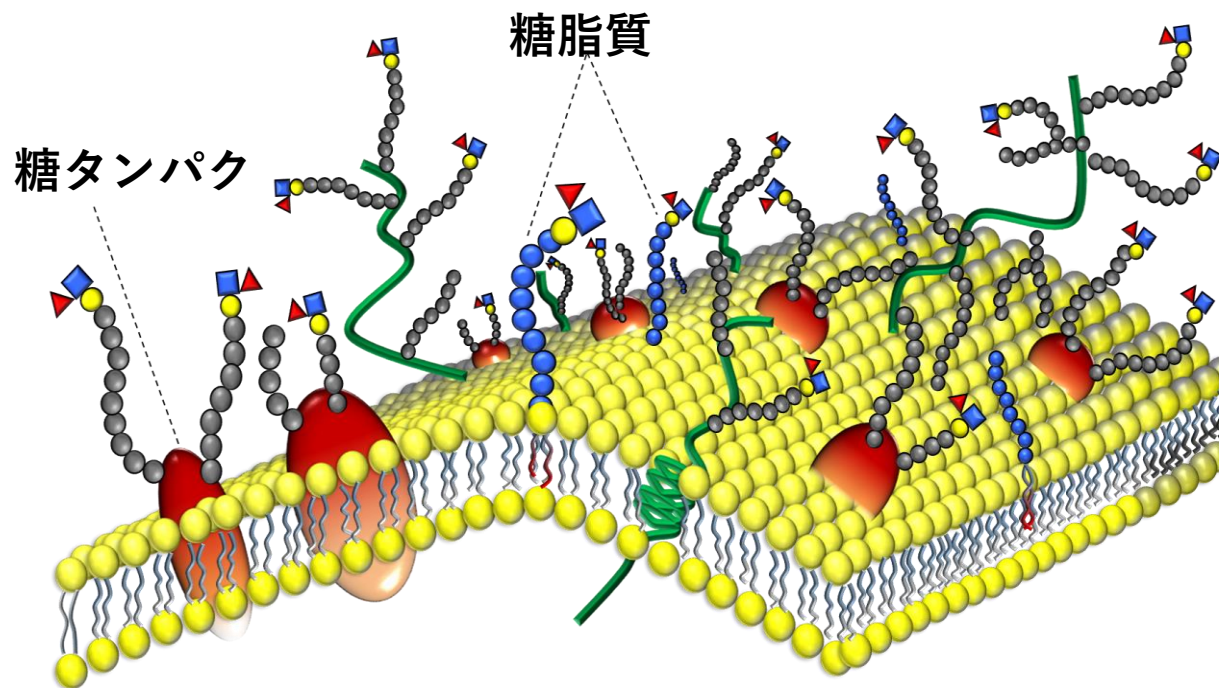
▶レクチンを用いた糖鎖標的治療研究 現状と課題



# がん細胞特有の「糖鎖」は、診断・治療の標的として魅力的



## 細胞の脂質二重膜



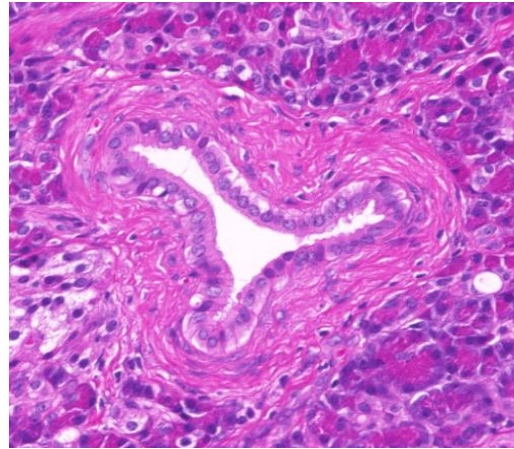
すべての細胞の最外層は「糖鎖」

糖鎖に着目した膵癌の診断、治療法開発

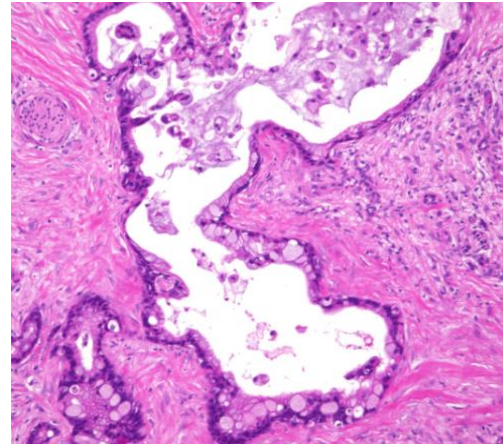


# 血清の腫瘍マーカーの多くは糖鎖の変化を検出

正常膵管



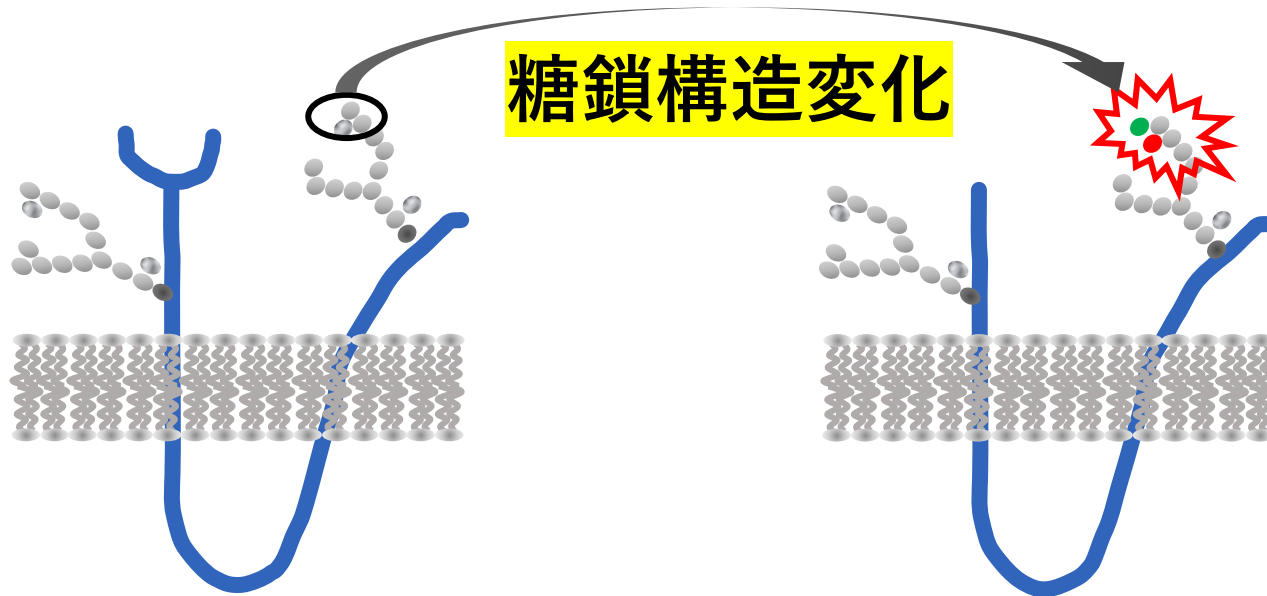
通常型膵癌



癌化



糖鎖構造変化



CA19-9  
DUPAN-2  
Span-1  
CEA  
CA125

• • • •



糖鎖の変化を検出

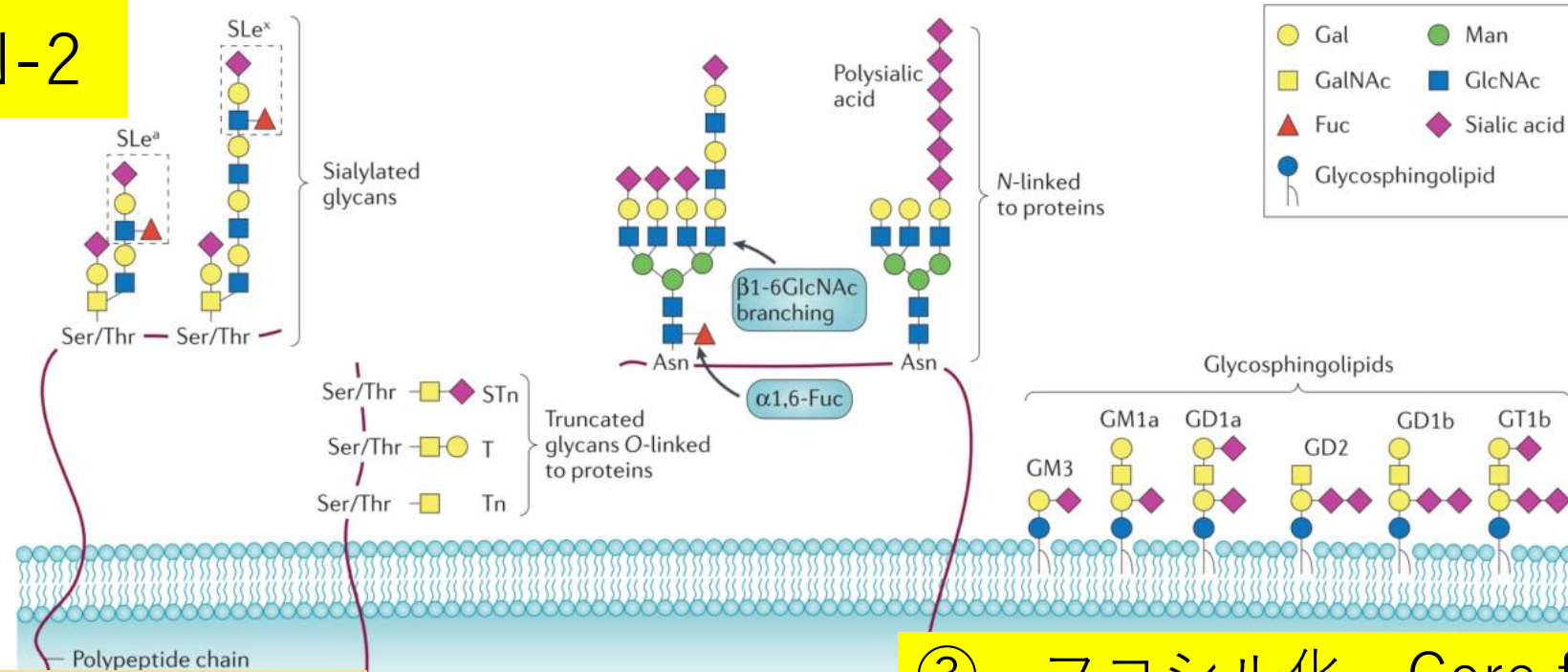


# 糖鎖解析の進歩による癌化に関わる糖鎖の解明

Pinho, S. S. and C. A. Reis (2015). Nat Rev Cancer 15(9): 540-555.

① シアル化  
CA19-9  
DUPAN-2

④ N型糖鎖の分岐の変化



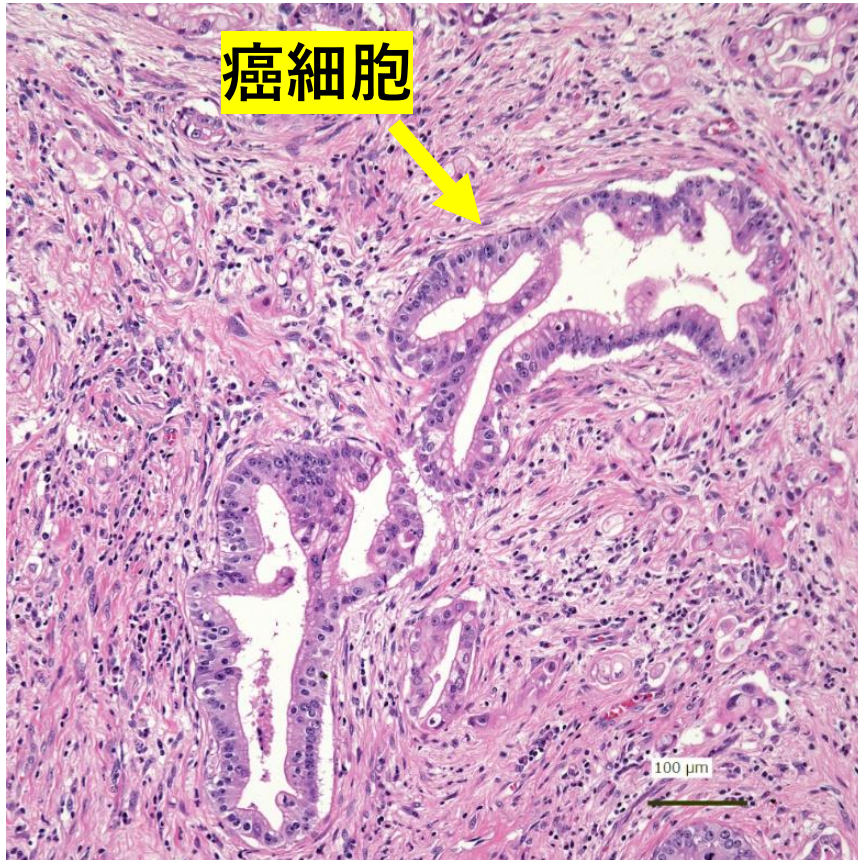
② O型糖鎖の短縮

③ フコシル化 Core fucosylation  
AFP-L3 = core fucosylation of AFP

癌化に伴う糖転移酵素、微小環境の変化による



# 膵癌の糖鎖解析に向けた課題



- ✓ 癌細胞のHeterogeneity
- ✓ 豊富に存在する間質

糖鎖解析にはこれらを考慮する必要があった

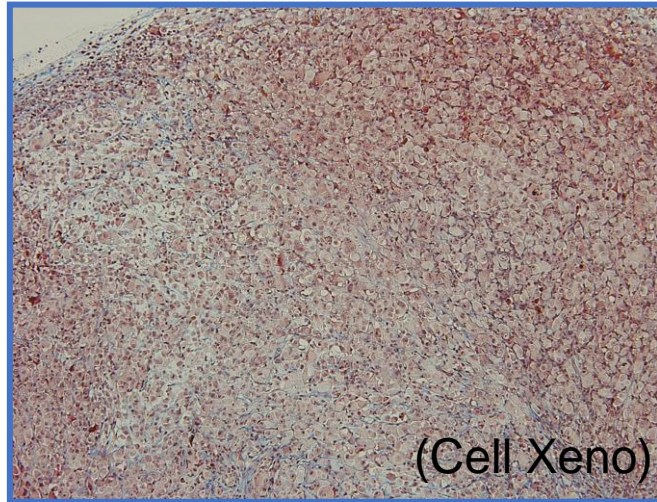
## 膵癌の組織像



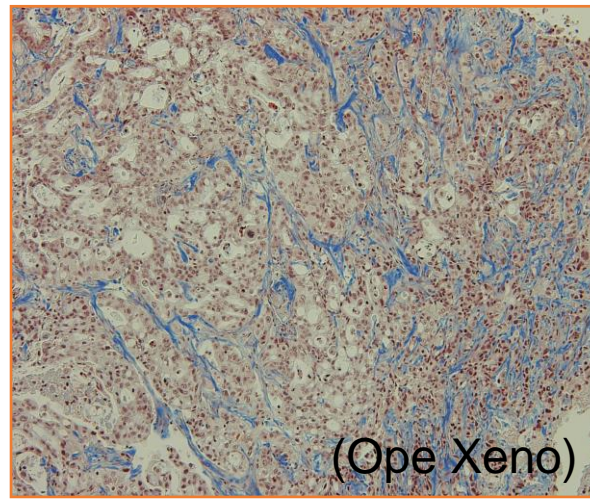
# 膵癌各種モデルの間質量

Akashi Y, Oda T et al., Pancreas 2013

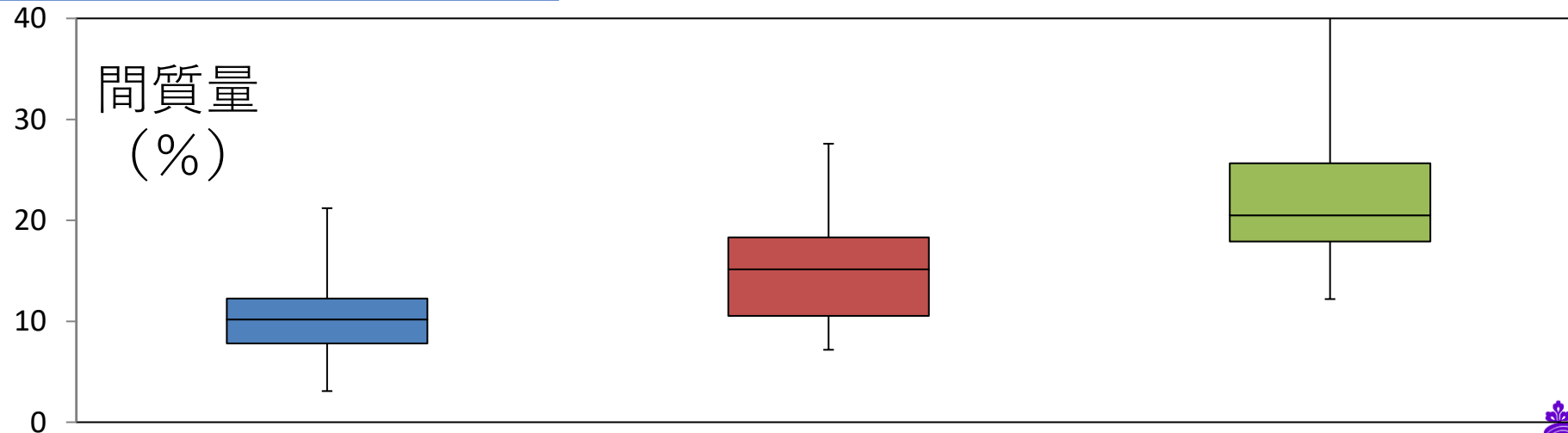
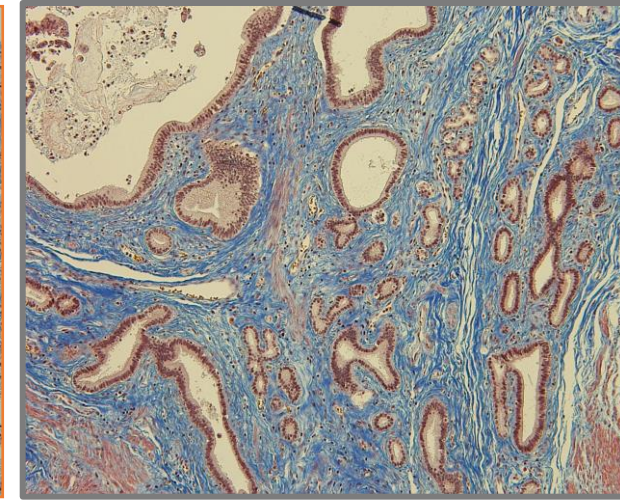
細胞株→マウス移植



ヒト膵癌→マウス移植



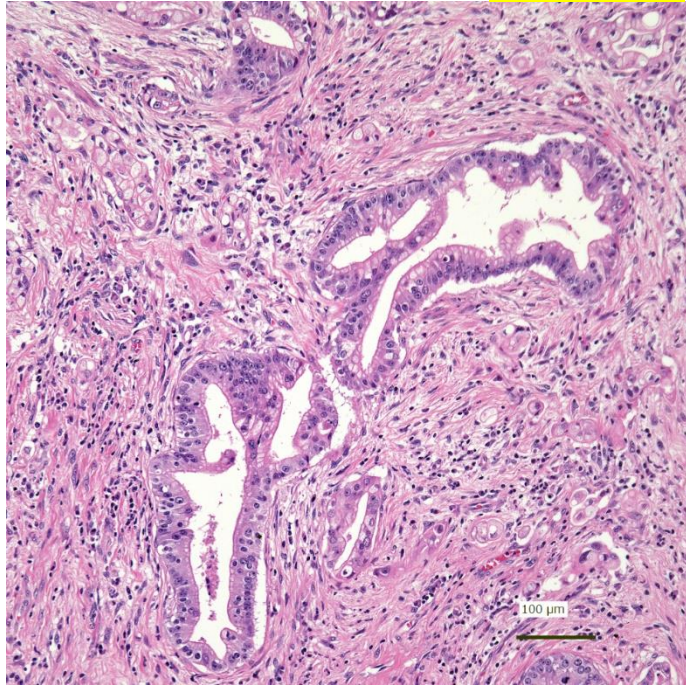
ヒト患者由来膵癌



# 膵腺癌(PDAC)に近い組織像を呈する “Capan-1”

臨床膵癌

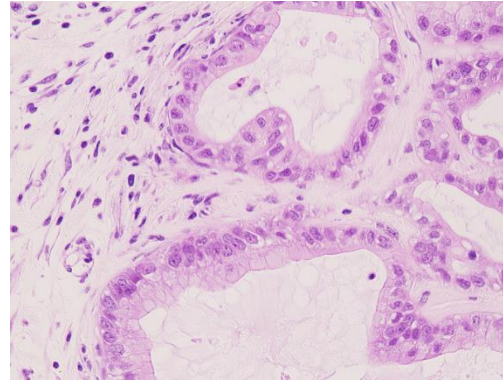
g++/s++



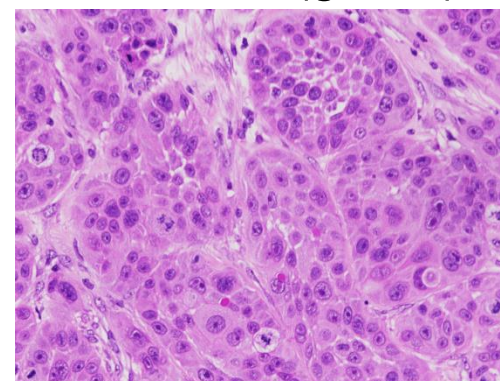
腺管形成能 (g -/+ /+++),  
間質増生 (s -/+ /+++)

膵癌細胞株マウス移植モデル

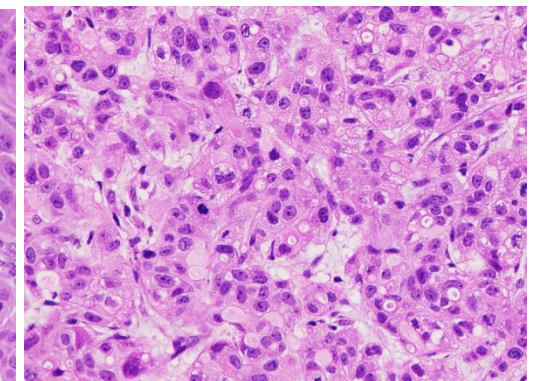
Capan-1 (g++/s++)



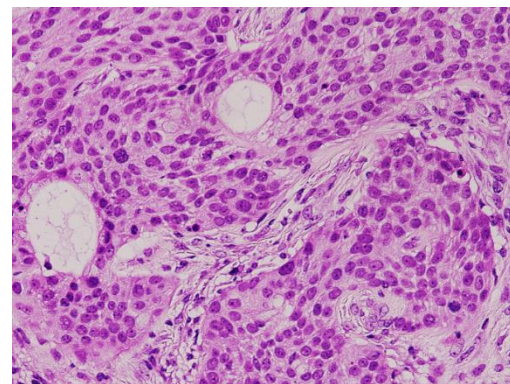
BxPC-3 (g+/s+)



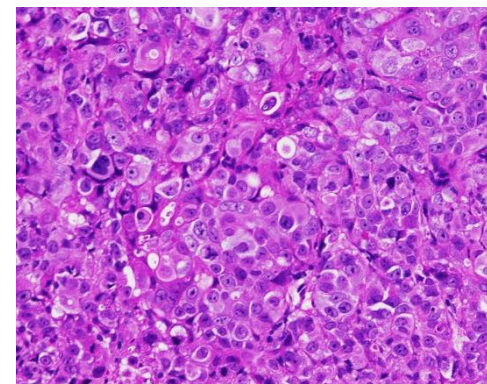
ASPC-1 (g+/s-)



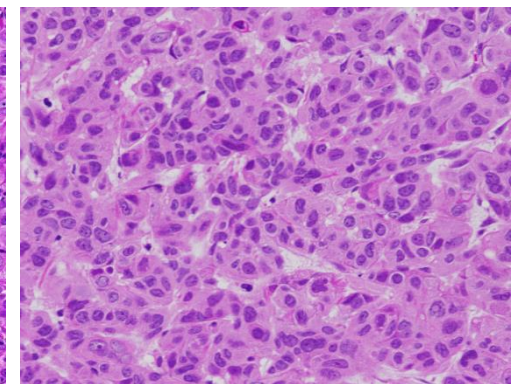
MIAPaCa-2 (g-/s+)



PANC-1 (g-/s-)

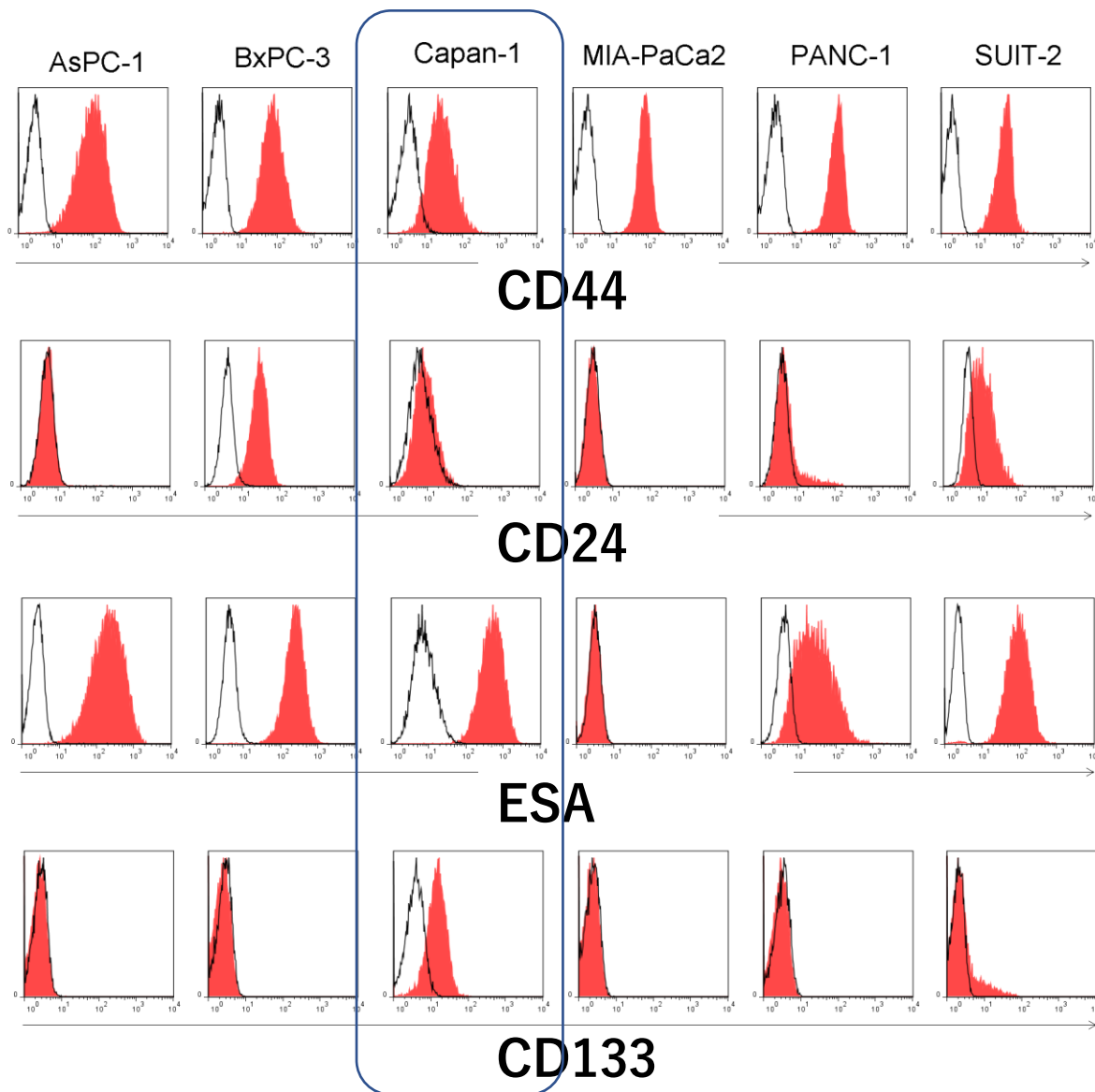


SUIT-2 (g-/s-)





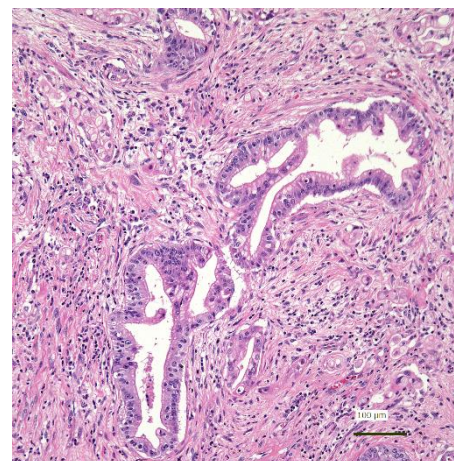
# 膵癌幹細胞発現解析



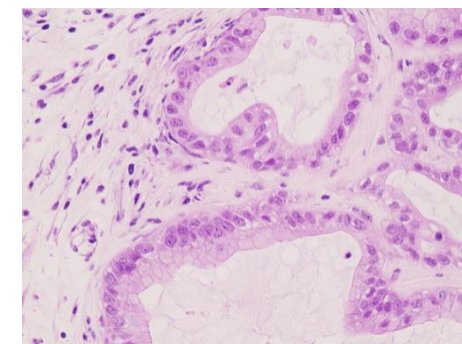
## 膵癌幹細胞マーカー

- 1 : CD24<sup>+</sup>、CD44<sup>+</sup>、ESA<sup>+</sup> Li et al. Cancer Res 2007
- 2 : CD133<sup>+</sup>、(CXCR4) Hermann PC et al. Cell Stem Cell 2007

PDAC g<sup>+++</sup>/s<sup>+++</sup>



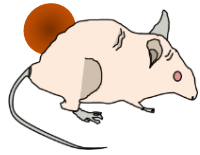
Capan-1 g<sup>+++</sup>/s<sup>+++</sup>



≡

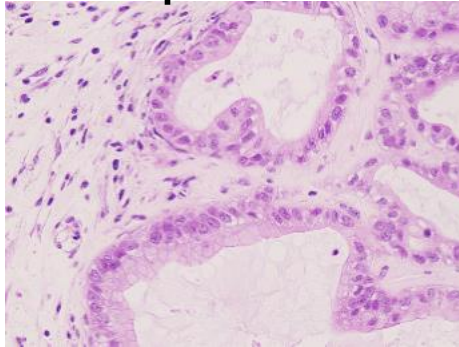


# レクチンマイクロアレイによる膵癌細胞株の糖鎖発現解析



Xenograft morphology

Capan-1



PDAC  
Cell lines

VS

各種膵癌細胞株

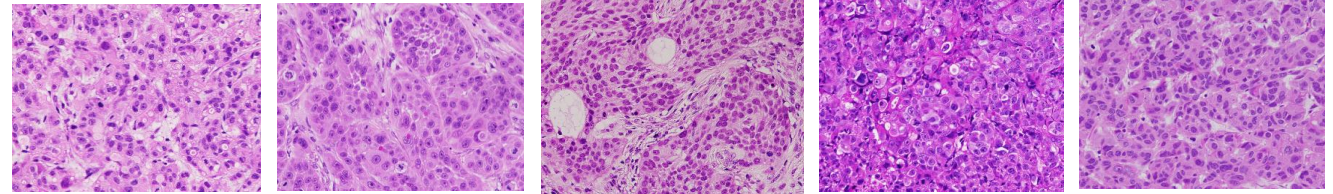
ASPC-1

BxPC-3

MIAPaCa-2

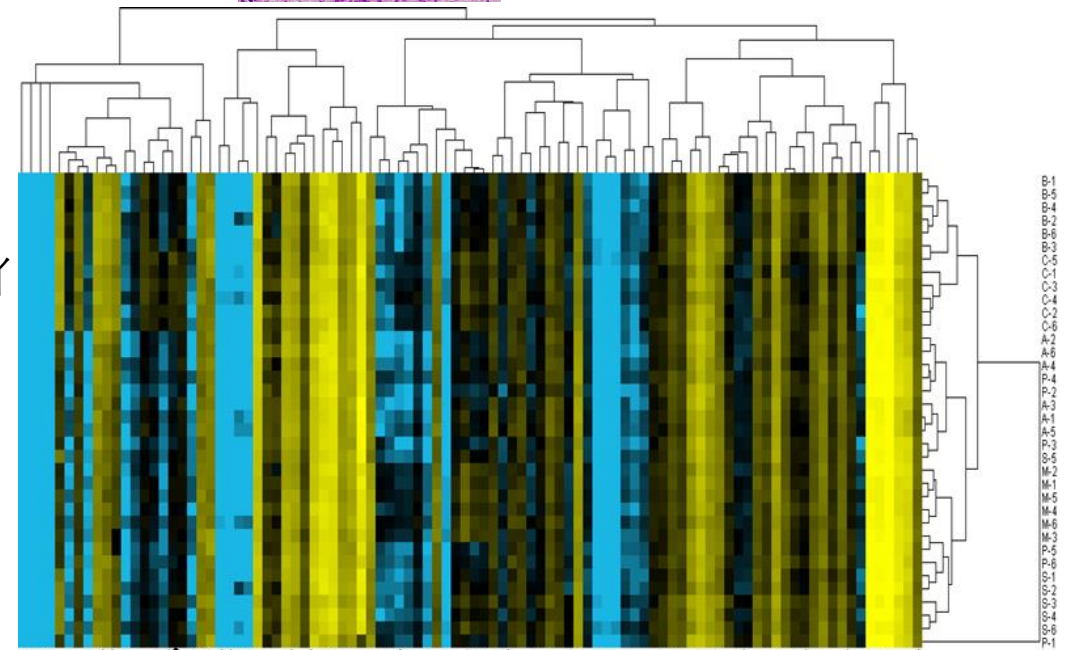
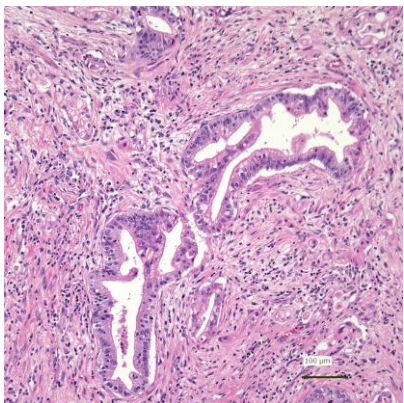
PANC-1

SUIT-2



臨床膵癌

96レクチンマイクロアレイ



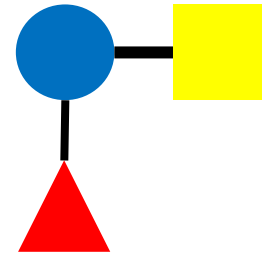
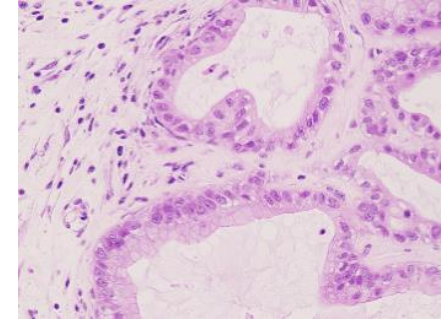
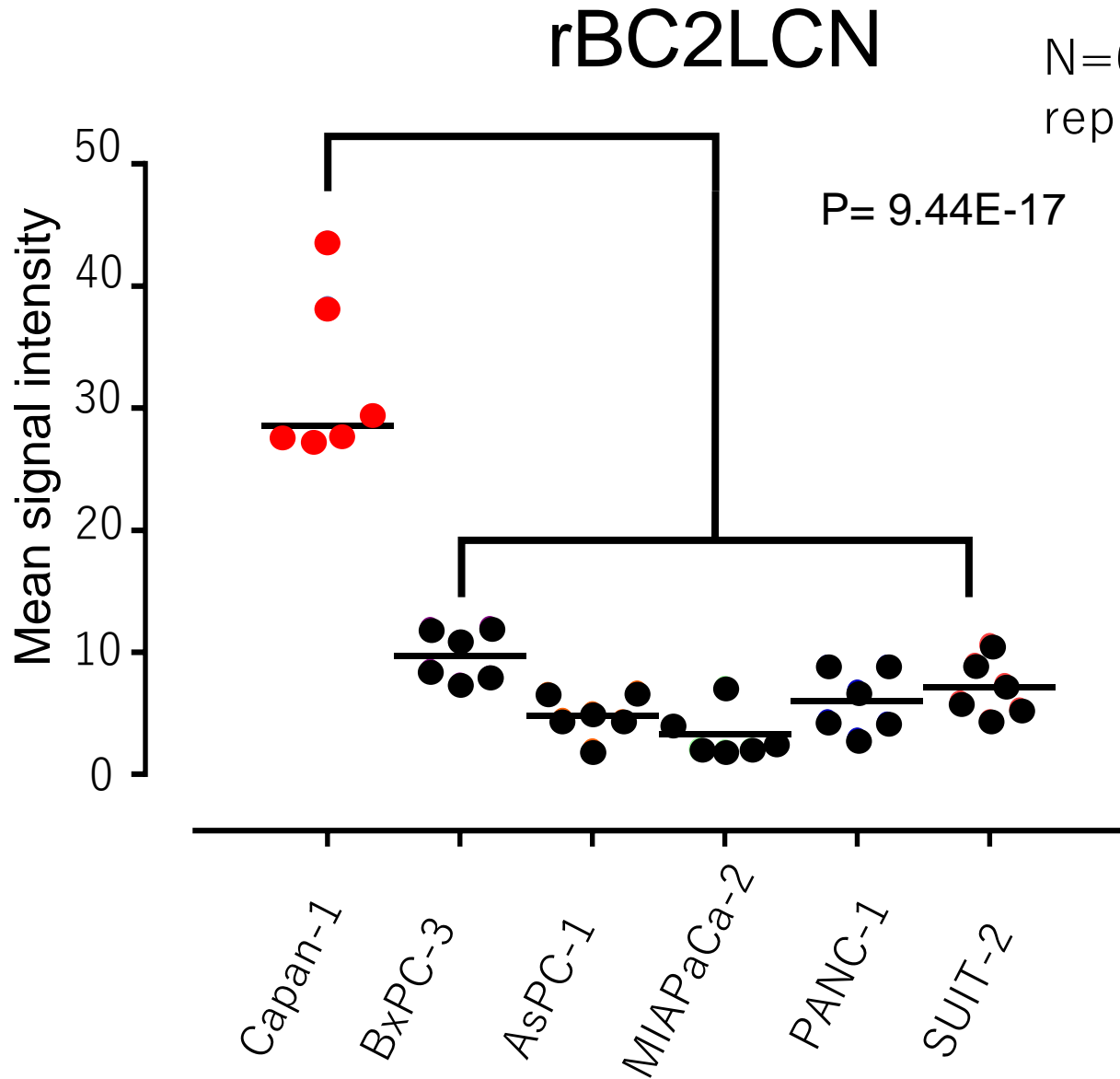
rBC2LCN lectin



96 lectins



# Capan-1に最も特異的に反応するrBC2LC-N



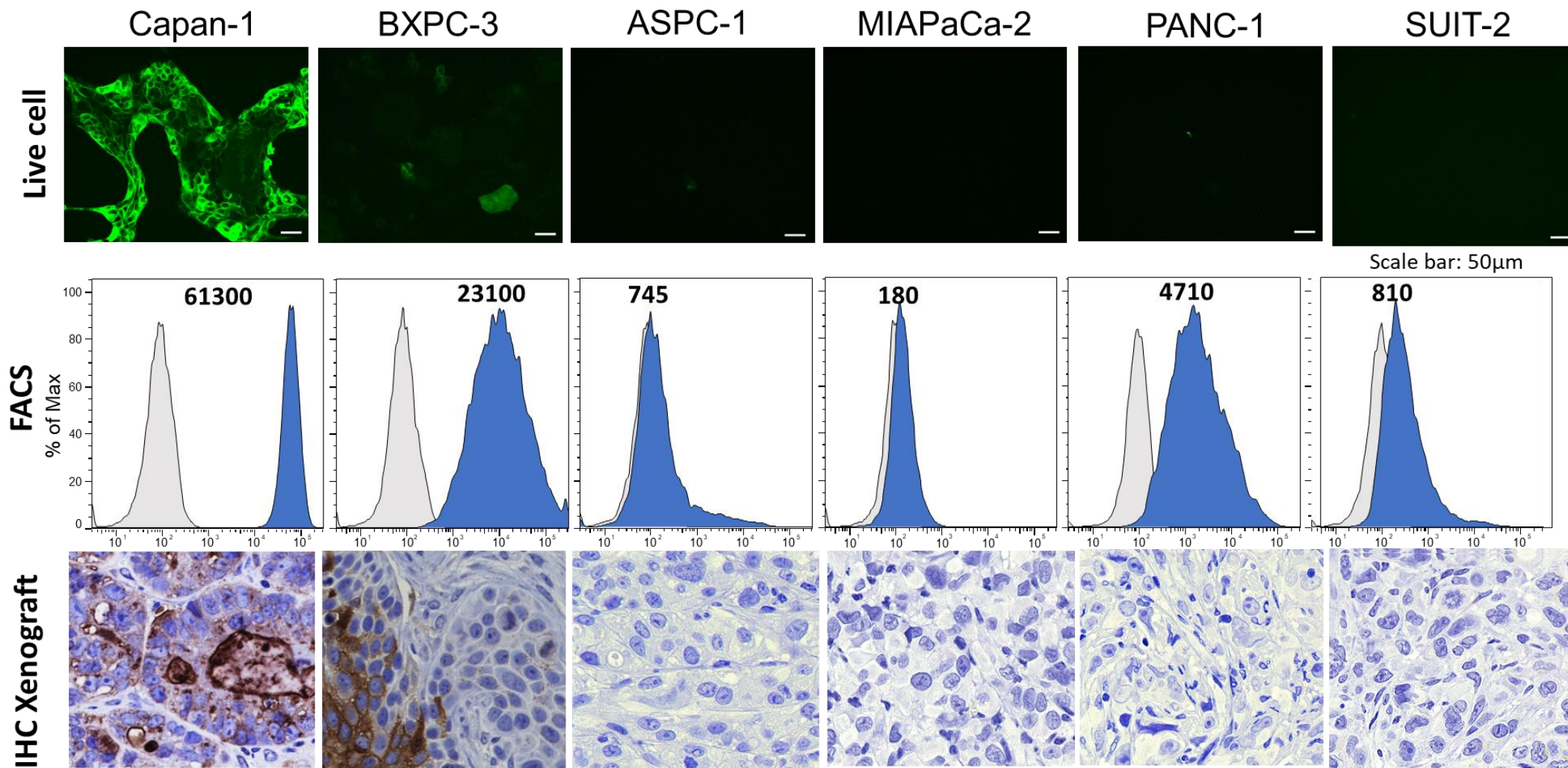
## H type 1/3/4構造糖鎖を認識

Fuc  $\alpha$  1-2Gal  $\beta$  1-3(GlcNac or GalNAc or Glc)  
H type 1, Lewis b and Lewis Y sequences

Sulak, O., et al. *Structure* 2010



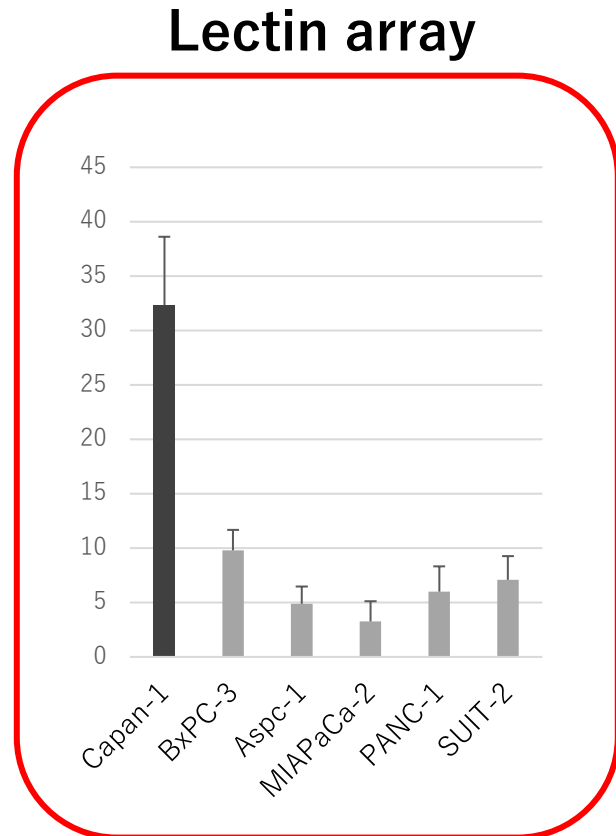
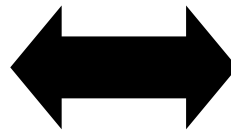
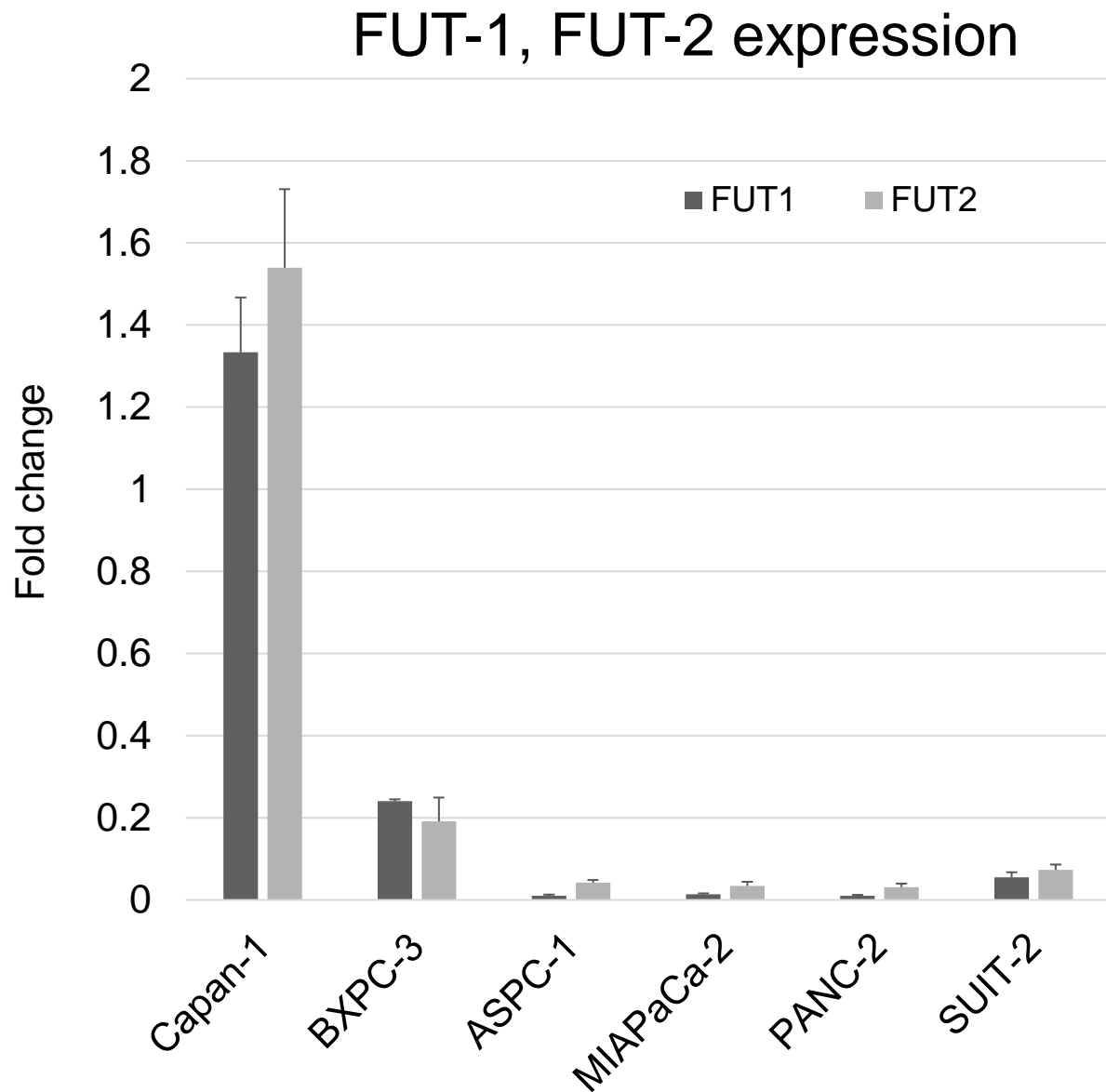
# rBC2LCNレクチンの各種膵癌細胞への反応性



マウス皮下腫瘍の染色



# 糖転移酵素の発現比がレクチンアレイ結果を再現

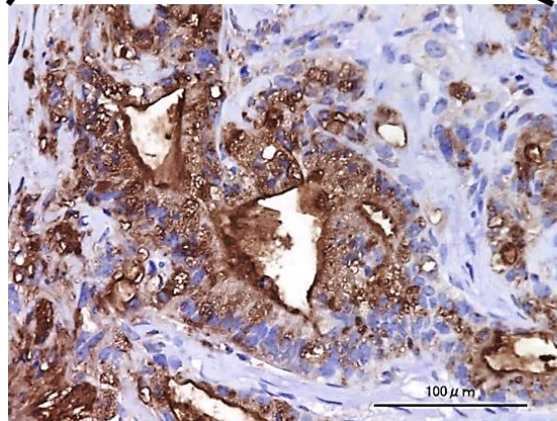
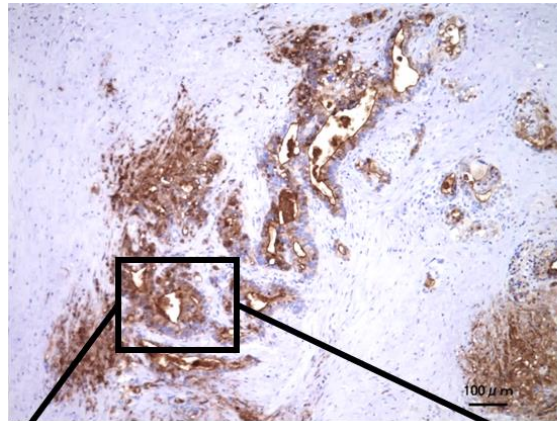


(RT-qPCR ; relative to GAPDH)

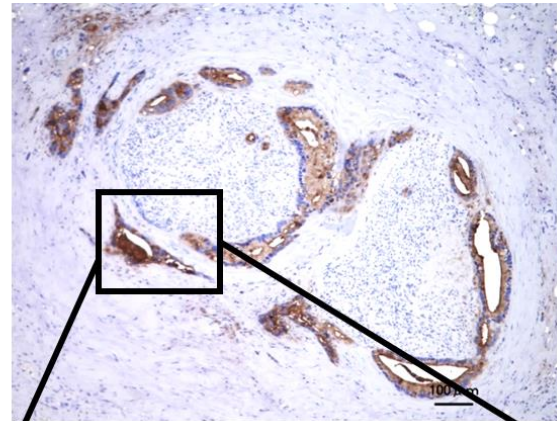


# rBC2LCN レクチンが 臨床膵癌に強く反応することを同定

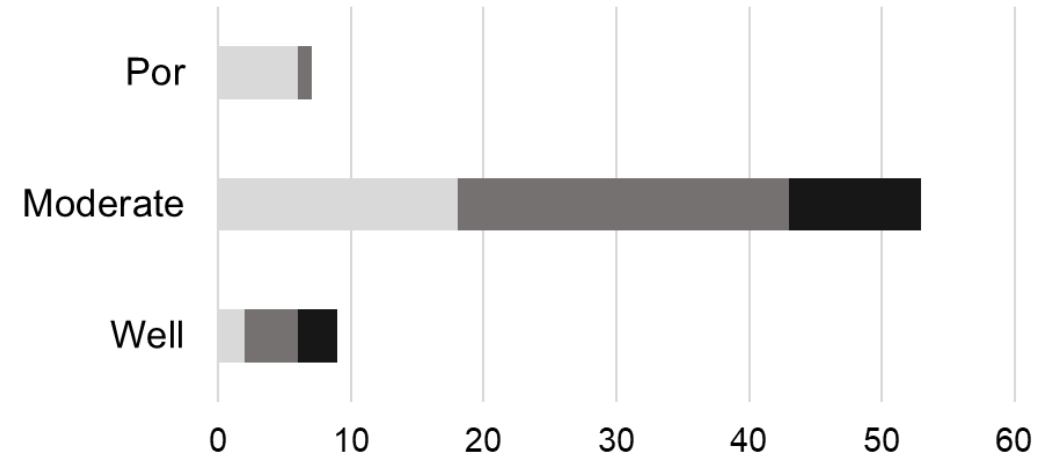
CASE1



CASE2



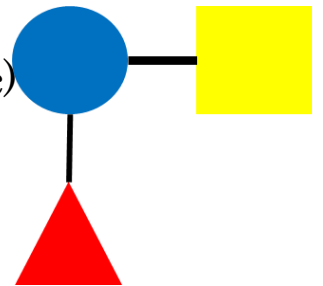
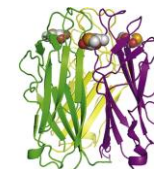
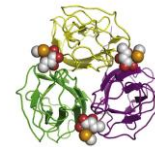
69/69全症例に陽性



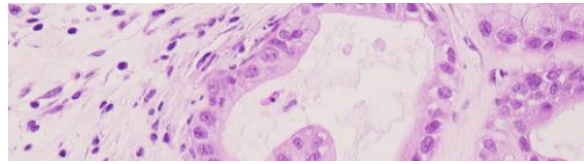
H type 1/3/4糖鎖構造を認識

Fuca1-2Galβ1-3(GlcNac or GalNAc or Glc)  
H type 1, Lewis b and Lewis Y sequences

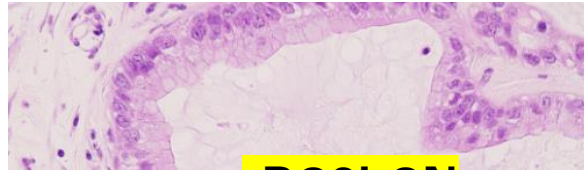
A



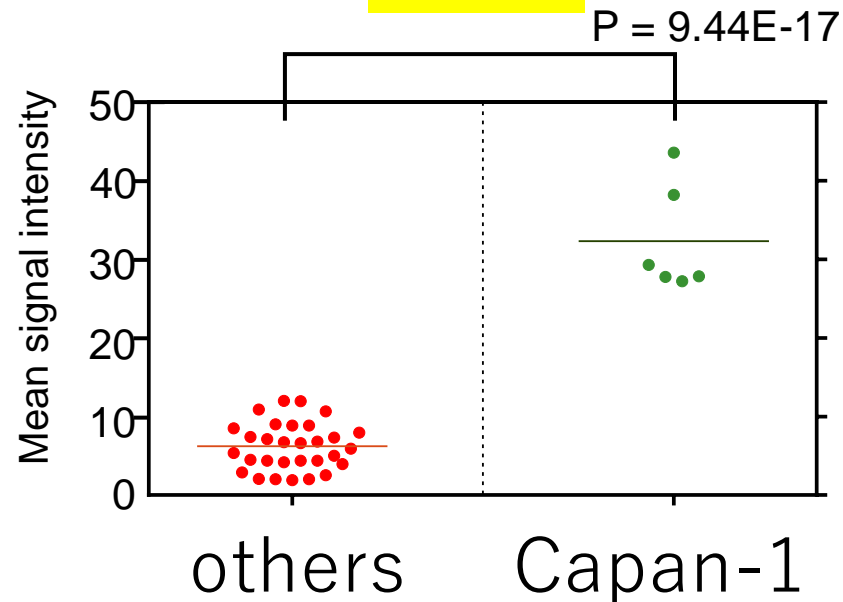
# rBC2LCNの反応性が、膵癌と未分化iPS細胞で偶然一致



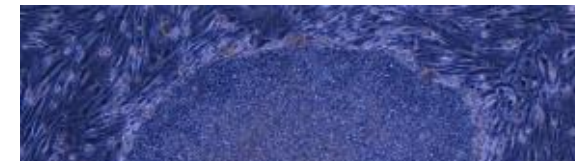
膵がん細胞



rBC2LCN



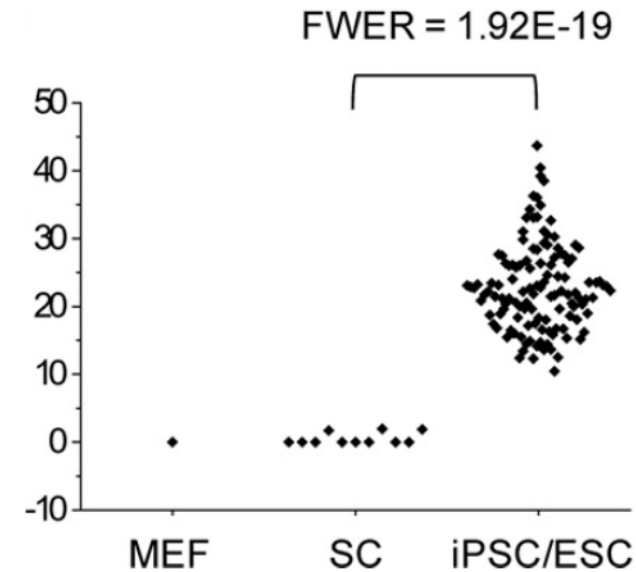
Shimomura et al. Mole Can Thera 2018



iPS/ES 細胞



rBC2LCN

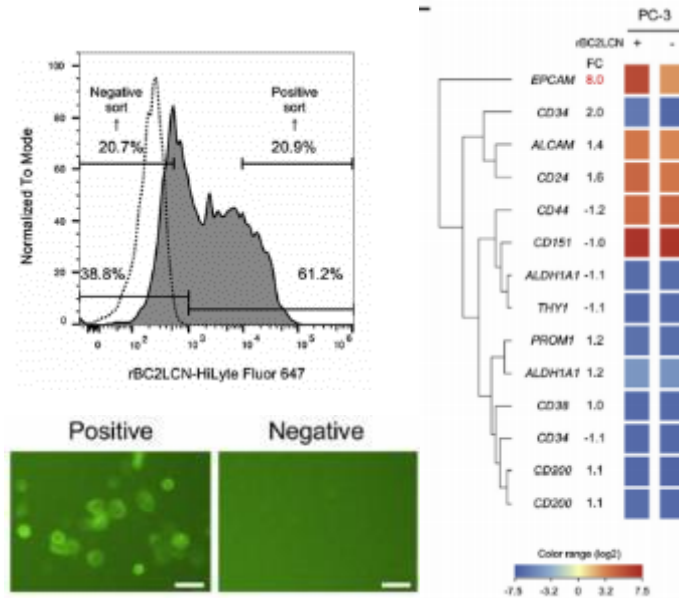


Tateno et al. JBC 2011



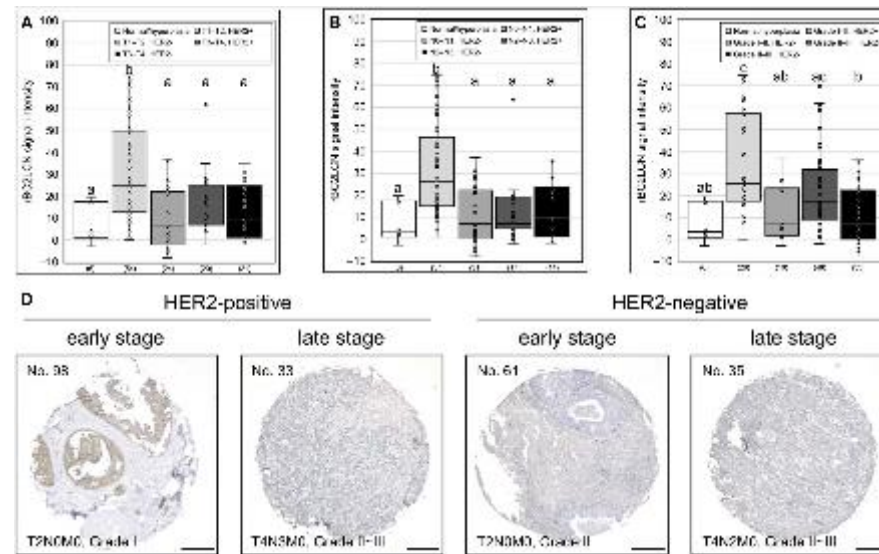
# 癌細胞におけるrBC2LCNレクチンの報告

## 前立腺癌



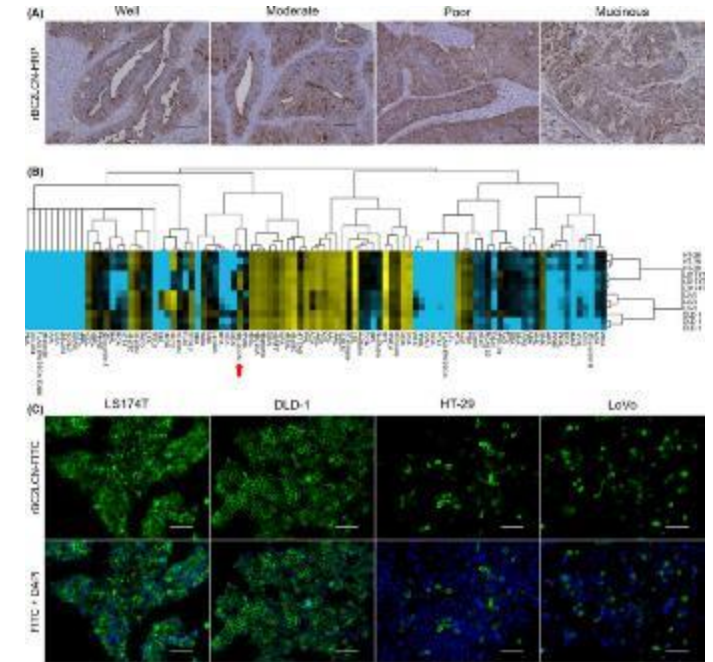
Mawaribuchi et al. BBRC 2019

## 乳癌



Mawaribuchi et al. FEBS open bio. 2020

## 大腸癌



Kitaguchi et al. Cancer sci. 2020

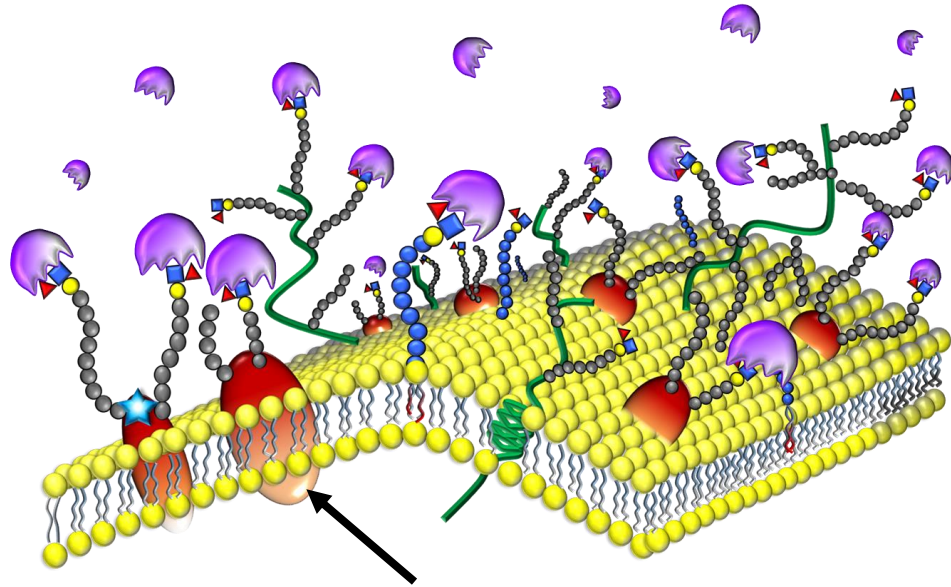
- ✓ 複数の癌種での報告
- ✓ rBC2LCN認識糖鎖と細胞の増殖速度の低下、細胞運動性の増加、薬剤耐性などの癌幹細胞に似た特徴との関連が示唆





# 膵癌細胞膜に存在するrBC2LCN陽性タンパクの追求

Furuta T, Shimomura O, Tateno H et al., Cancer Science 2021



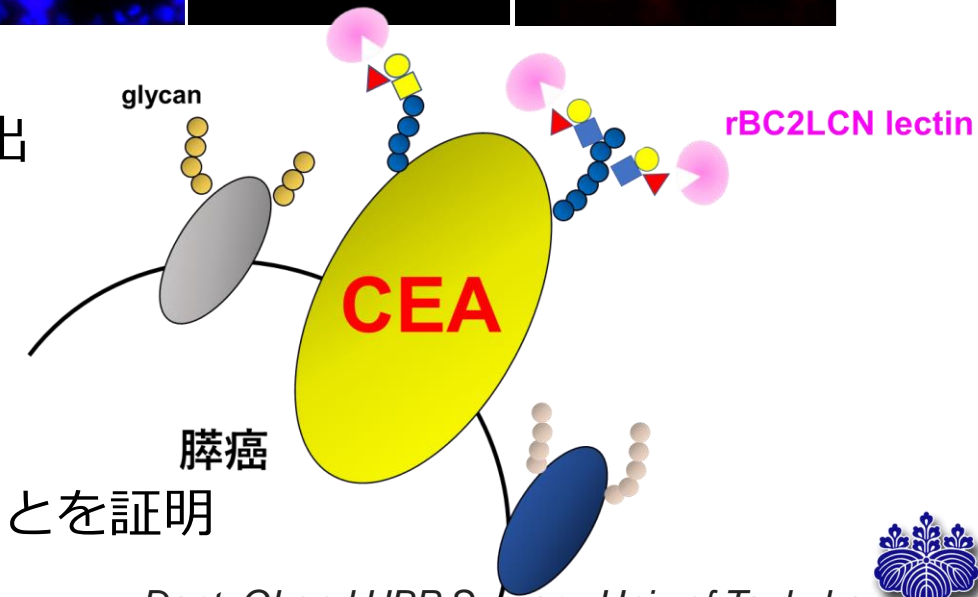
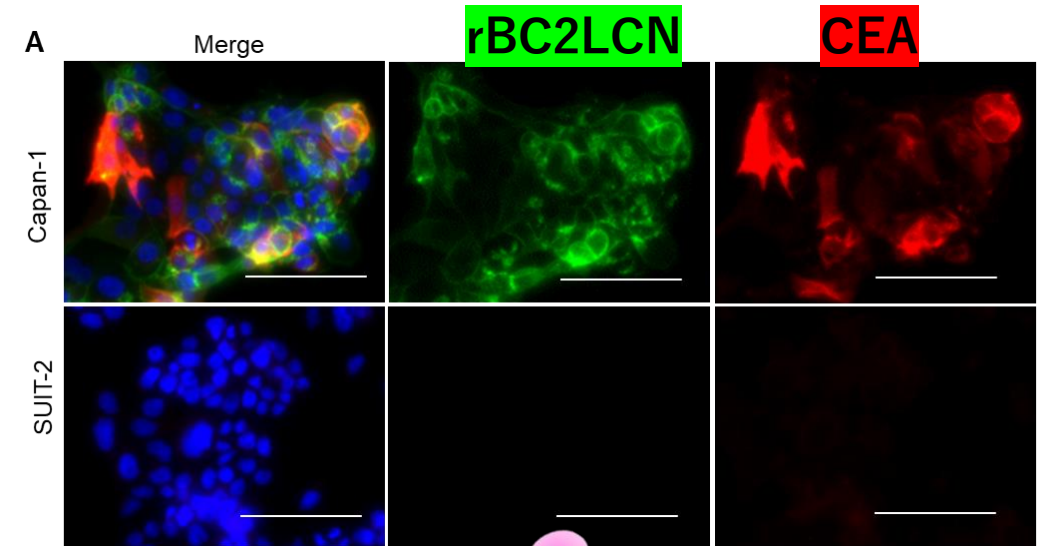
## 膵癌細胞膜のコアタンパクの解析

膵癌細胞より抽出したタンパクからrBC2LCN陽性分画を抽出



プロテオミクス解析の結果、40の候補糖タンパク質を同定

膵癌細胞表面では、**CEA**が一つのKeyタンパクであることを証明



# 本セミナー内容

▶膵癌について 現状と課題

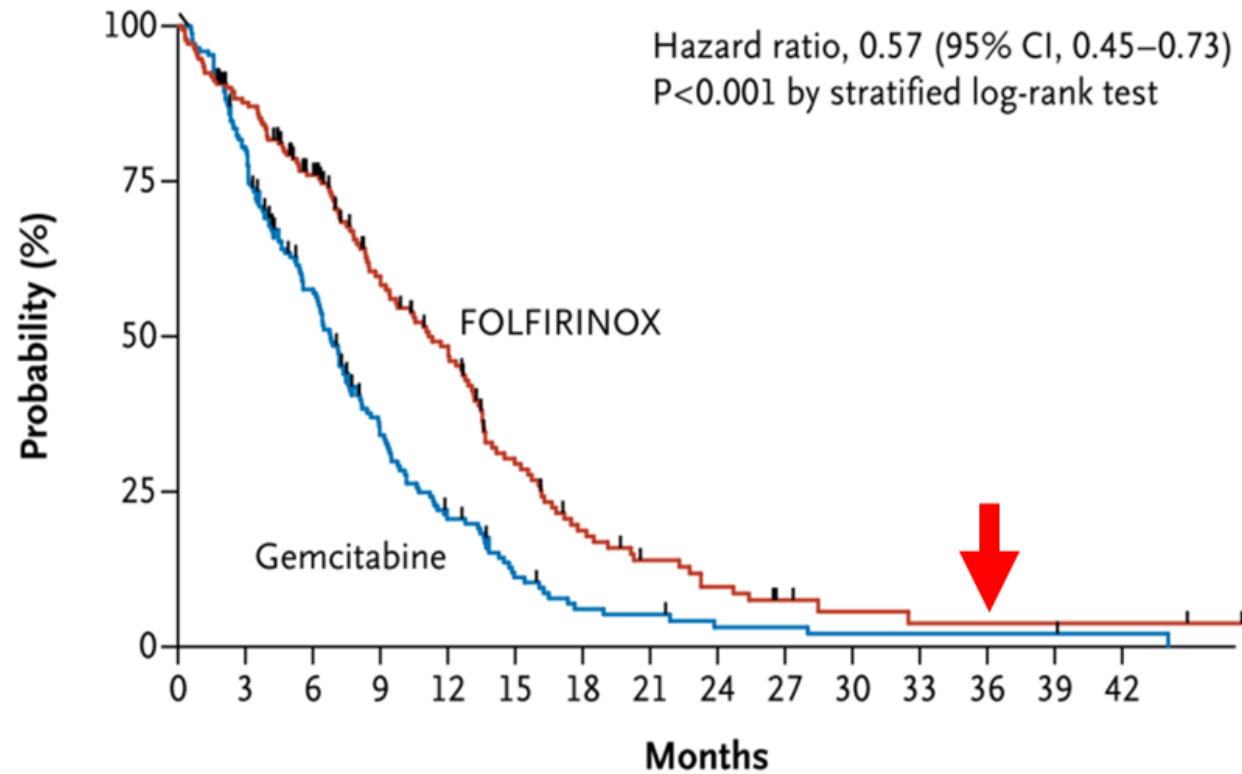
▶膵癌の糖鎖解析

▶レクチンを用いた糖鎖標的治療研究 現状と課題



# 転移を有する膵癌 抗がん剤の効果

ステージ4（転移）膵癌に対するFOLFIRINOX療法



ACCORD11試験

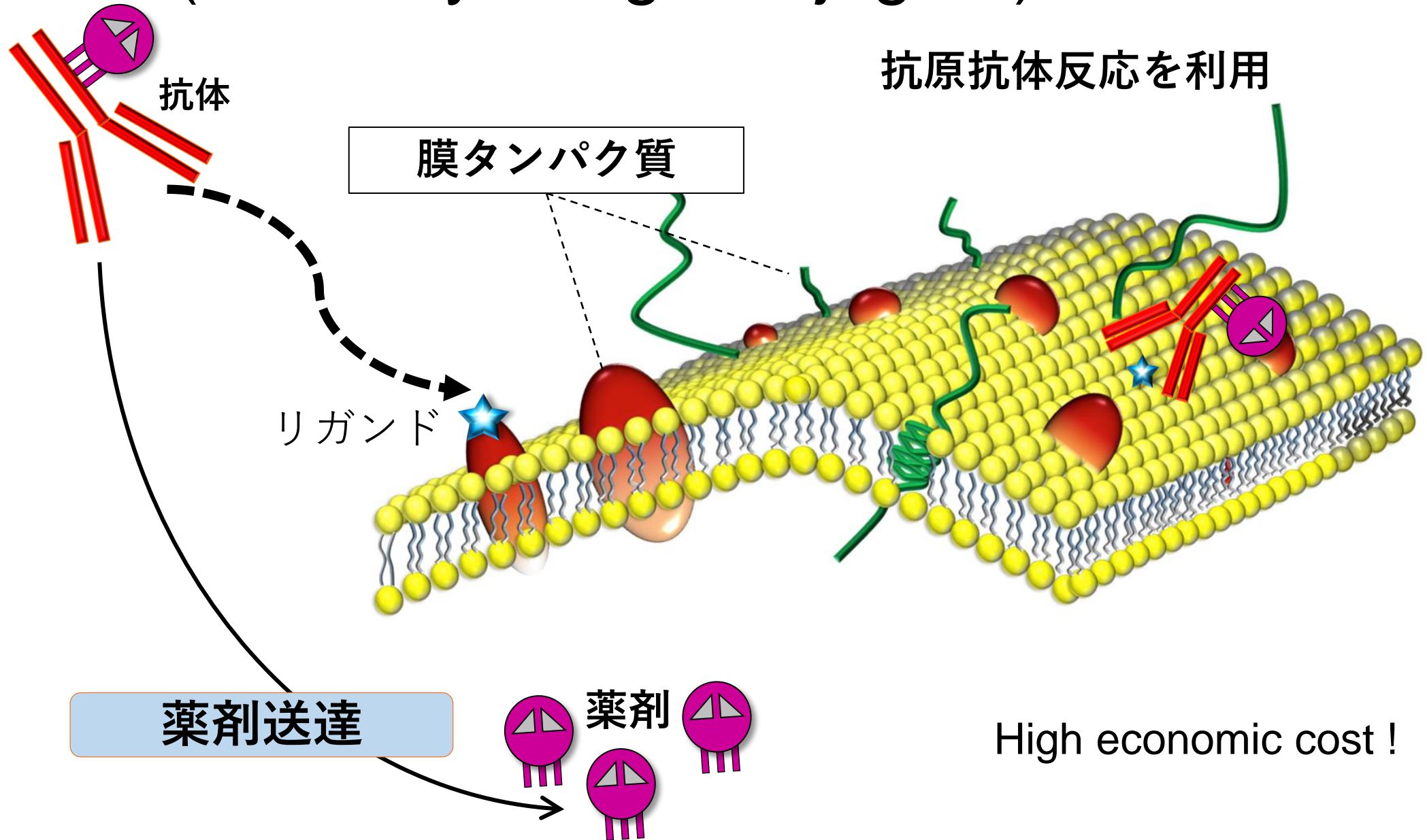
Conroy, T., et al. *N Engl J Med* 2011

**生存期間（中央値）  
11.1ヶ月**

**3年生存率 ≒ 0%**



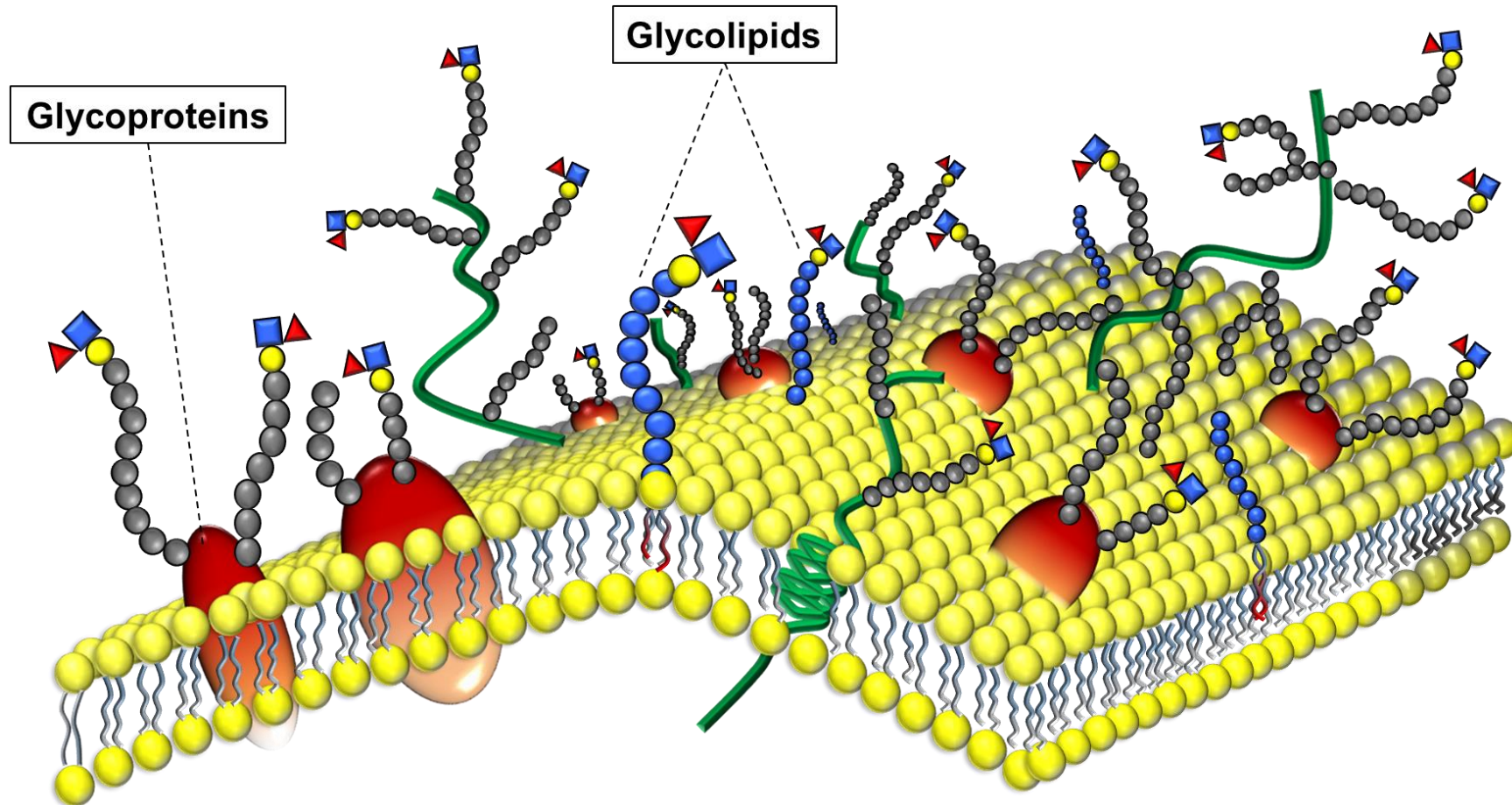
# ADC (Antibody-Drug Conjugate)



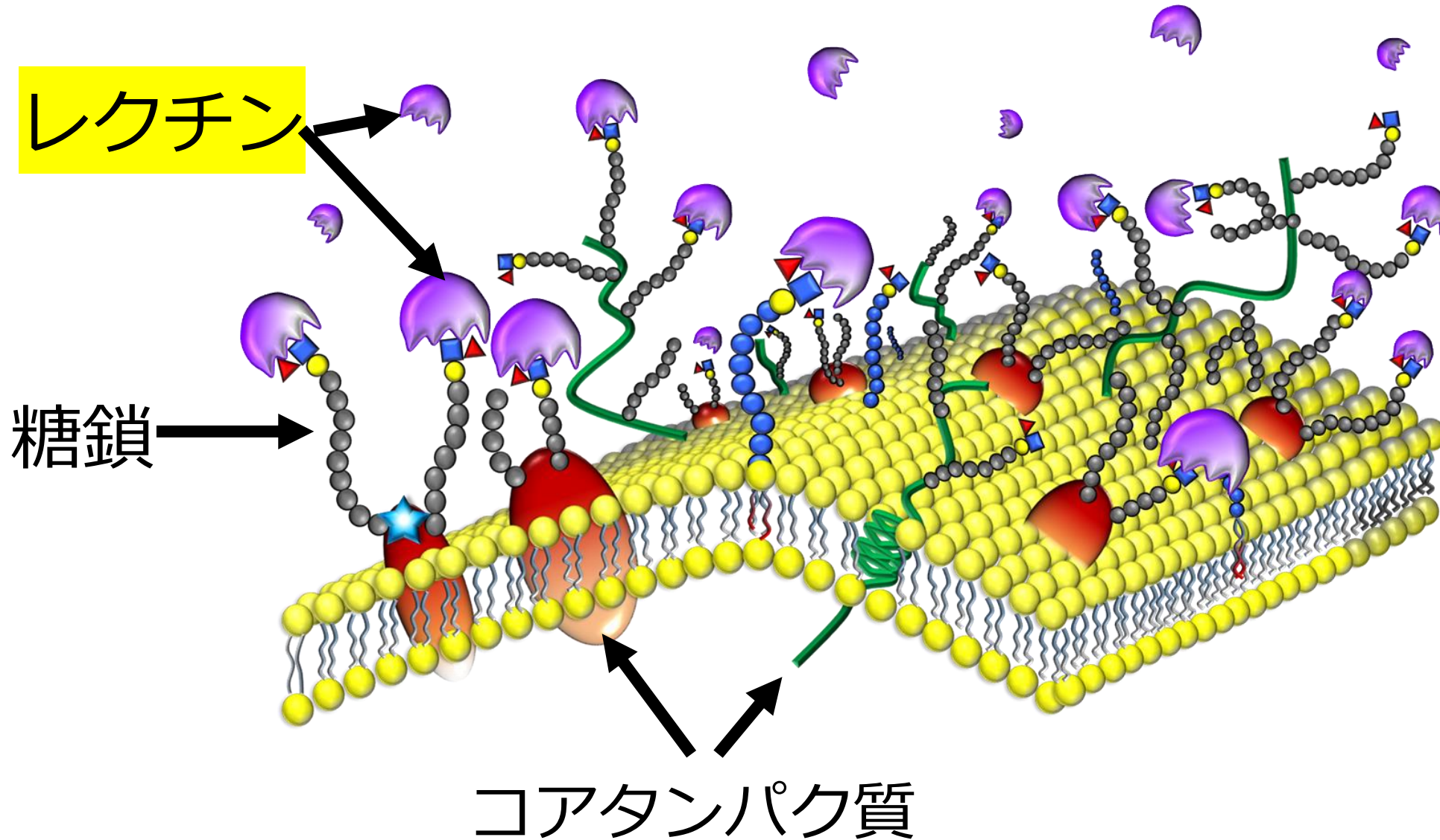
High economic cost !



# 癌特異的な糖鎖は、治療の標的となりうる？

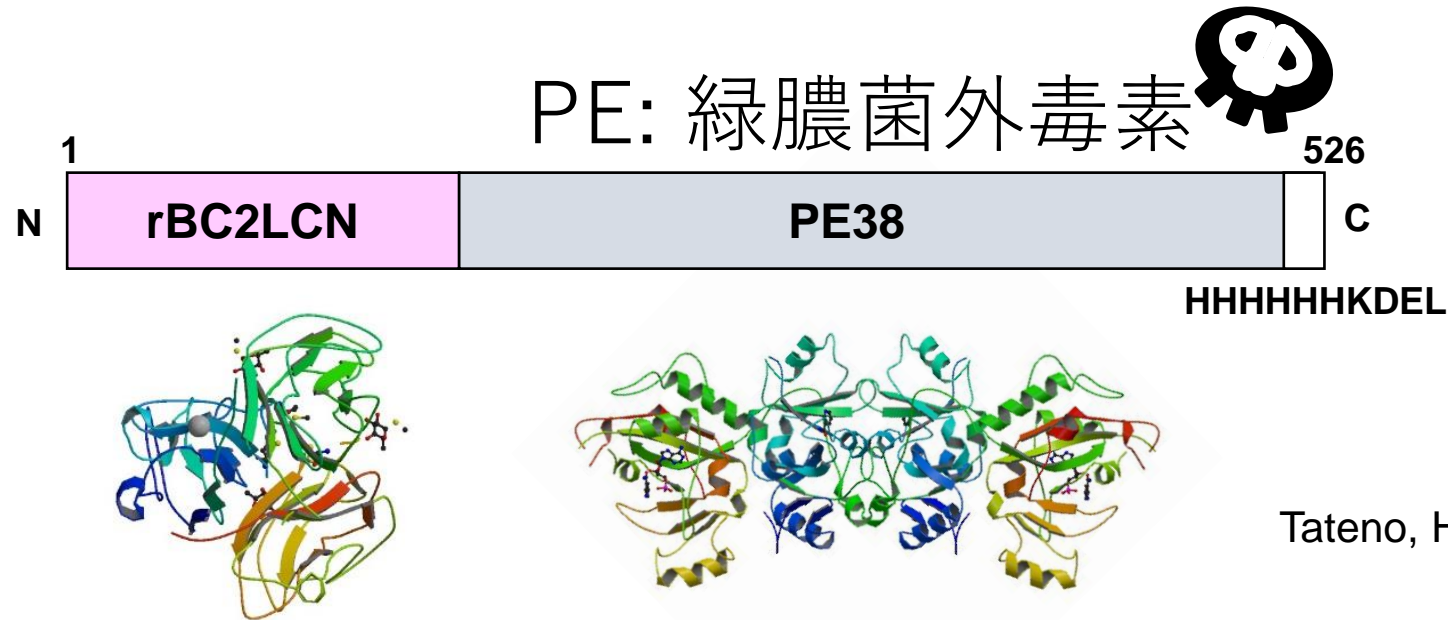


# レクチンを薬剤担体として使用できないか？



# レクチンー薬剤融合薬の精製

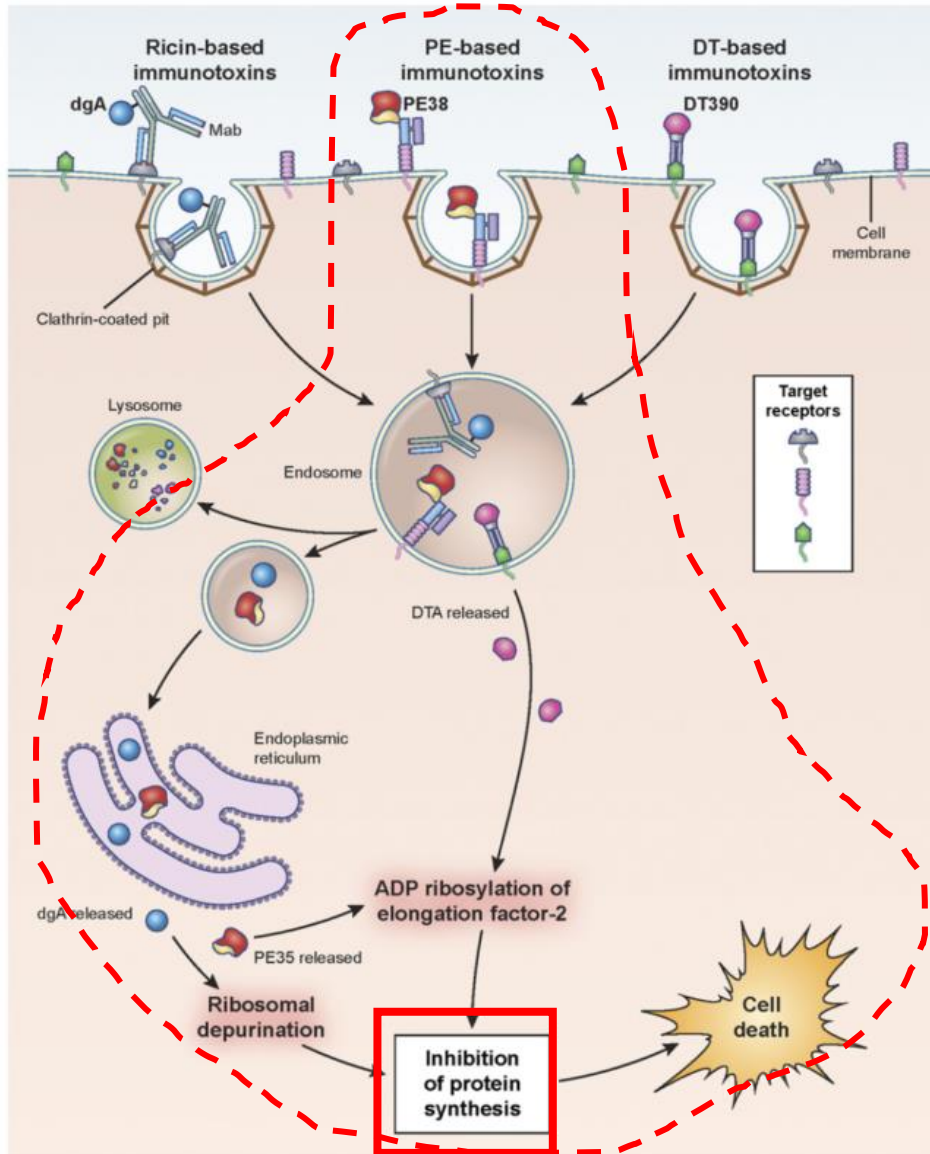
Lectin-Drug Conjugate “LDC” ( rBC2LCN-PE conjugate)



Synthesized fusion protein in E.coli system



# Pseudomonas aeruginosa Exotoxin A (PEA)



PE based Antibody-Drug Conjugate(ADC)  
Cytotoxic effect by inhibition of protein synthesis

Binding to membrane protein

↓

Internalized by endosome

↓

Transport to ER and released PE35

↓

elongation Factor-2 (ADP ribosylation)

↓

**タンパク合成阻害**

↓

Cell death



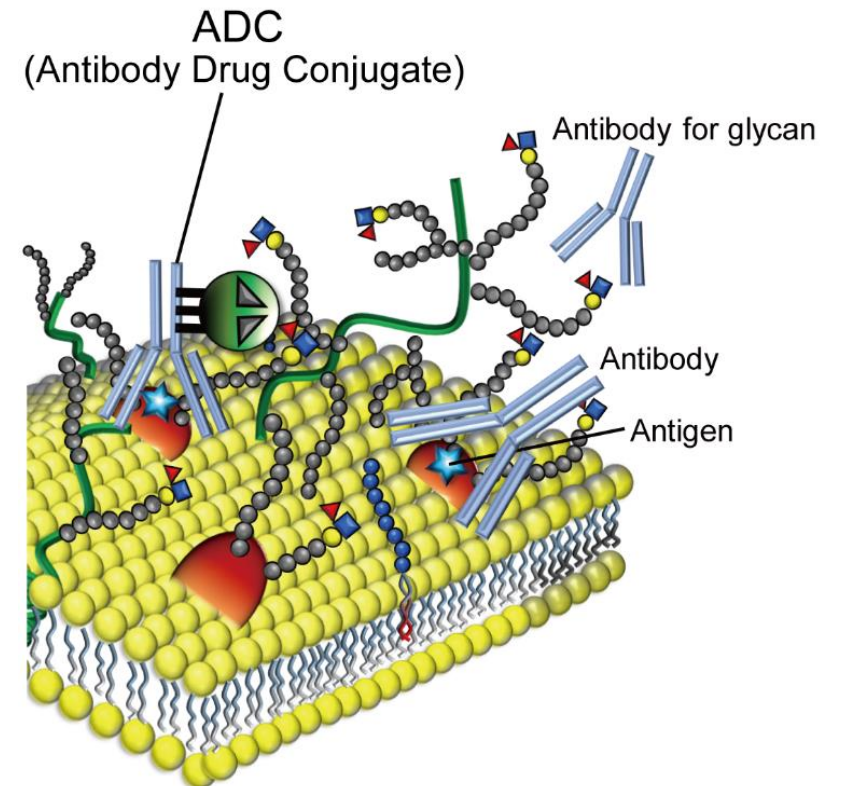


# Lumoxiti (Moxetumomab pasudotox-tdfk)

抗CD22抗体-PE38 (FDA 承認2018/9/13)

ADC (Antibody-drug conjugate)

➔ 再発・難治性有毛細胞白血病治療薬

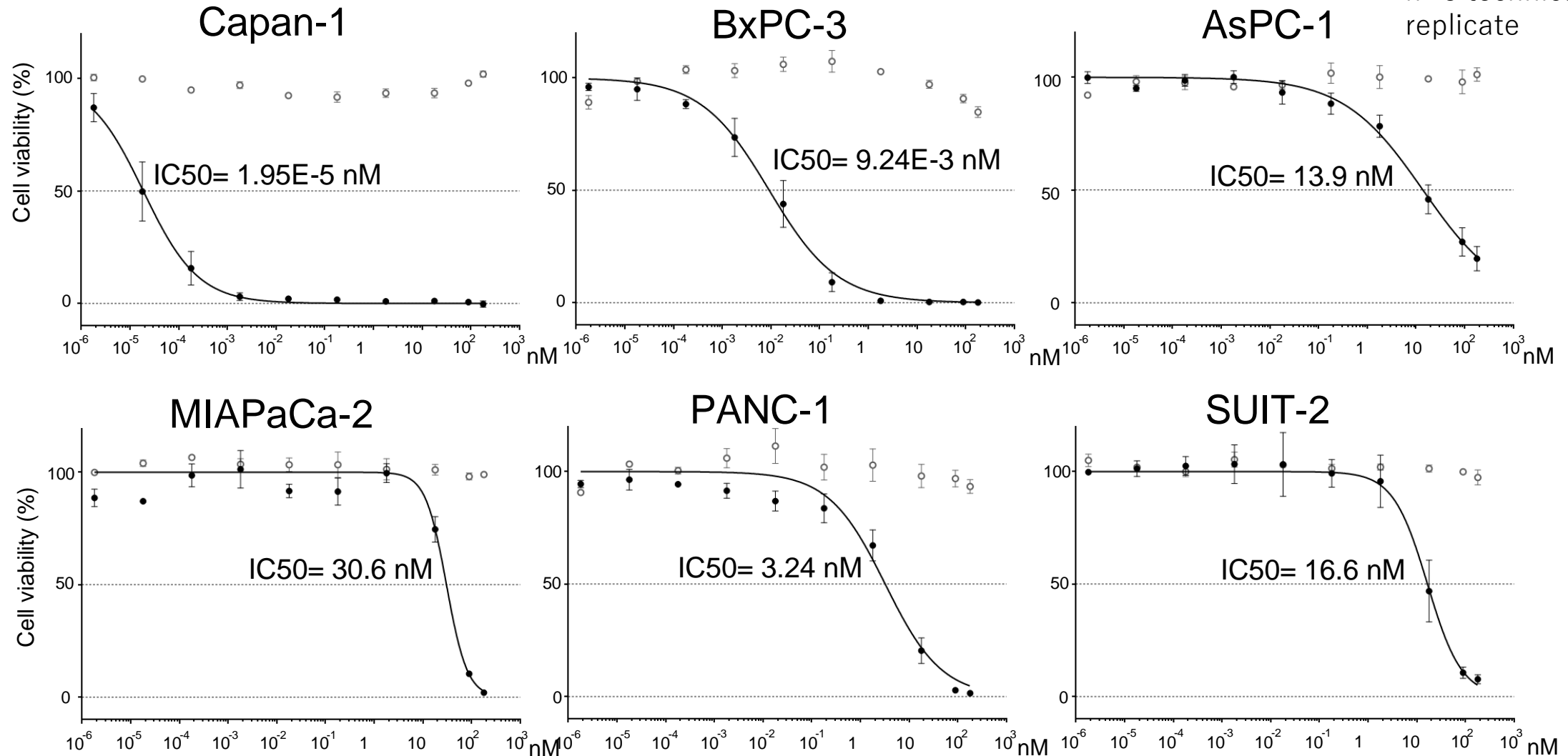


# LDC (rBC2LCN-PE38) 抗腫瘍効果 MTT (WST-8 test)

細胞株によって効果が異なる

○ : rBC2LCN only    ● : rBC2LCN-PE38

n=3 technical replicate

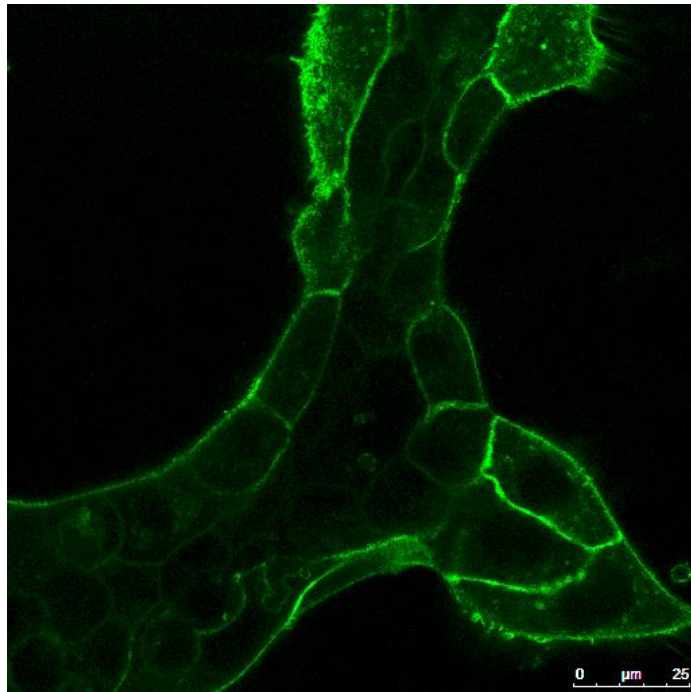




# 膵癌細胞へのレクチンの取り込み

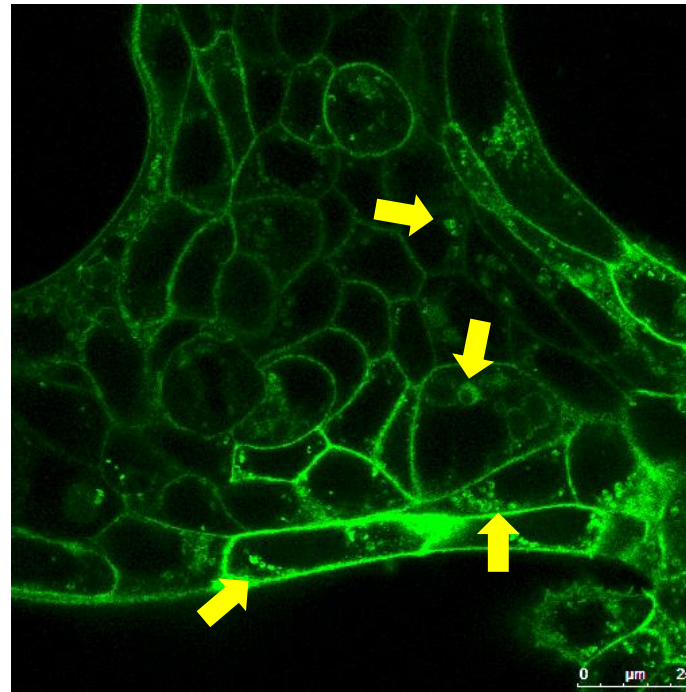
Green: rBC2LCN-FITC

1 Hour



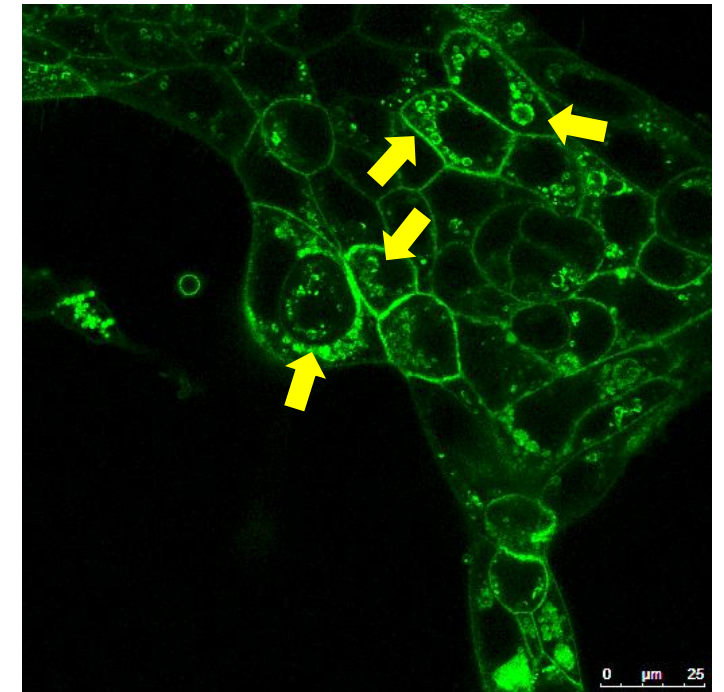
Bind to cell surface

24 Hour



lectins was detected  
inside the cell

48 Hour



The amount of  
lectin increased



# LDC の抗腫瘍効果 *in vivo* マウス皮下腫瘍

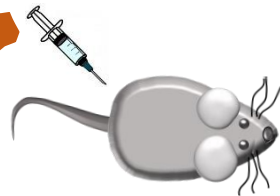
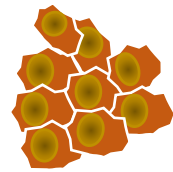
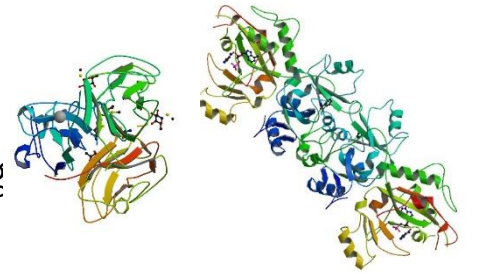
膵癌細胞株

Capan-1, SUIT-2

2M S.C

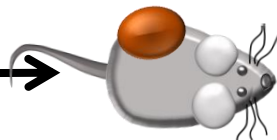
N=6

- 0: Control (normal saline) 100 $\mu$ l
- 1: LDC 40ng/100 $\mu$ l/body (2 $\mu$ g/kg)
- 2: LDC 1 $\mu$ g/100 $\mu$ l/body
- 3: LDC 5 $\mu$ g/100 $\mu$ l/body



BALB/c nu nu ♀  
6week

14d



ランダム化



Day0



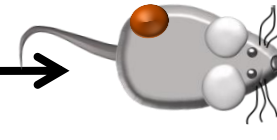
Day4



Day8



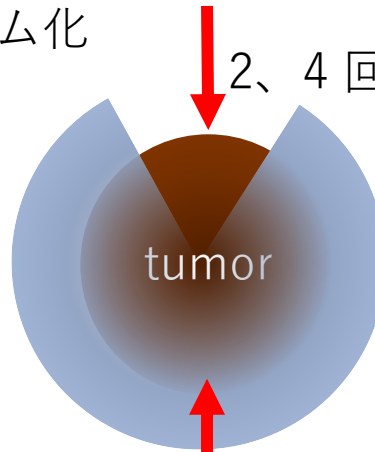
Day12



Day16  
腫瘍摘出

腫瘍局所投与 4回

2、4回目 注射

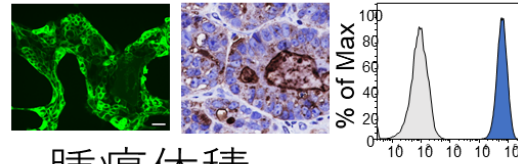


1、3回目 注射



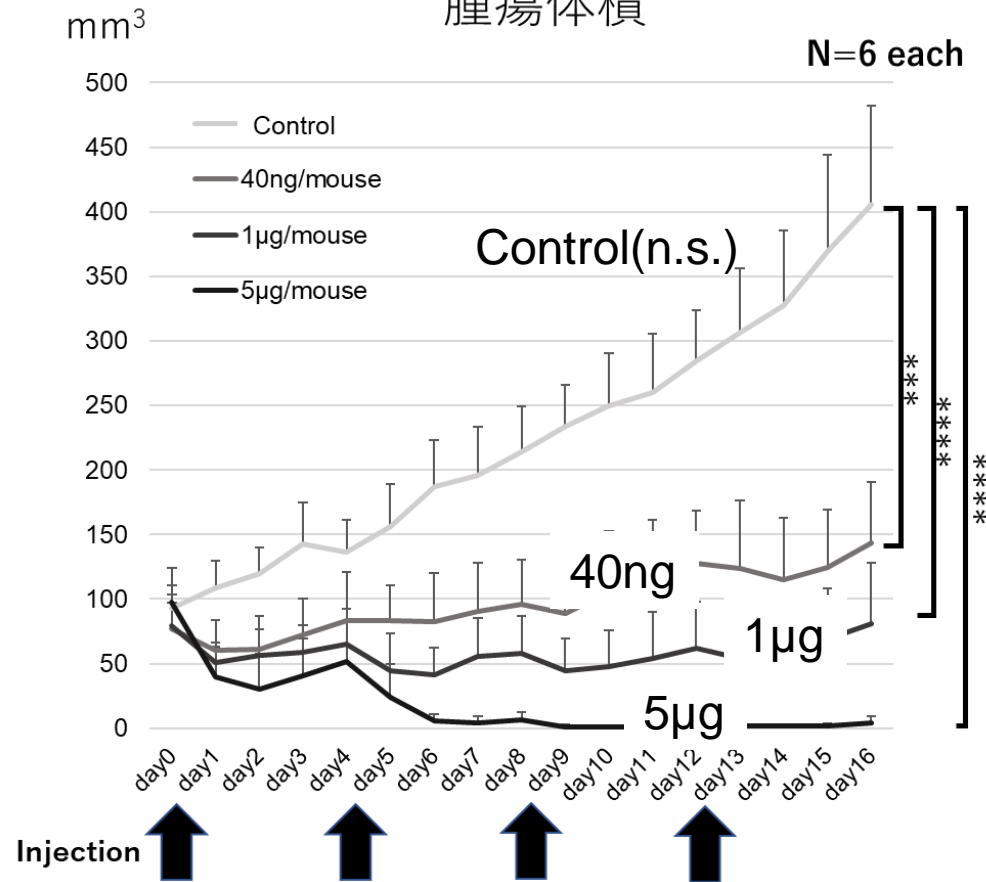
# LDC のマウス膵癌モデルへの効果

Capan-1

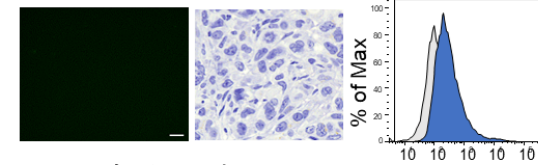


腫瘍体積

N=6 each

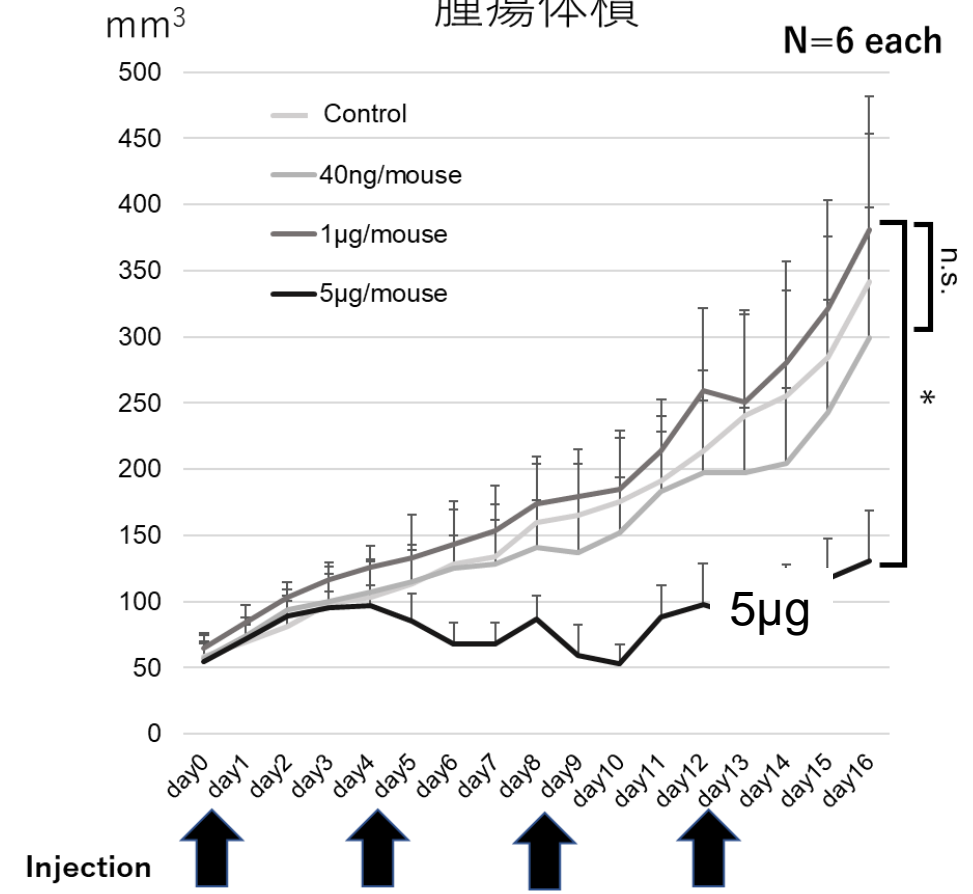


SUIT-2



腫瘍体積

N=6 each



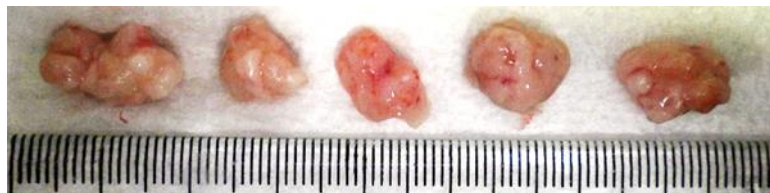
N=6 biological replicate \* : P<0.05, \*\*\*:P<0.001, \*\*\*\*:P<0.0001 n.s.: not significant Relative to control



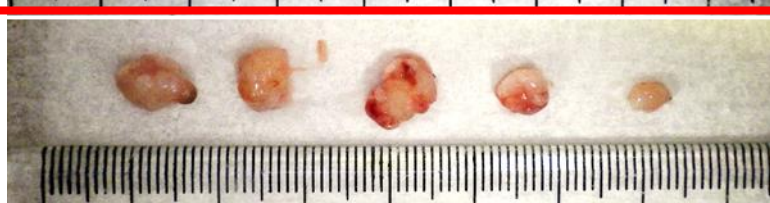
# 膵癌細胞株同所移植モデルへのLDCの効果

Pancreas tumor

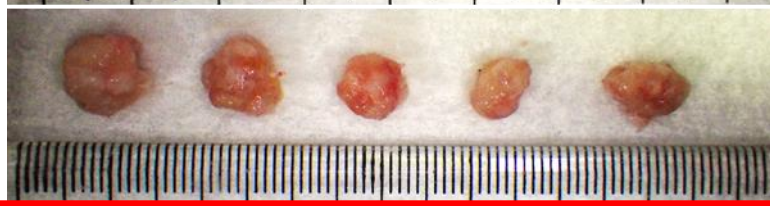
Control



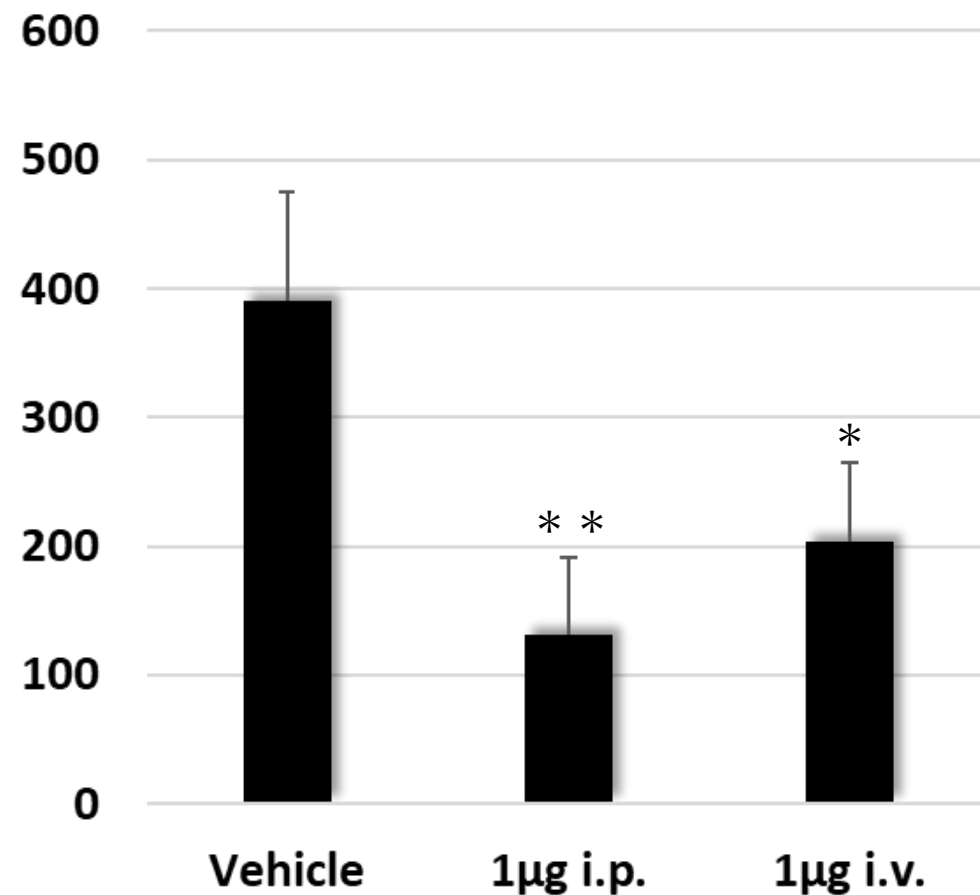
LDC 1ug i.p.



LDC 1ug i.v.



## Tumor weight



N=5 biological replicate

\*: P<0.05, \*\*:P<0.01

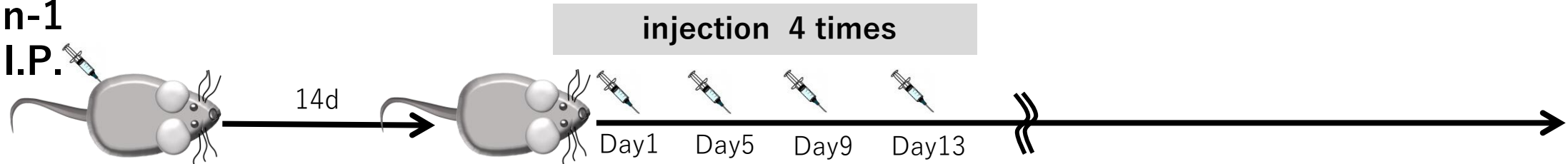
Relative to control by ANOVA (Turkey)

	Dissemination	Liver Metastasis	Spleen Metastasis
Vehicle	4	4	3
1µg i.p.	0	0	1
1µg i.v.	1	0	2

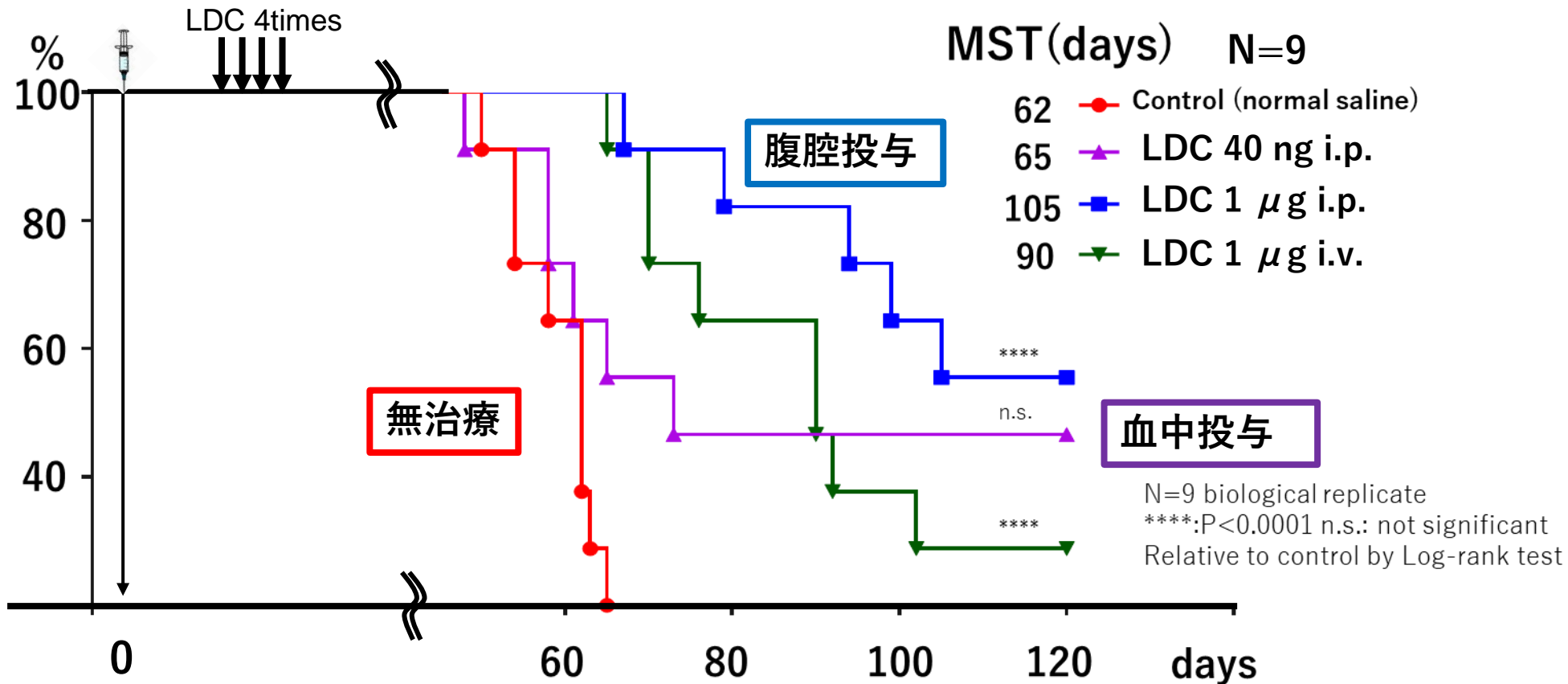


# LDC4回投与で膵癌腹膜播種マウスの予後を改善

Capan-1  
2M I.P.

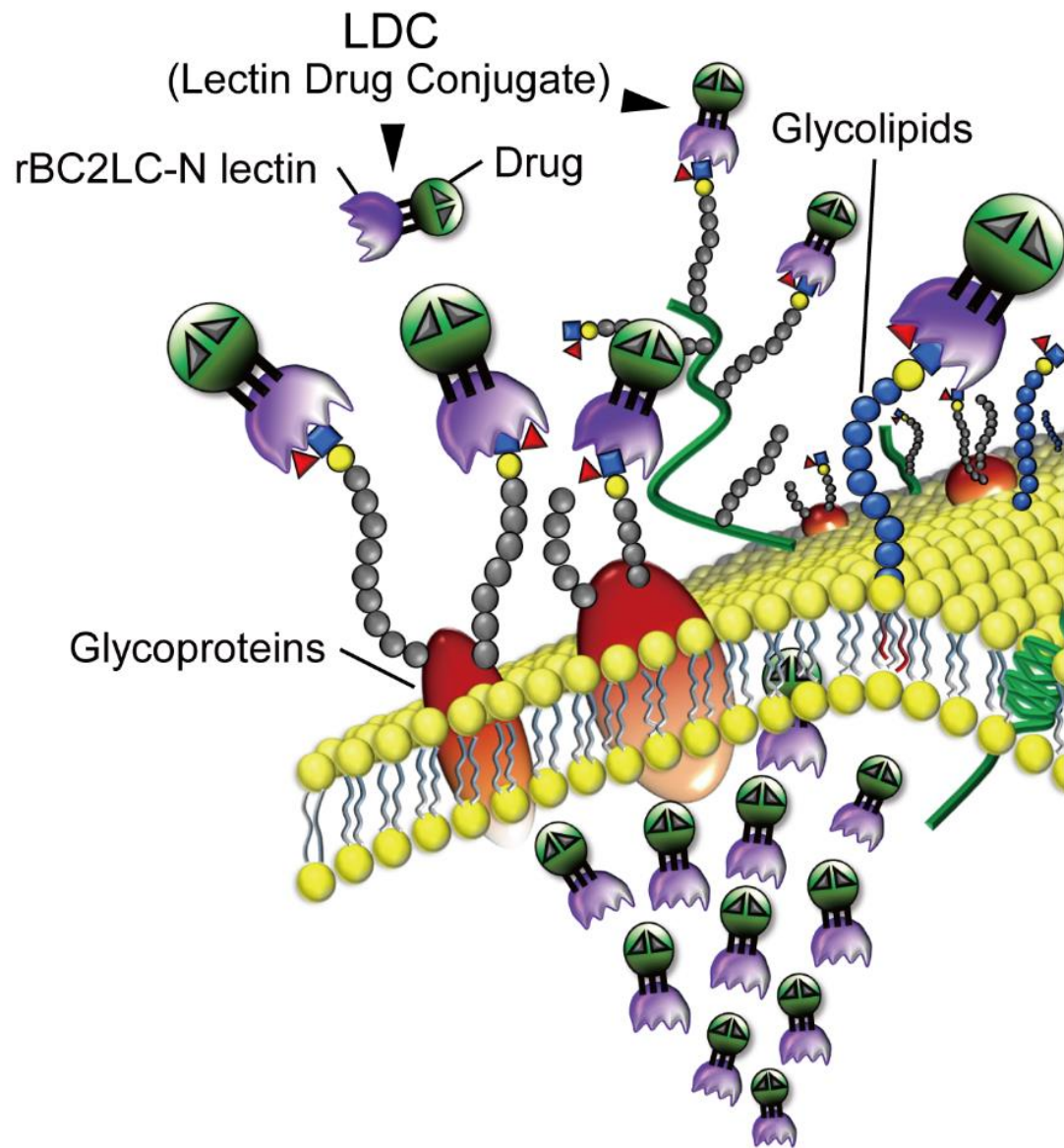


BALB/c nu nu ♀ 6week

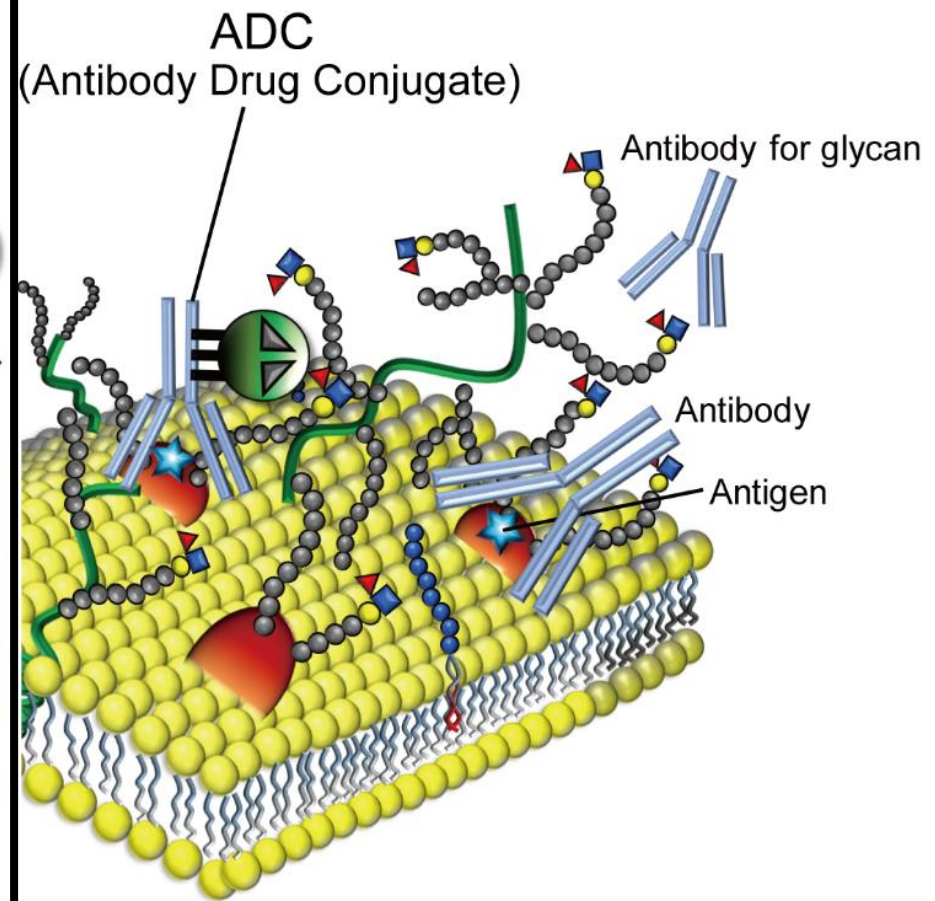




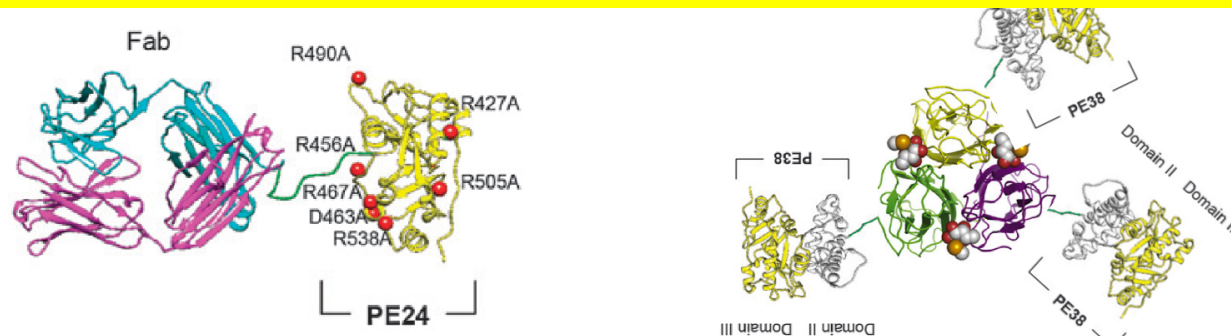
# レクチンによる薬剤送達



# 抗体による薬剤送達



# ADC vs. LDC for Pancreatic Cancer



	RG7787	rLDC
施設	米国 NCI (Ira Pastan et.al.)	日本 AIST-筑波大 (館野他)
Targeting bullet	Fab of antibody	rBC2LC-N lectin
Target	Mesothelin	H type 1,3,4 glycan
Payload	PE24	PE38
MW (kDa)	72	54 (162 at trimer)
Disease	Mesothelioma, Panc ca. (Ovarian ca., Lung ca., Gastric ca., Bile duct ca.)	Panc ca. (Ovarian ca., Lung adeno ca., Gastric ca., Colon ca.)
IC50 (ng/mL)	1.38	0.00104 (1.04 pg/ml)
(pmol/L)	18.9	0.0195 (=2 fmol/L)
LD 50 (mg/Kg)	10	0.36



# 抗体薬剤複合薬より約1,000倍高い殺細胞効果

Target antigen	malignancy	Agents name	Toxin	IC50	Reference
IL-2R	CLL	Anti-Tac(Fv)-PE40KDEL	PE	1.2~9 ng/ml	Kreitman, R. J., et al. 1992
GP185/HER2	Breast Ovary	Saporin Anti-GP185/HER2 IT	SAP	0.43~1.1 nM	Tecce, R., et al. 1993
IL-2R	ATL CLL	Anti-Tac(FV)-PE40	PE	0.04~>1000 ng/ml	Kreitman, R. J., et al. 1994
NCAM	Lung cancer	SEN7-PE	PE	22~85 pM	Zangemeister, U., et al. 1994
CD80	Hodgikin's	Anti-B7-1-saporina	SAP	3.2 ng/ml	Vooijs, W. C., et al. 1997
GRP	Lung	DAB389GRP	DT	9.5 pM	vanderSpek, J. C., et al. 1997
E4	Prostate	E4PE35-KDEL	PE	0.3-20 ng/ml	Essand, M. and I. Pastan 1998
CD30	Hodgikin's	Ki-4(scFv)-ETA	PE	43 pM	Klimka, A., et al. 1999
CD22	lymphoma	RFB4(dsFv)-PE38	PE38	0.4 ng/ml	Kreitman, R. J., et al 1999
IL-13R	Head and Neck	IL13-PE38	PE38	3~7 ng/ml	Kawakami, K., et al. 2001
IL-4R	Pancreas	IL4-PE38	PE38	0.3~0.5 ng/ml	Kawakami, K., et al. 2002
EpCAM	Head and Neck	4D5MOCB-ETA	PE	0.005~0.2 pM	Di Paolo, C., et al. 2003
GCSF	AML	DTU2GSF	DT	5.8~34.7 pM	Abi-Habib, R. J., et al. 2004
CD19	Blood	FMC63(Fv)-PE38	PE38	0.6-14 ng/ml	Du, X., et al. 2008
CD22	Blood	RFB4(Fv)-PE38	PE38	50-550 ng/ml	Du, X., et al. 2008
CD22	Blood	B3(dsFv)-PE38	PE38	0.1-2.5 ng/ml	Weldon, J. E., et al. 2009
CD30	Lymphoma	SGN-35	MMAE	1.3 ng/ml	Okeley, N. M., et al. 2010
mesothelin	Pancreas	RG7787	PE24	1.38~33.28 ng/ml	Hollevoet, K., et al. 2014
HER2	Breast	4D5scFv-ETA	PE40	22 nM	Sokolova, E. A., et al. 2014
CD71	Pancreas	HB21(Fv)-PE40	PE40	3~3.7 ng/ml	Hollevoet, K., et al. 2015
Glypican-3	liver	HN3-PE38	PE38	0.068 nM~	Gao, W., et al. 2015
<b>H type 1/3/4</b>	<b>Pancreas</b>	<b>rBC2-PE38</b>	<b>PE38</b>	<b>1.04 pg/ml (0.0195 pM)</b>	<b>Author</b>

既報のADCの報告

IC50 = 1~1000 ng/ml

1,000~  
10,000  
Times

LDC: IC50 = 1.04 pg/ml

CLL; chronic lymphocytic leukemia, ATL: adult T-cell leukemia, PE; pseudomonas aeruginosa exotoxin, DT; diphtheria toxin, SAP; plant toxin saporin 6, MMAE; monomethylauristatin E,

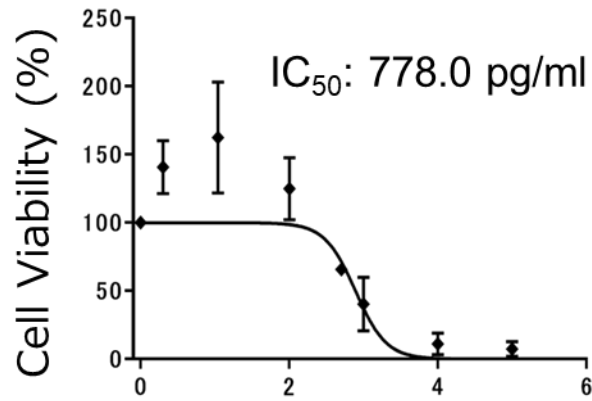


# LDC 其他の癌腫への応用

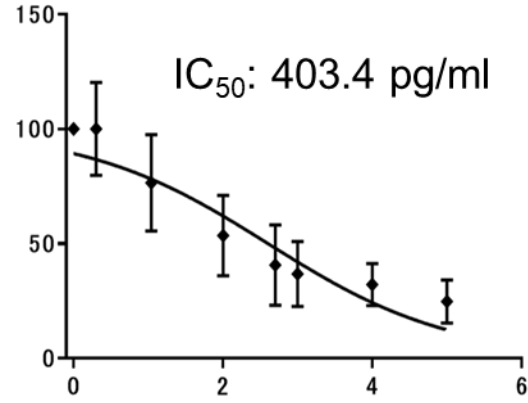
胃癌、大腸癌



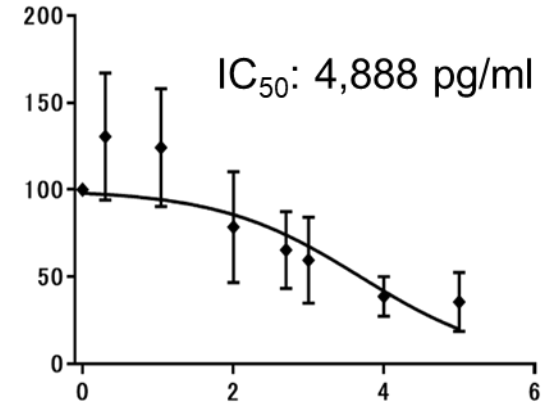
# LDC (BC2-PE38) for colon ca. cell lines



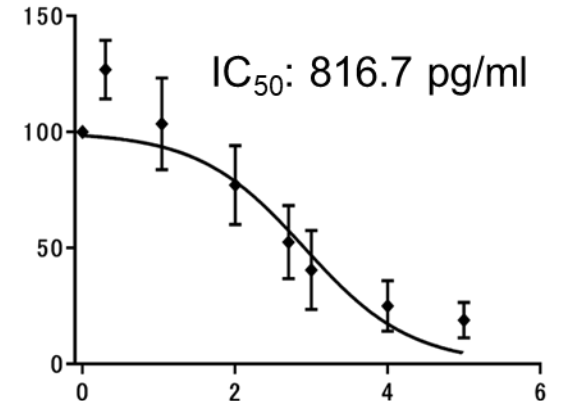
**LS174T**



**DLD-1**

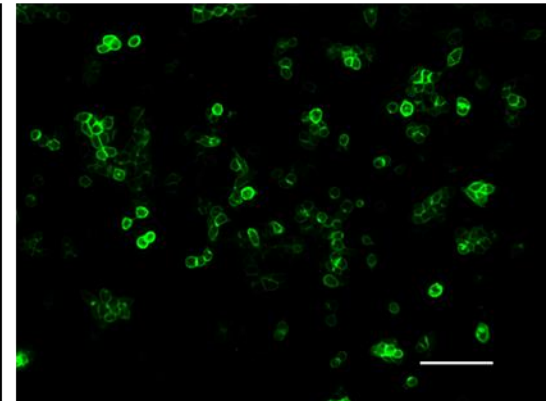
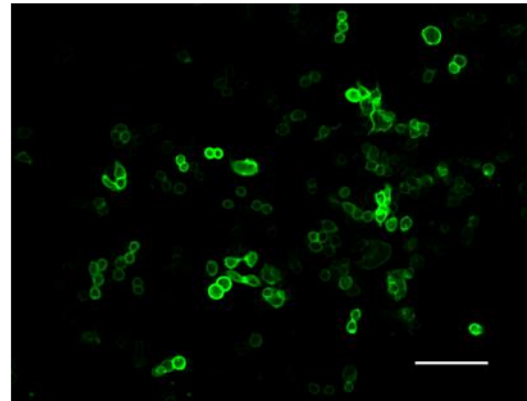
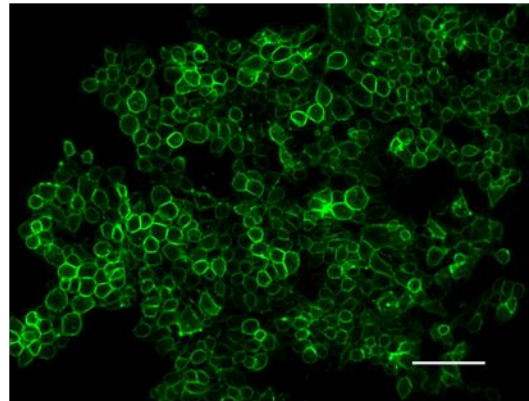
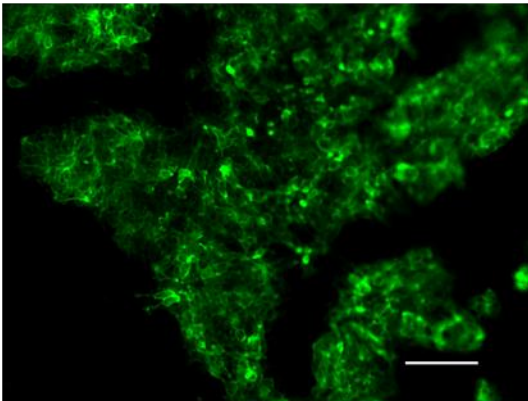


**HT-29**



**LoVo**

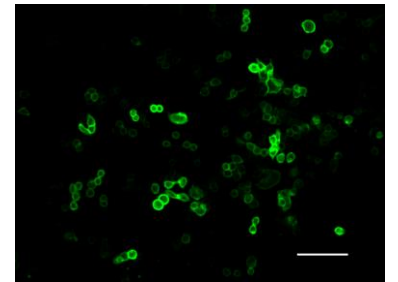
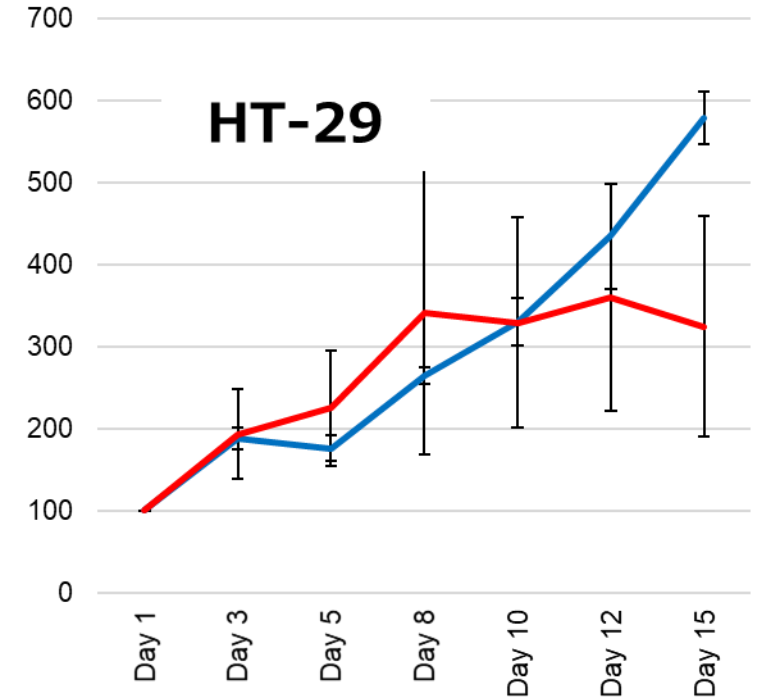
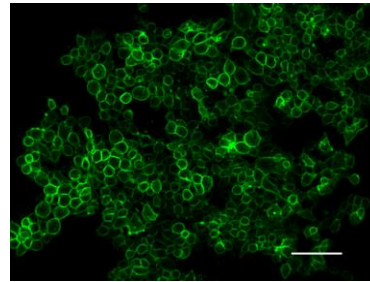
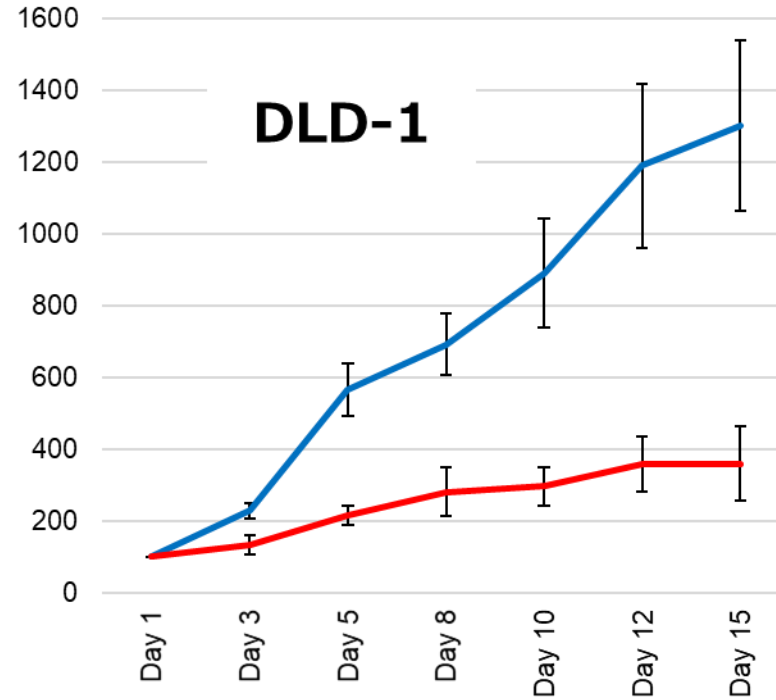
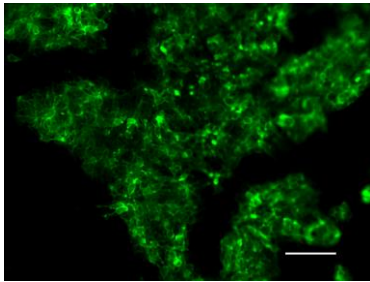
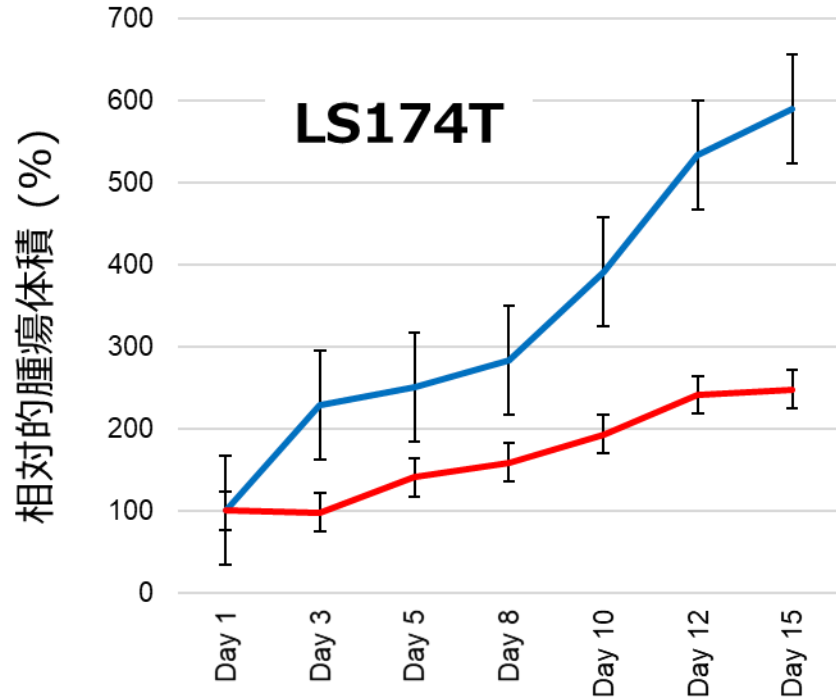
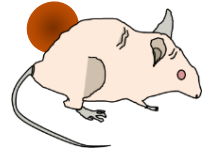
rBC2LCN-FITC



Kitaguchi D, Shimomura O, Tatenno H et al., Cancer Science 2020



# rBC2LCN-PE38 effect for colon ca. xenograft models



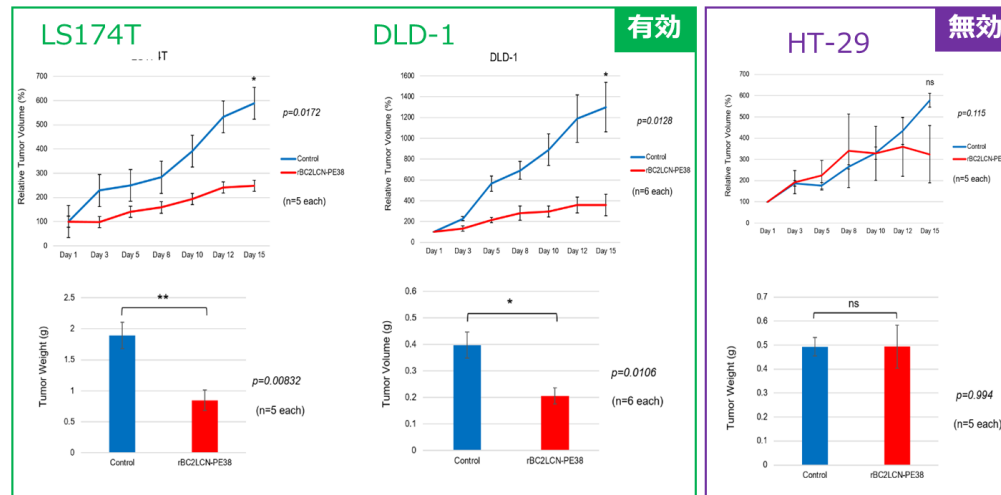
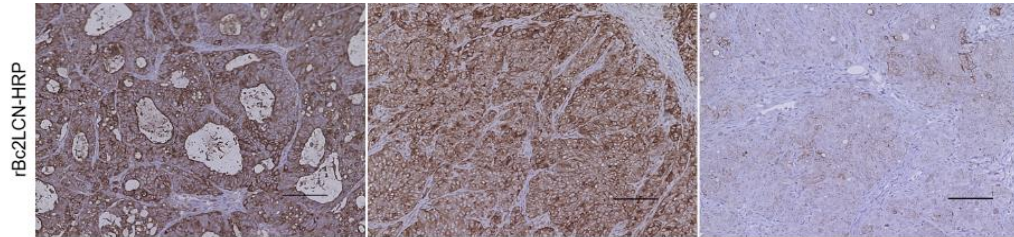
# LDCの大腸癌への効果

大腸癌細胞株を用いたLDCの抗腫瘍効果の検証

LS174T

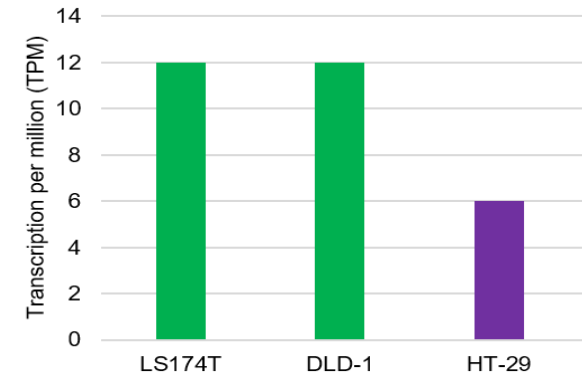
DLD-1

HT-29

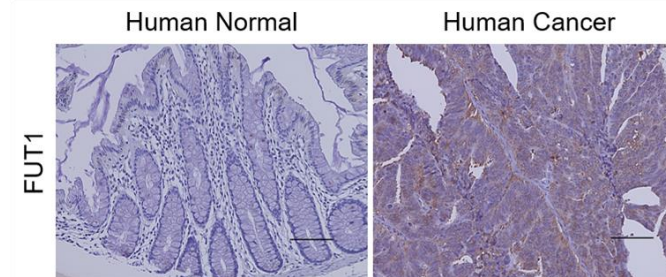
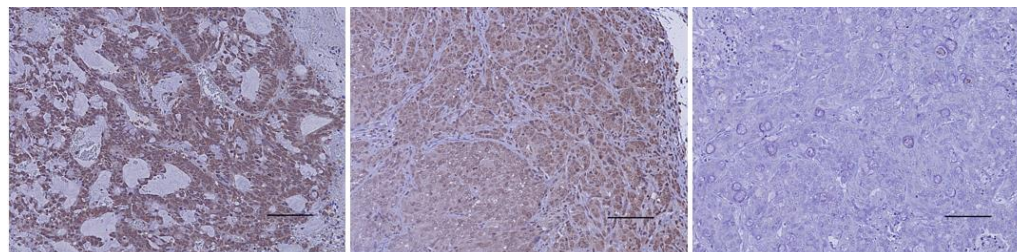


Kitaguchi, Shimomura, Oda, et al., Cancer Science 2020

FUT1遺伝子発現の違い



FUT1発現



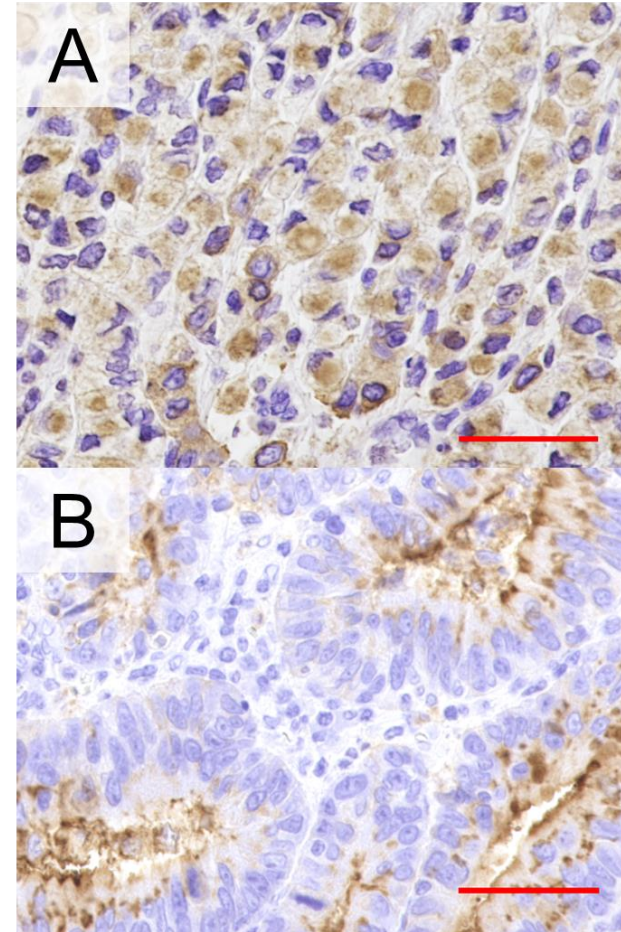
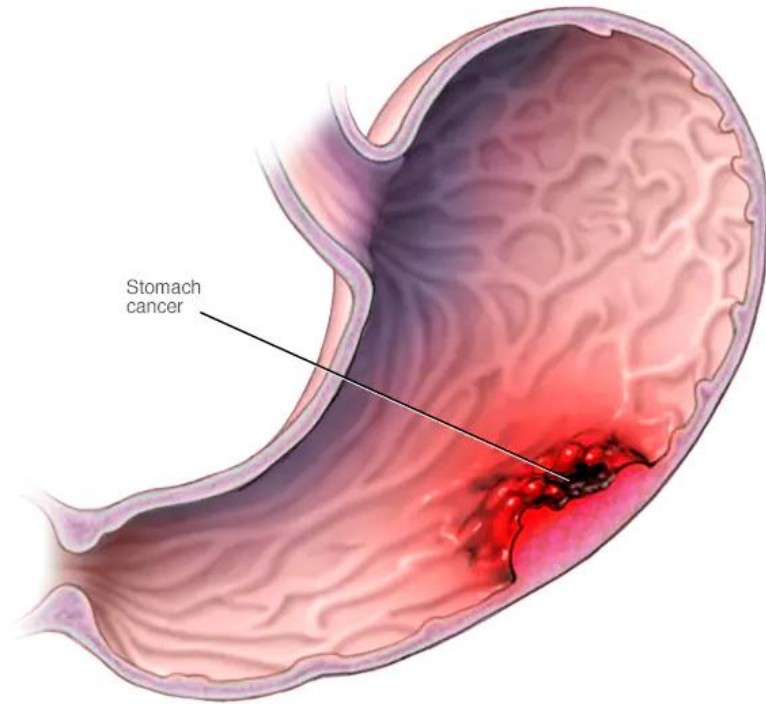
正常組織 << 腫瘍組織

有意なFUT1染色性の差

Dept. GI and HBP Surgery, Univ of Tsukuba



# LDCの胃癌への効果



Scirrhus Type

Moderately differentiated

rBC2LCN lectin-HRP

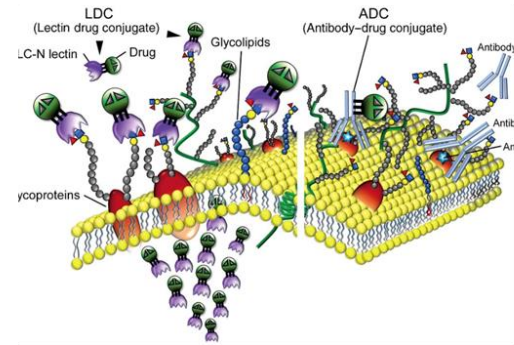
Yang Y, Akashi Y, Shimomura O, Tateno H, *et al*: Gastric Cancer 2022





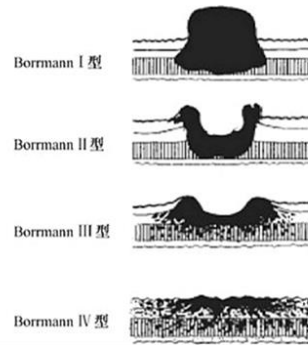
# LDC (レクチン融合薬) の胃癌への効果の検討

## Is lectin a potential drug carrier for GC?



—A Novel Therapeutic Strategy for Pancreatic Cancer: Targeting Cell Surface Glycan Using rBC2LC-N Lectin-Drug Conjugate (LDC). O. Shimomura. *Mol Cancer Ther*. 17(1): 183-95. ©2017 AACR

Apply to GC?



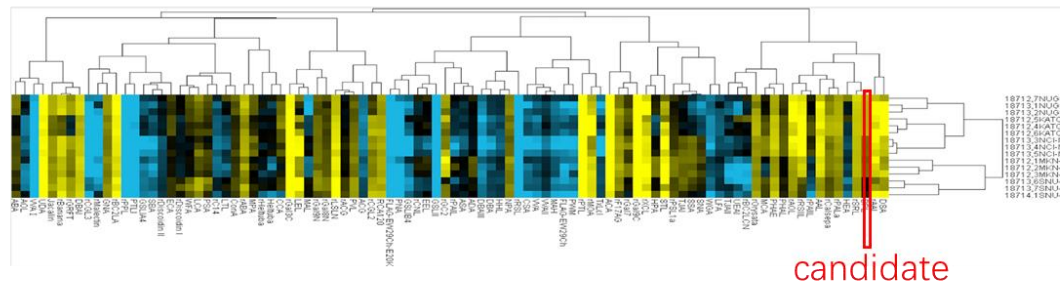
method



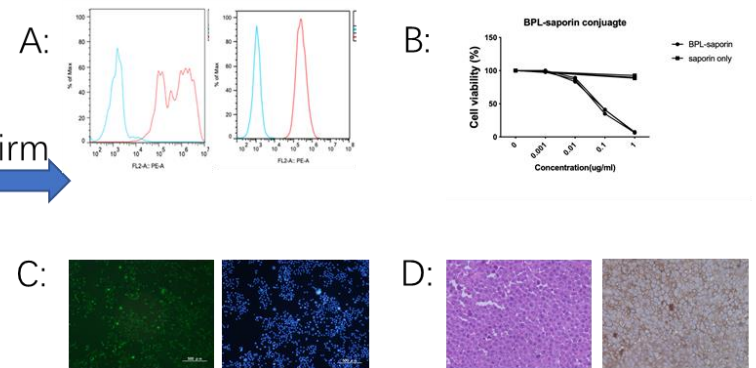
- Protein extraction
- Lectin array: find the lectin
- Lectin blotting: check the affinity
- FACS: check the affinity of cell lines
- Lectin staining
- MTT assay
- Hemagglutination
- Check in vivo

Aim: To develop a novel therapy for refractory gastric cancer.

Results:



confirm



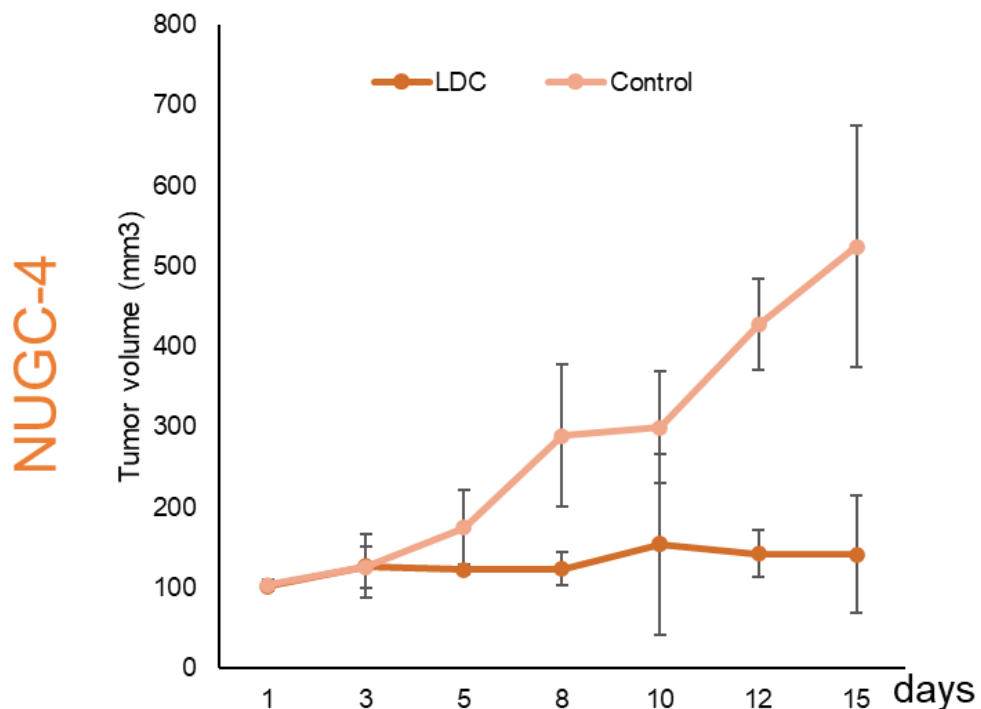
Yang Y, Akashi Y, Shimomura O, Tateno H, *et al*: Gastric Cancer 2022



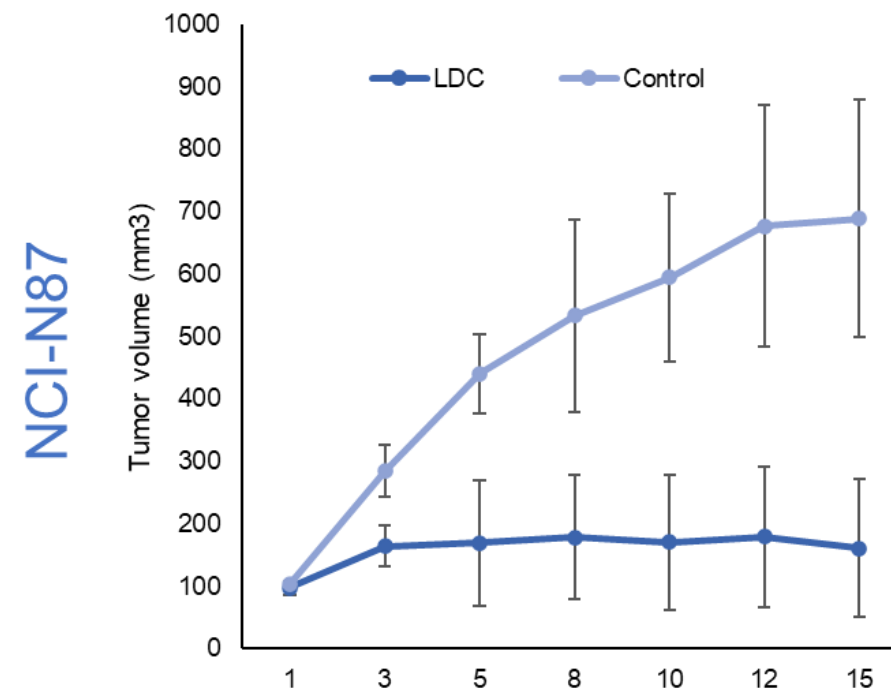


# 胃癌に対してもrBC2-PE38(LDC)は有効

## Scirrhouous type



## Non-Scirrhouous type



Yang Y, Akashi Y, Shimomura O, Tateno H, *et al*: Gastric Cancer 2022



# レクチンの生体投与 血液凝集活性が最大の問題点

Lectin was found as proteins cased  
**erythrocyte aggregation.** 1888 H. Stullmark



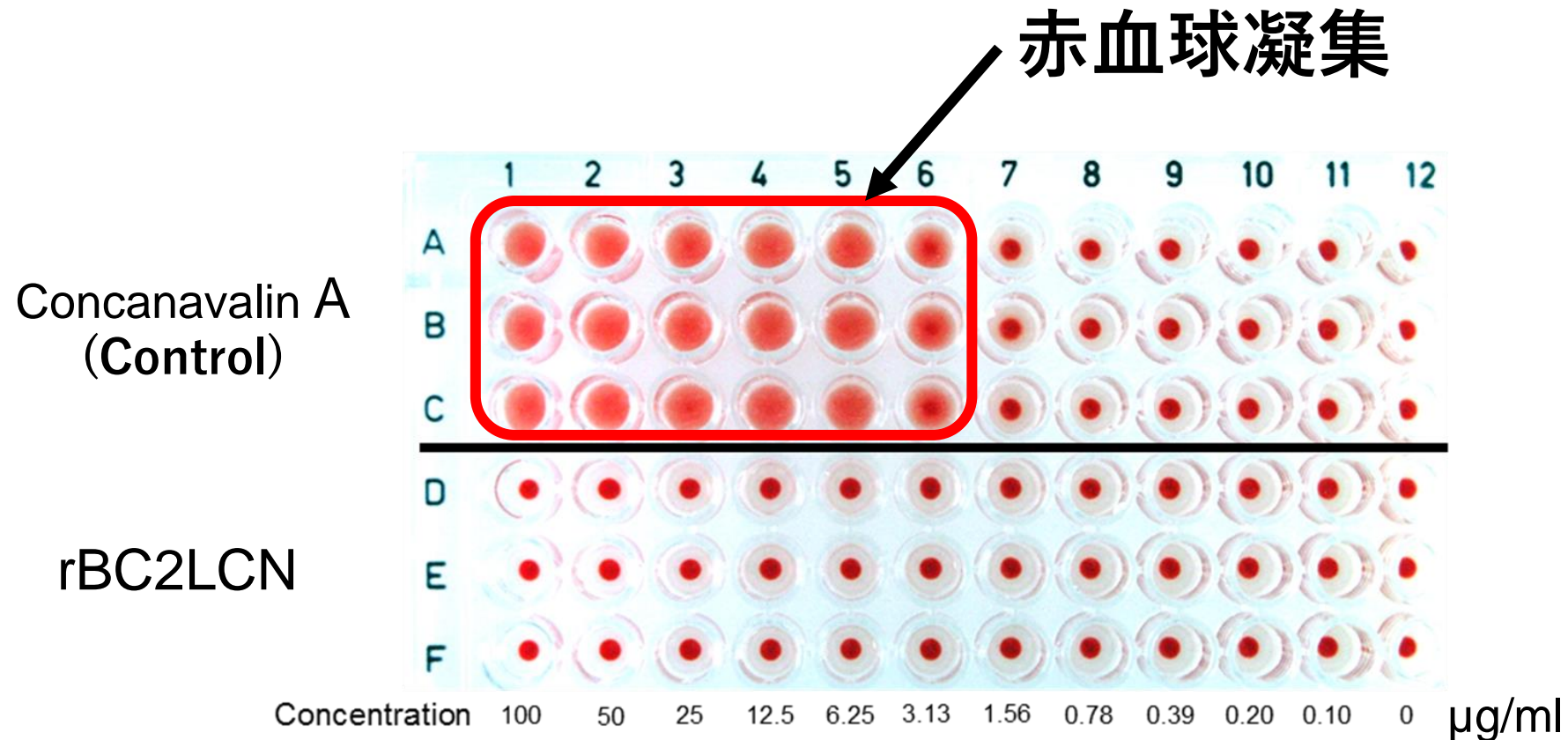
Posionous lectin; Ricin (RCA1) → **lethal dose 30 $\mu$ g/kg (human)**  
Olsnes S et al., Nature. 1974



一般的にレクチンは生体毒である・・・？



# rBC2LCN は赤血球の凝集反応が見られなかった



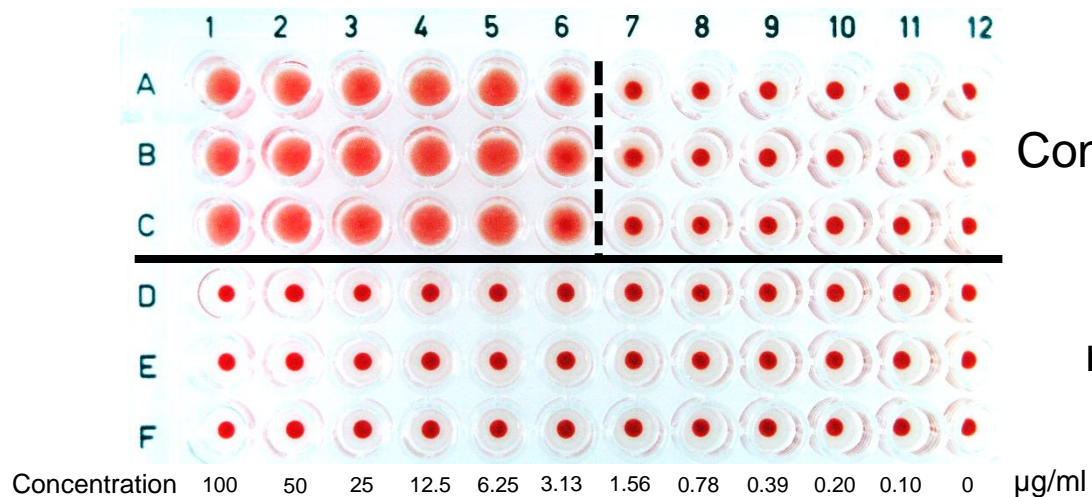
Human Blood type A



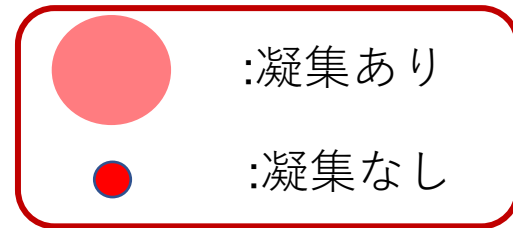
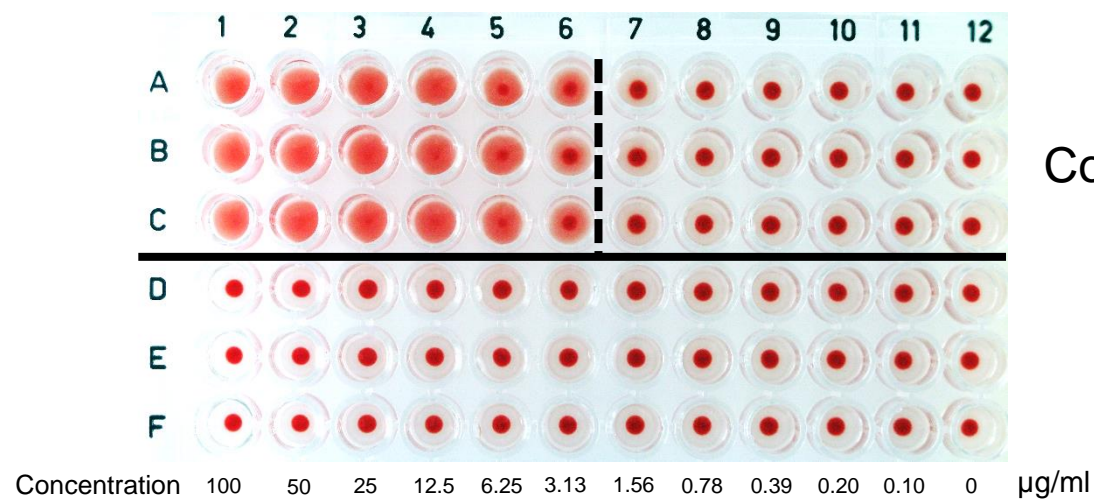
# ヒト赤血球での凝集反応試験

Sialydase処理ヒト赤血球

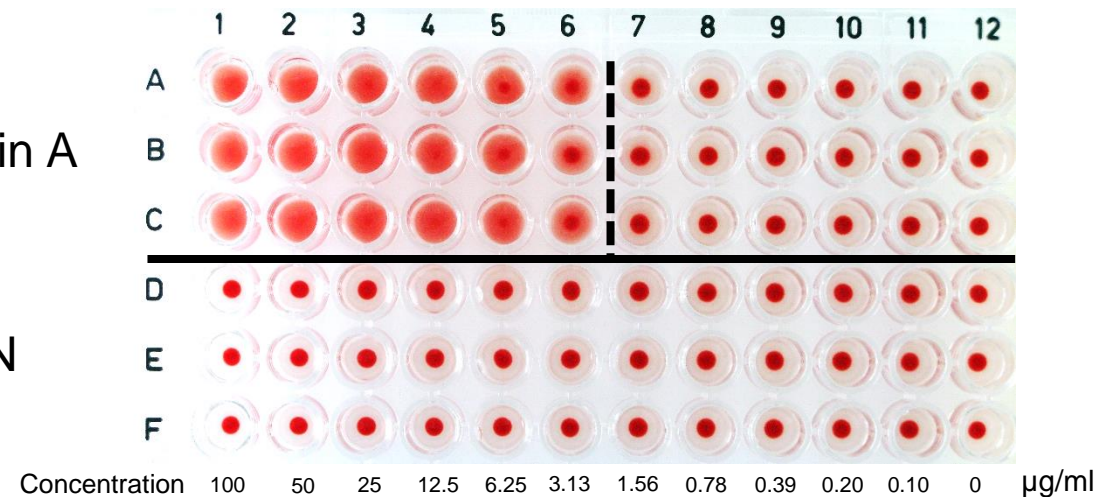
A



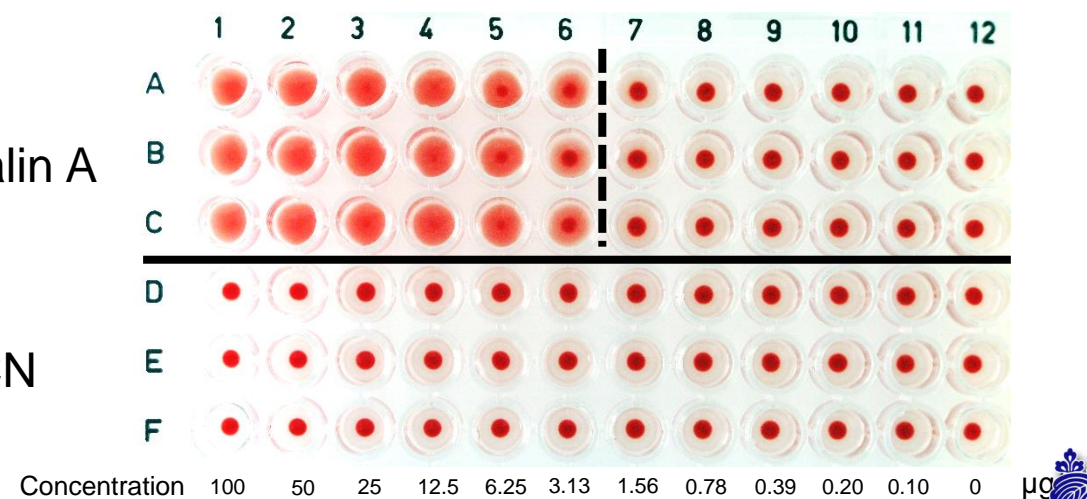
B



O



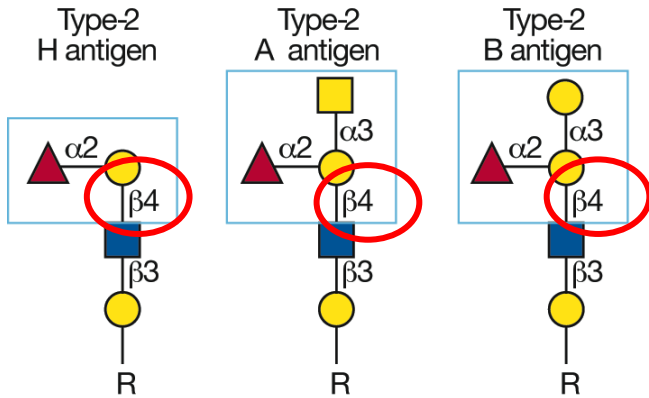
AB



# Blood type A,B,H determinants expressed by the epidermis are primarily constructed from type-2 units

Blood type

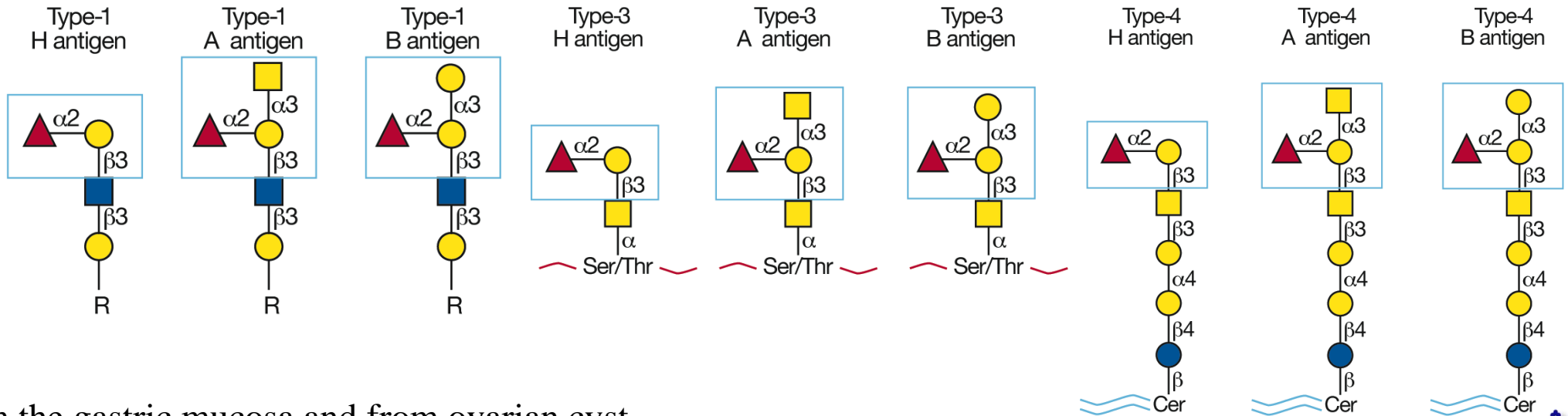
Type 2



Essentials of Glycobiology  
Second Edition

rBC2LCN recognize

H Type 1/3/4

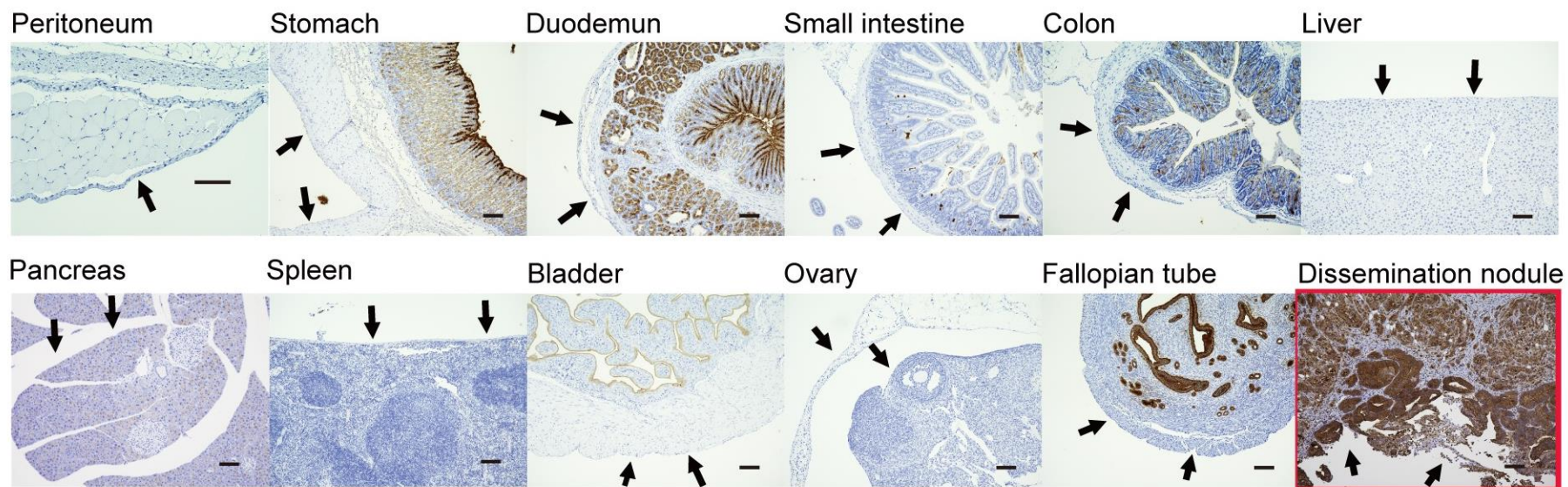


Mucins derived from the gastric mucosa and from ovarian cyst fluid express A, B, and H antigens on type-3 units

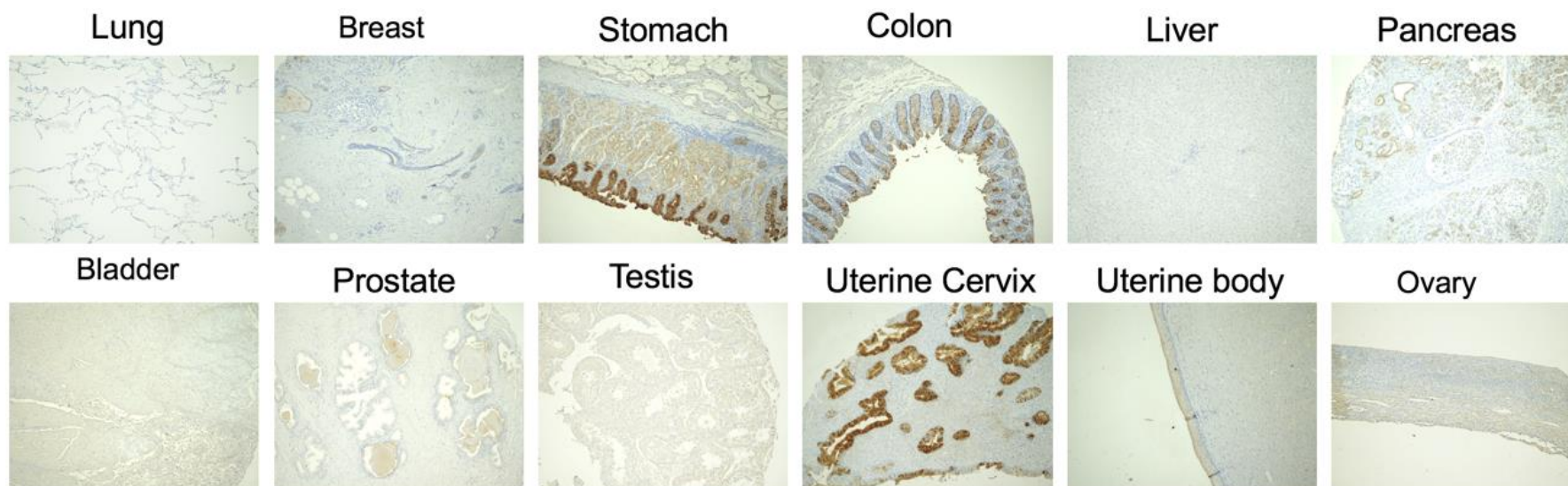


# 正常組織へのrBC2LC-NのOff site結合

Mouse



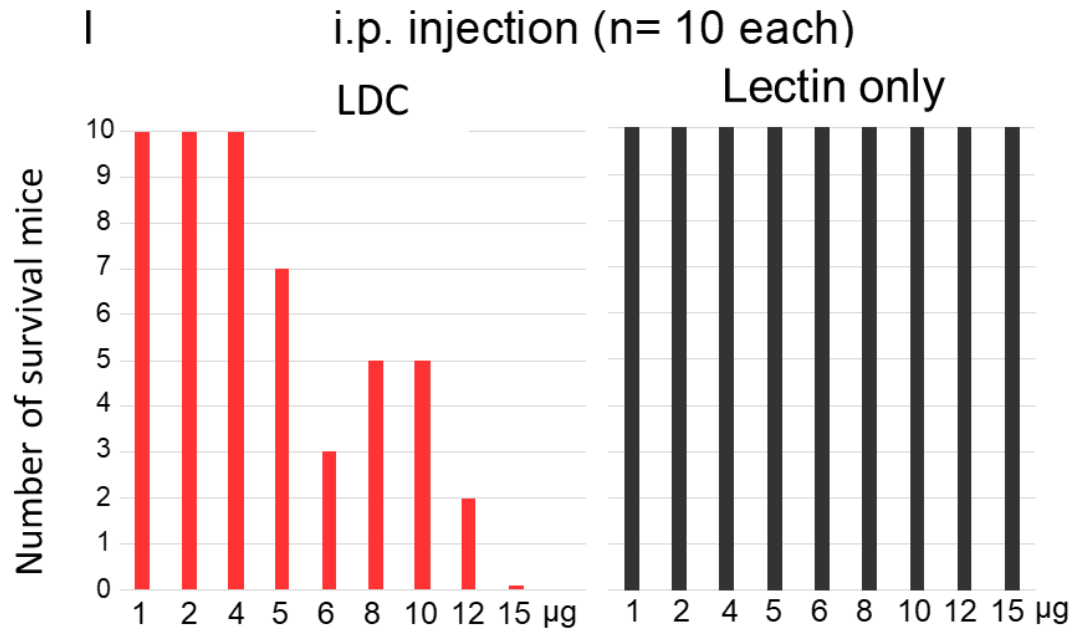
Human



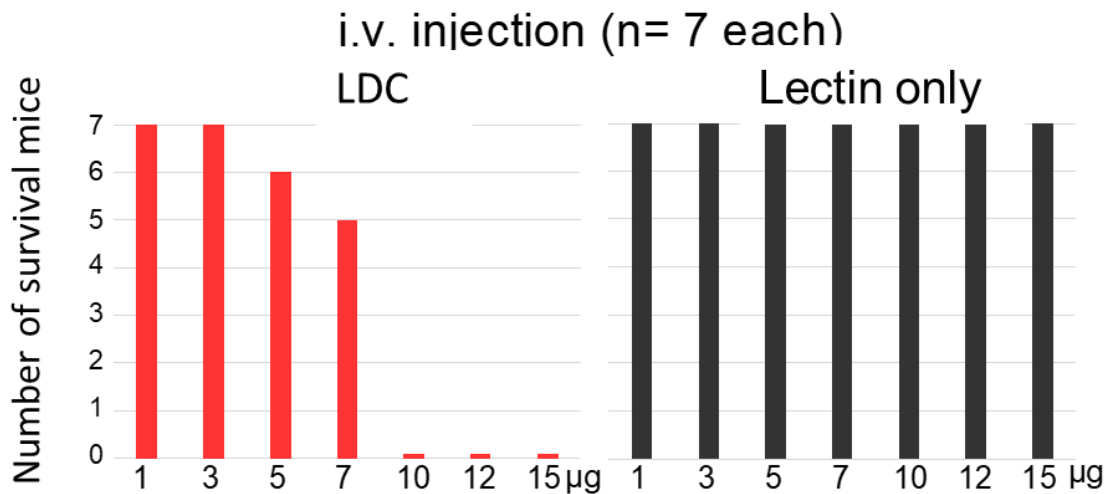


# マウスへの毒性試験

# LD50 test of LDC



50%致死量 LD50=  
約7μg/mouse



レクチン単独では  
15 μg/mouseまで毒性なし



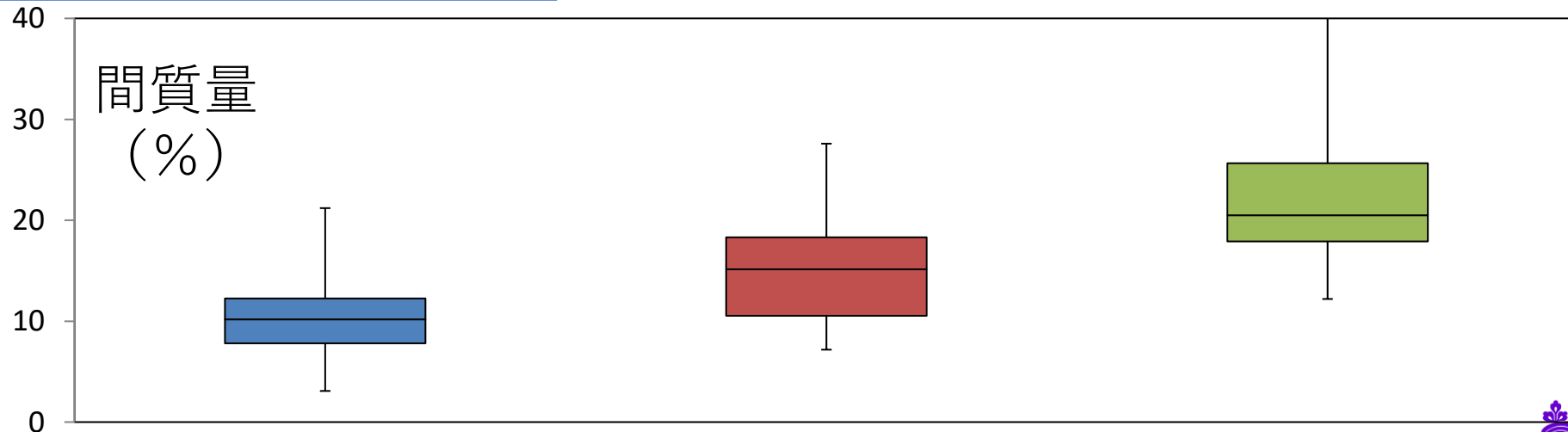
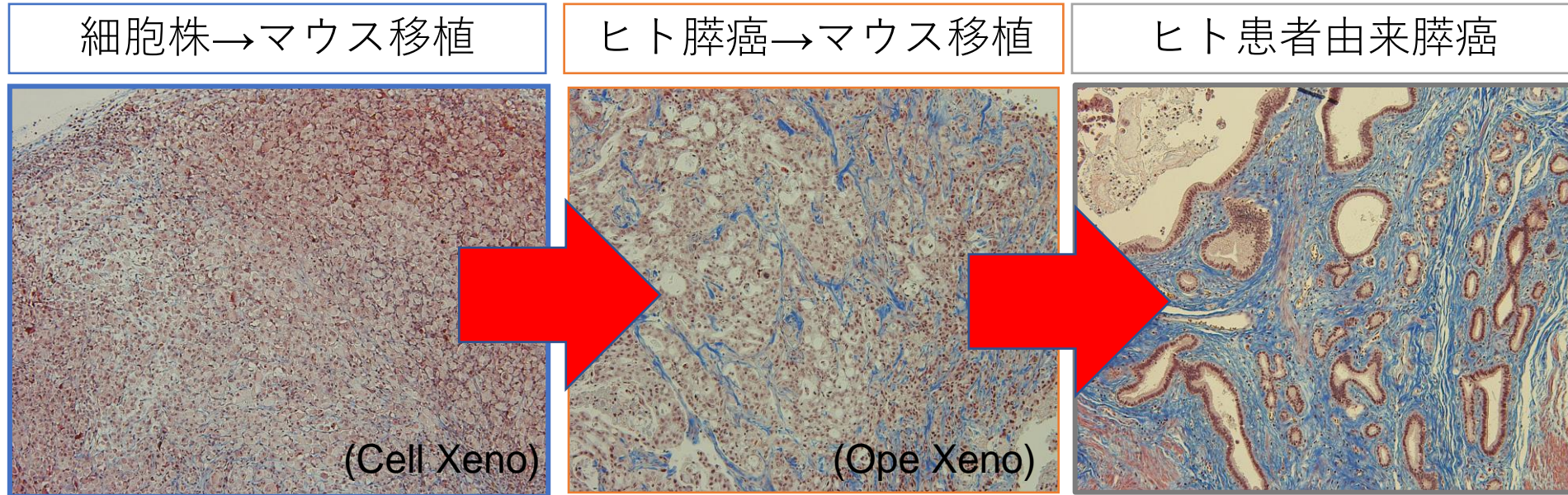
# 大型動物でのLDCの毒性評価

- ✓ LDC投与による肝障害、腎障害が確認された。
- ✓ レクチン単独投与では、副作用はなし。

➔ PE38はげっ歯類に対して猛毒、哺乳類にも一部毒性あり



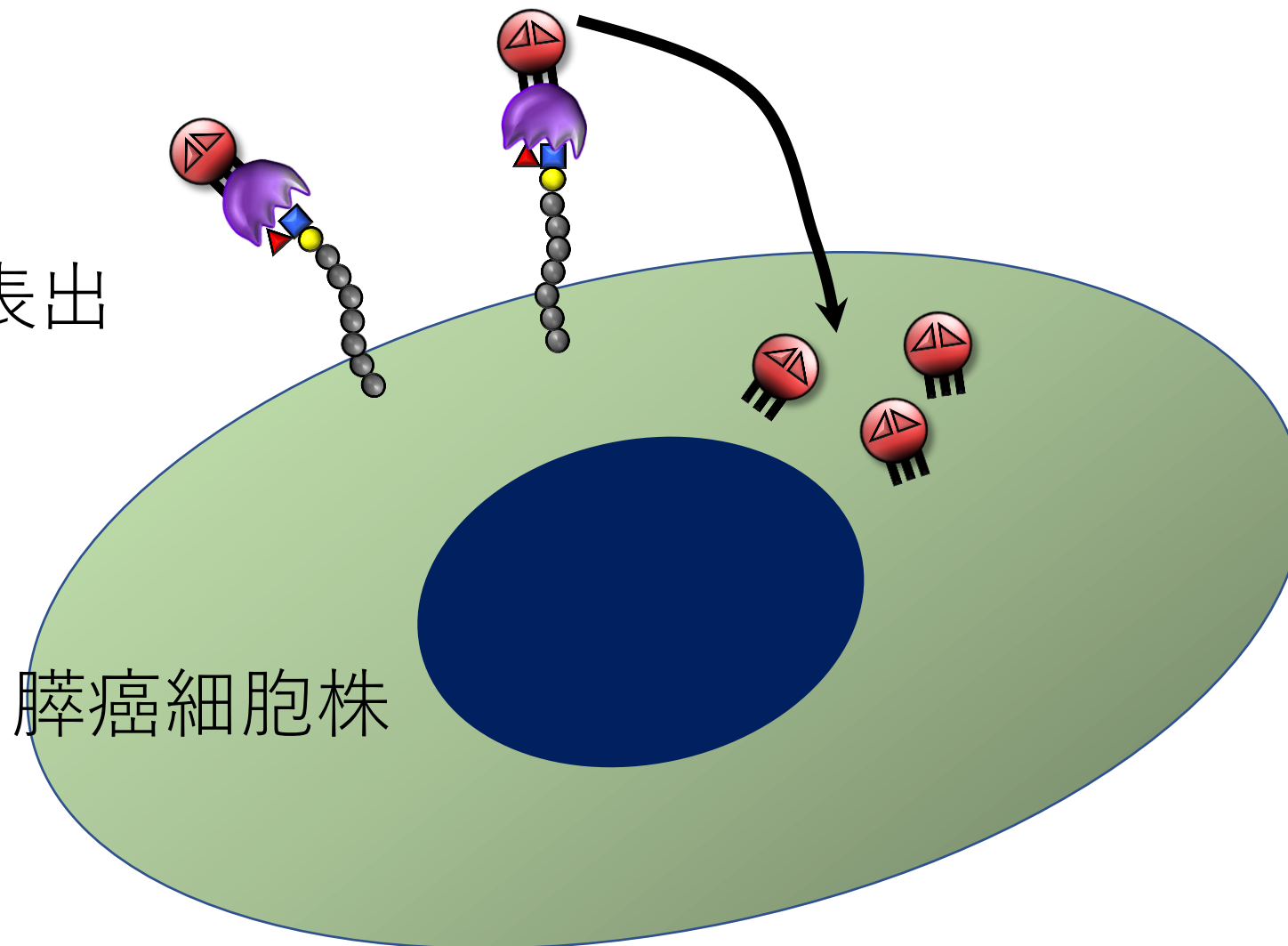
# より臨床膵癌に近いモデルでの検証



# 膵癌細胞株を用いた治療実験

糖鎖（標的）が表出

→治療は容易



# 膵癌の薬剤送達の難しさ

低分子のレクチンでも間質のバリアを通過は困難...

臨床膵癌への治療

細胞外マトリックス

がん関連繊維芽細胞CAFs

膵癌細胞

間質を操作して、薬剤送達を改善しようとする研究は無数にある。しかしこの間質をいじるのは非常に難しい。

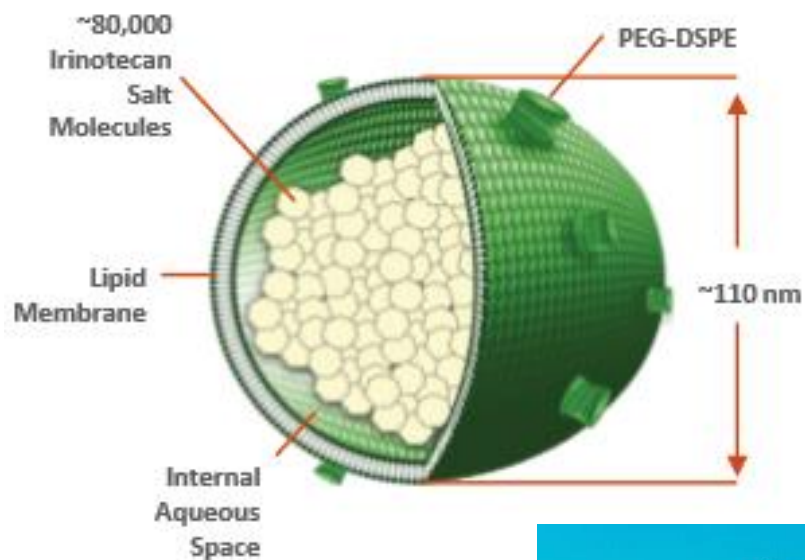


# BC2-PE38の副作用の回避を目指した取り組み

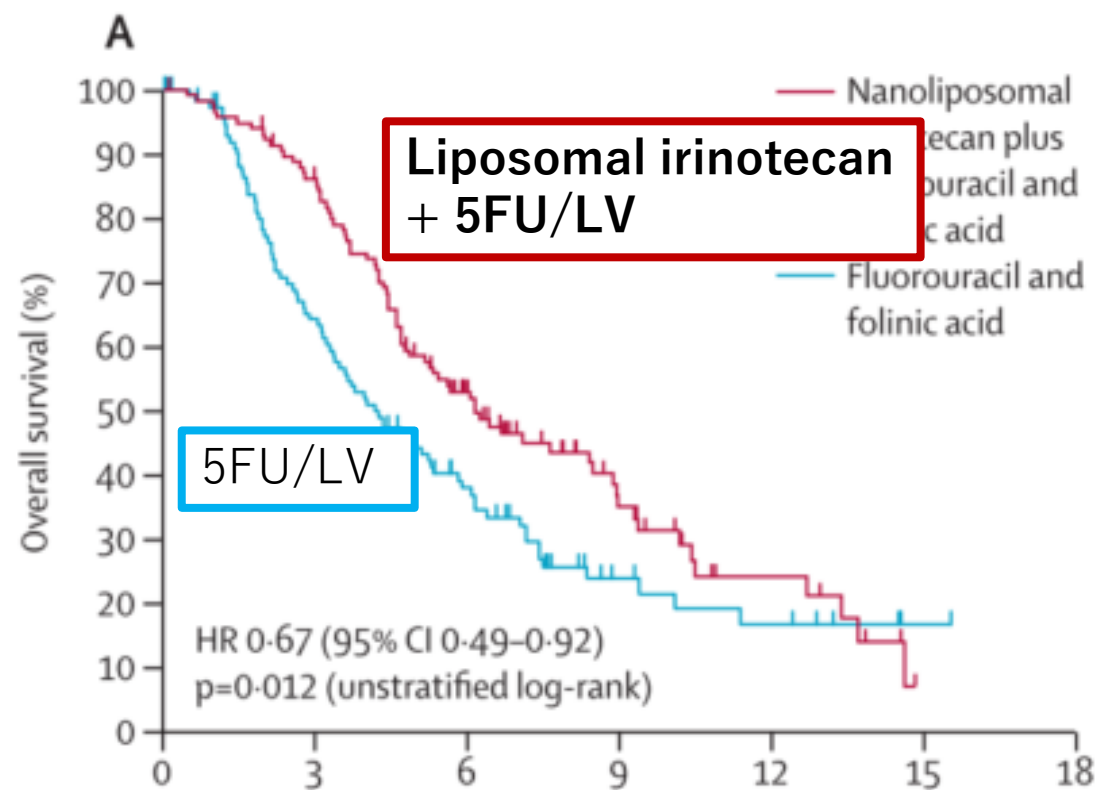
- リポソーム製剤への応用
- 光免疫療法との融合



# 膵癌に承認された抗がん剤含有リポソーム製剤



NAPOLI-1: a global, randomized, open-label, phase 3 trial.  
Stage4 膵癌患者に対する効果

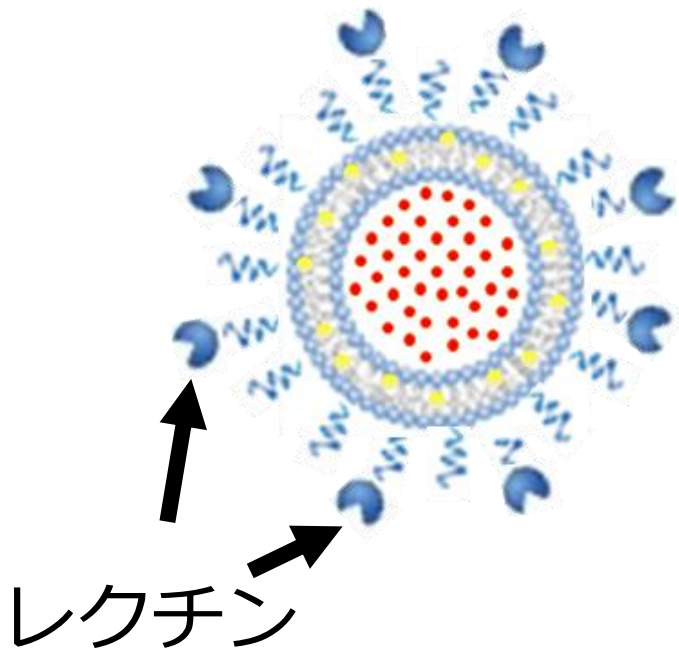


Wang-Gillam A, et al. Lancet. 2016;387(10018):545-57



# リポソーム製剤のレクチンコーティングによる Active Targeting

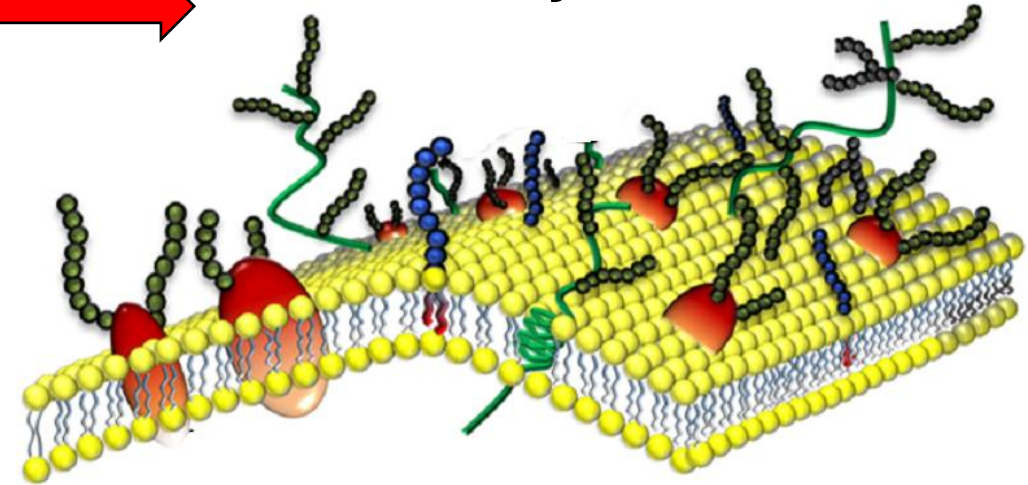
## Lectin coating liposome



*Active  
Targeting*



Cancer-Glycan

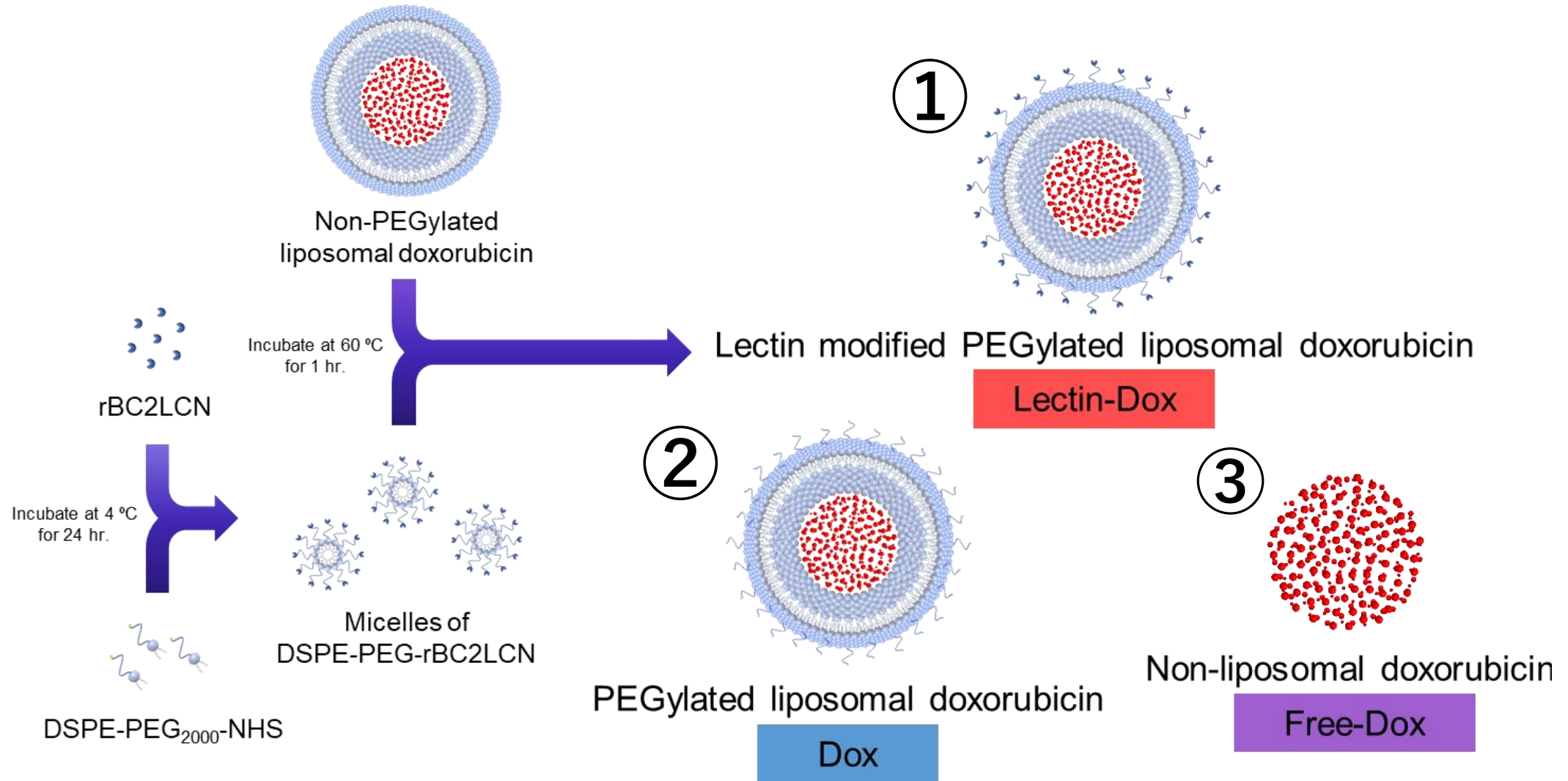


細胞接着性↑, 抗腫瘍効果↑, オフターゲット効果↓





# レクチンコーティングリポソーム製剤による癌治療



# レクチンCoatingリポソーム製剤による治療

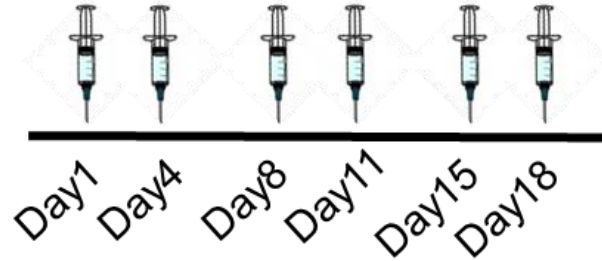
Capan-1/SUIT-2  $2 \times 10^6$  /mouse  
subcutaneous injection



14 days



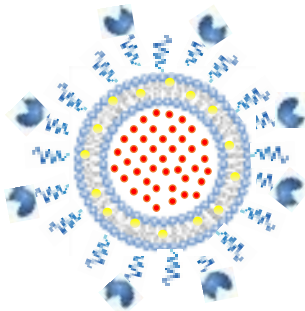
Intravenous injection twice per week  $\times$  3 weeks  
(doxorubicin concentration 2 mg/kg)



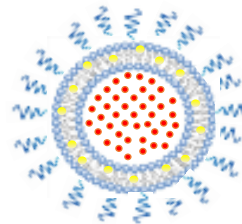
Sacrificed at Day 22

BALB/c nude mouse ♀ 7-8w

① レクチンコーティングDOX



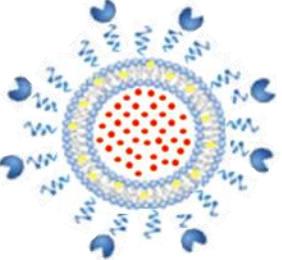
② ドキソルビシン  
リポソーム製剤 (DOX)



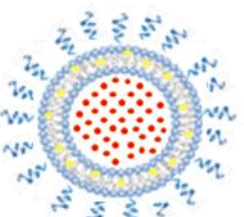
③ Free Dox




# レクチンコーティングによりマウス腫瘍へDDS改善

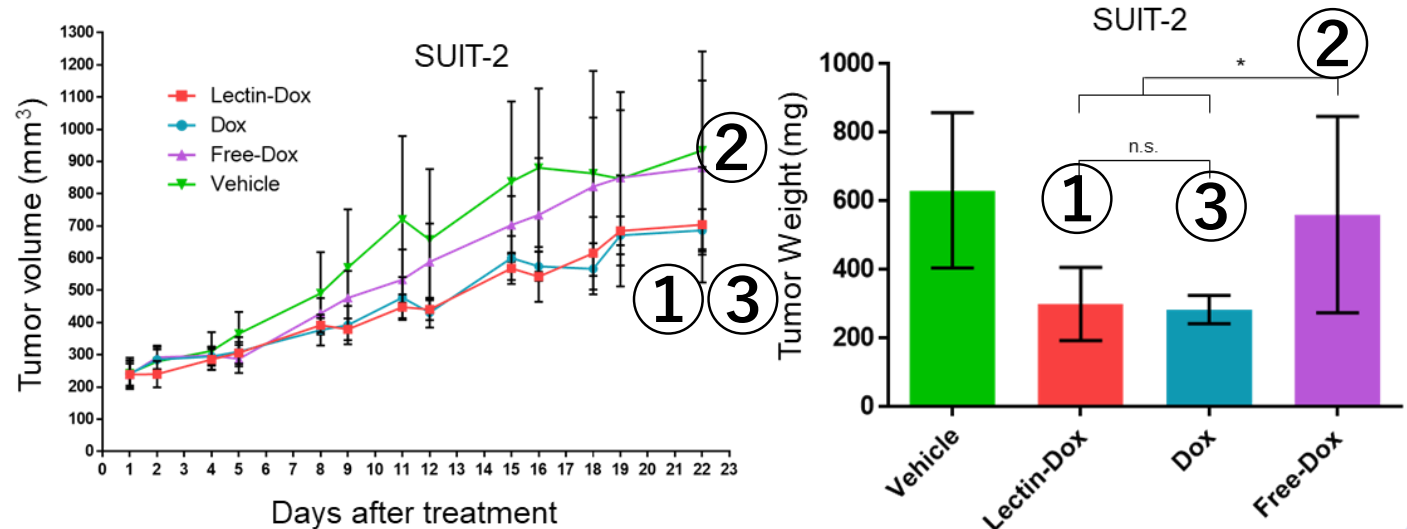
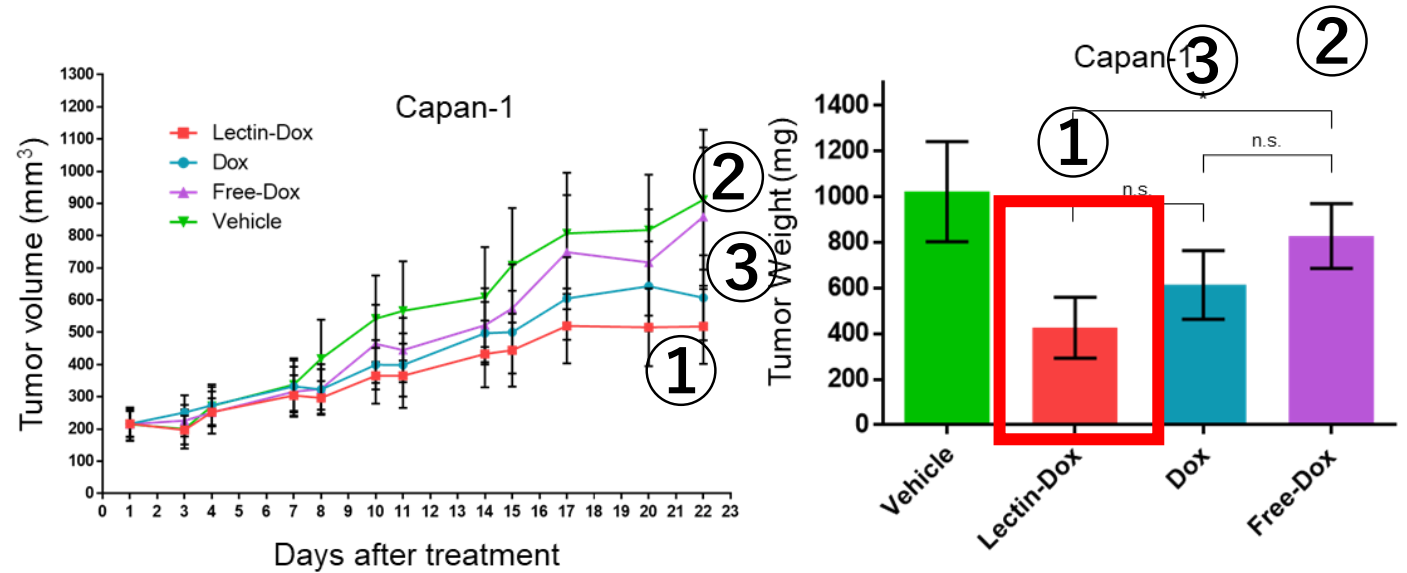
①  
  
 レクチンコーティング

**Capan-1**  
 標的糖鎖あり

②  
  
 リポソーム単体

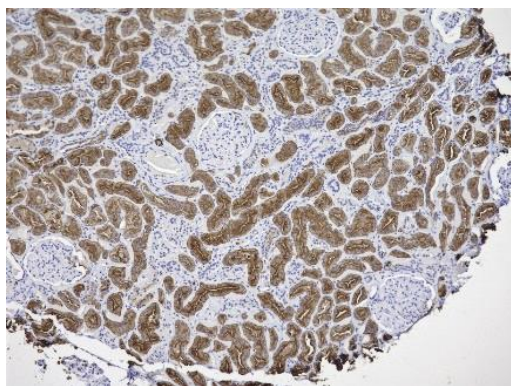
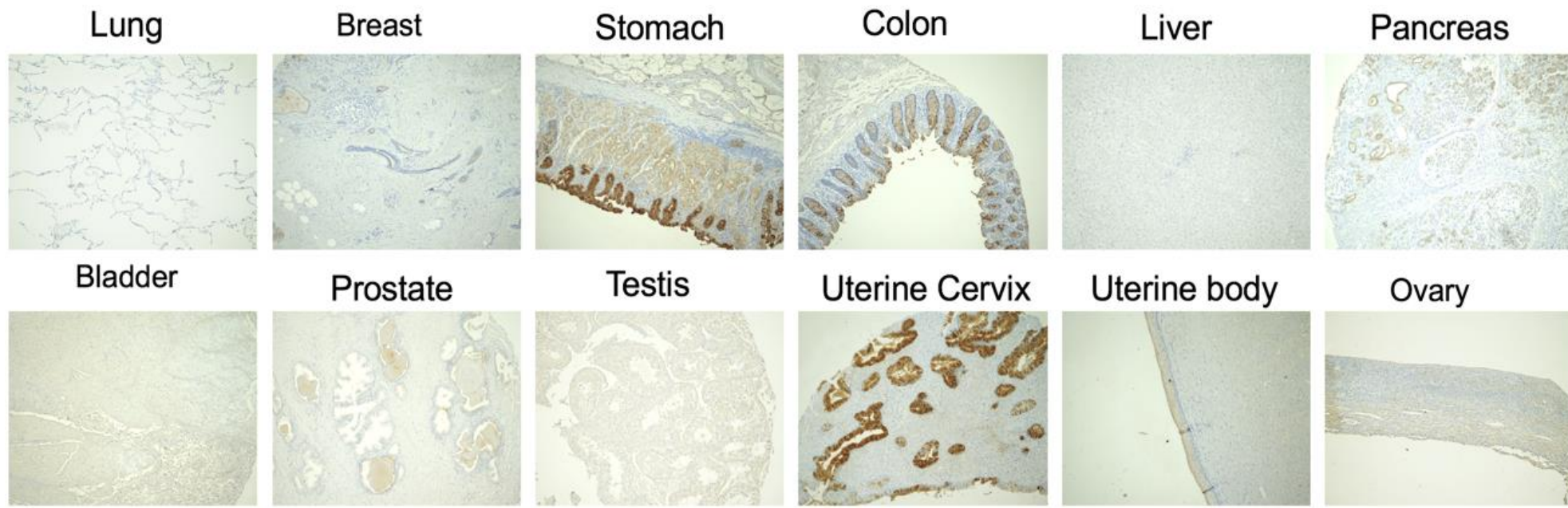
**SUIT-2**  
 標的糖鎖なし

③  
  
 ドキソルビシン



# rBC2LCNのOff site結合

ヒト  
正常部



腎臓(尿細管)

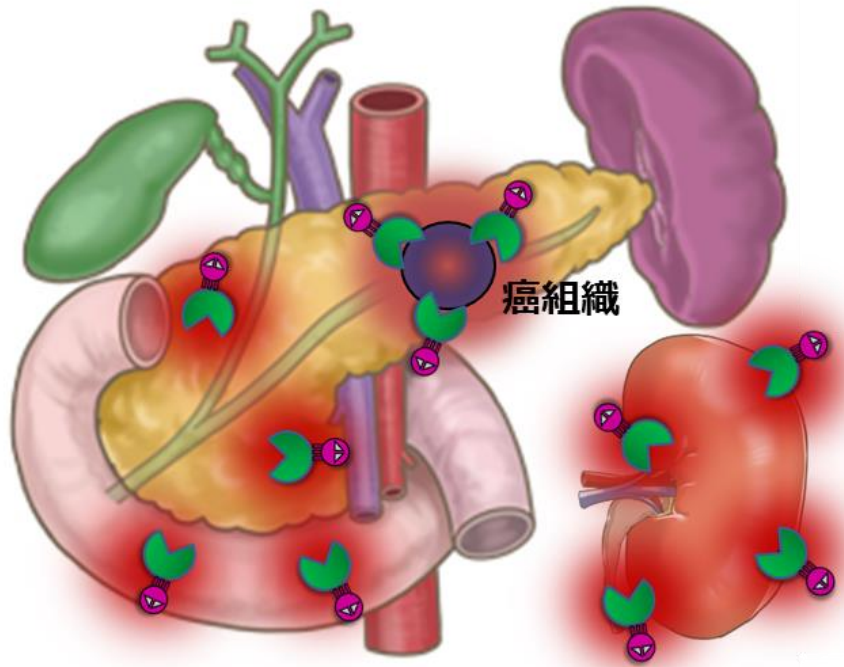
全身投与では副作用の懸念



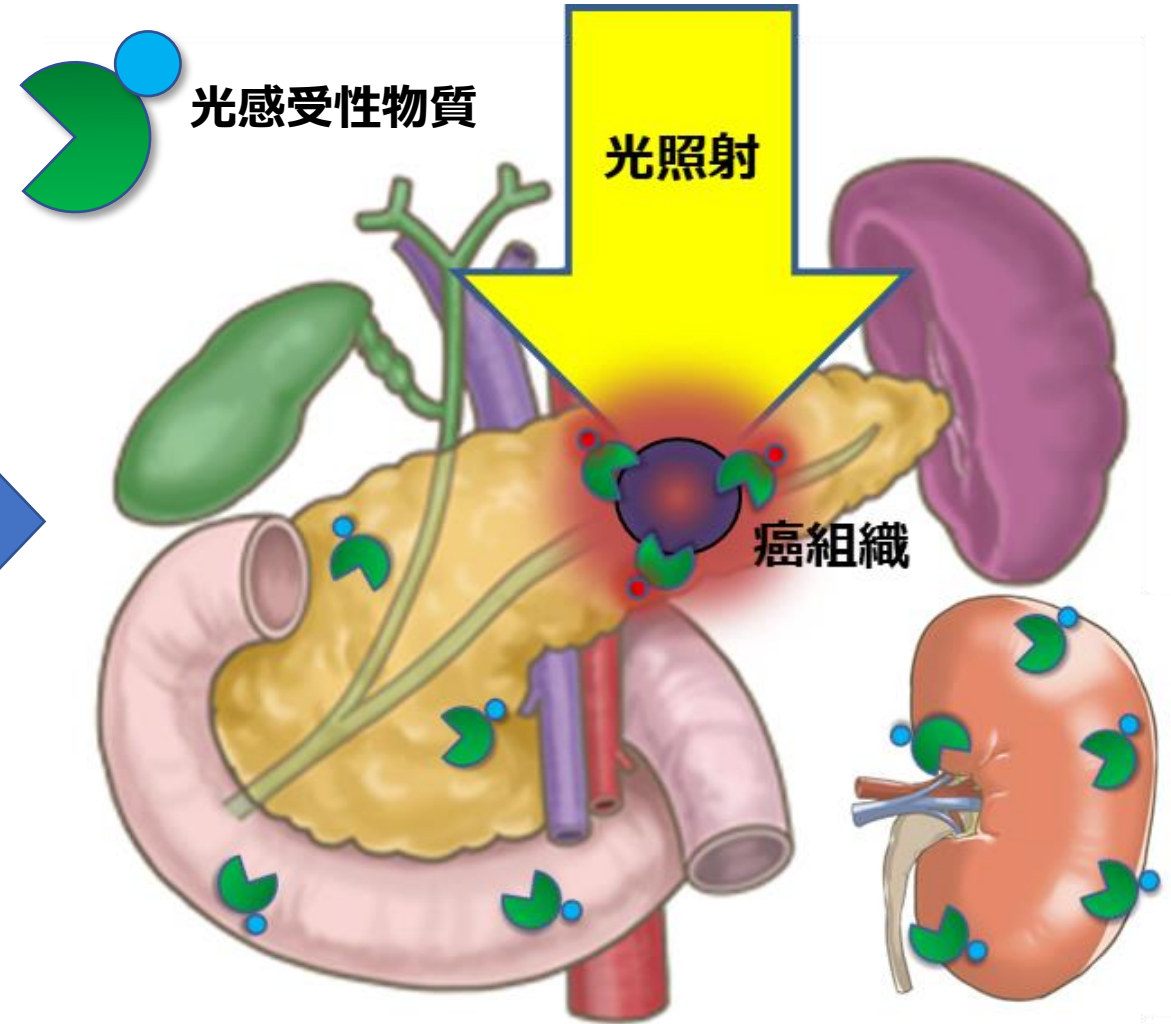
# 正常組織へのレクチンの反応を抑える

→光免疫療法との融合

LDC全身投与の場合



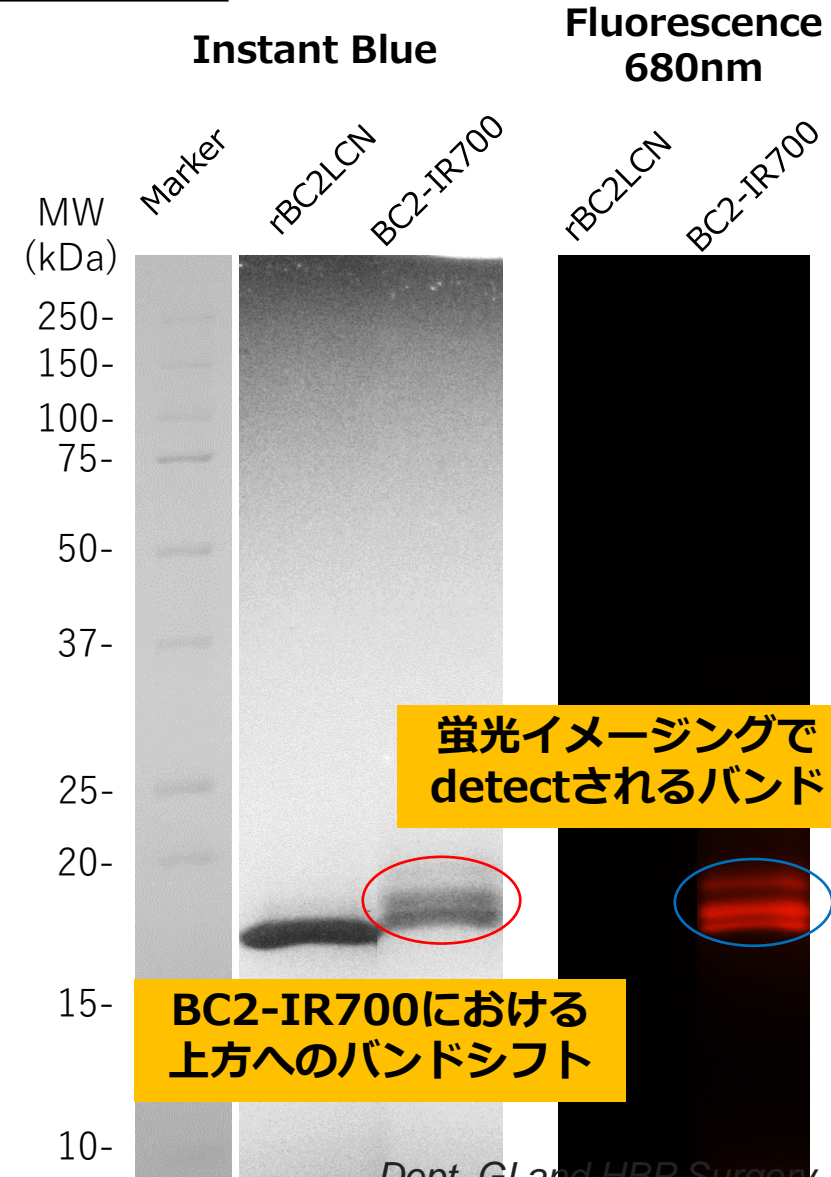
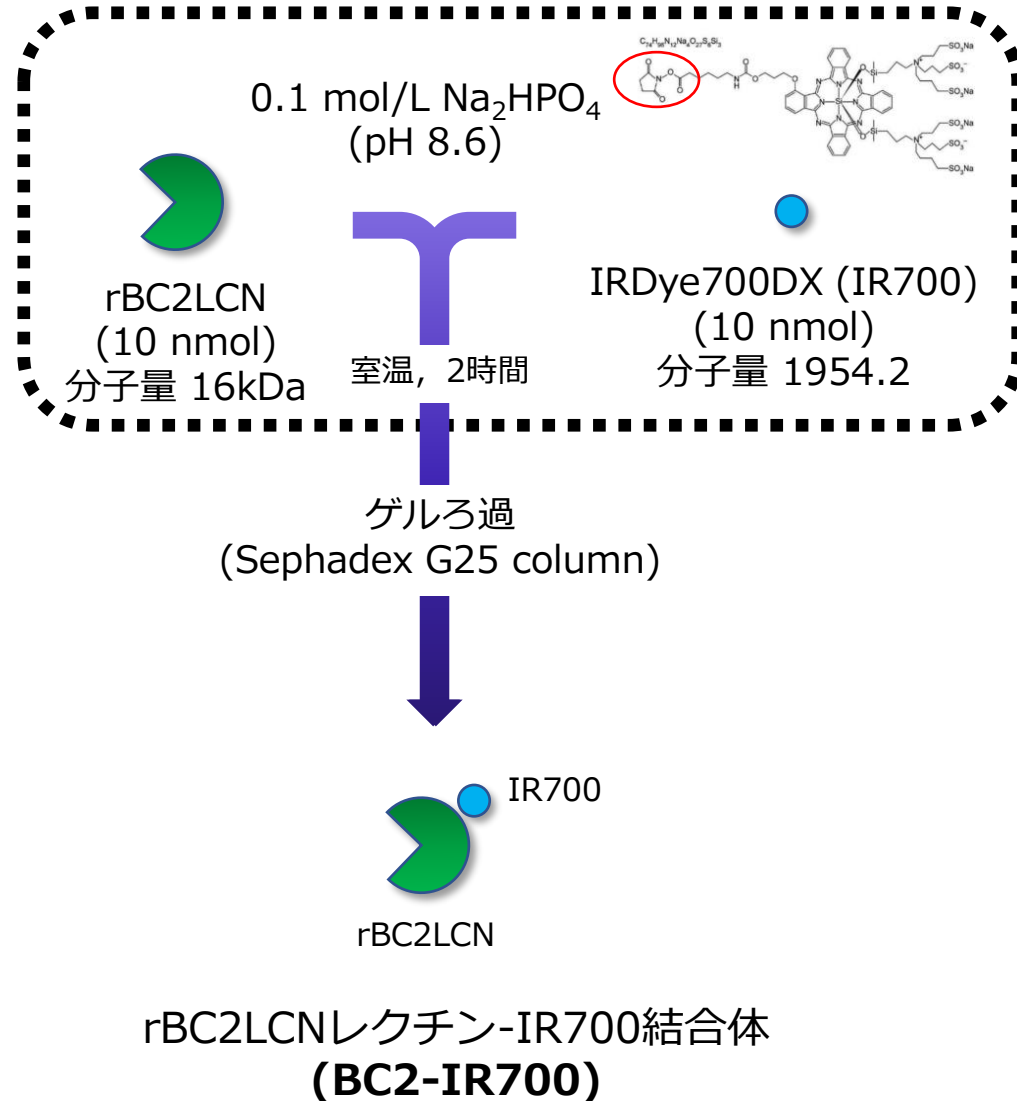
正常臓器への有害事象



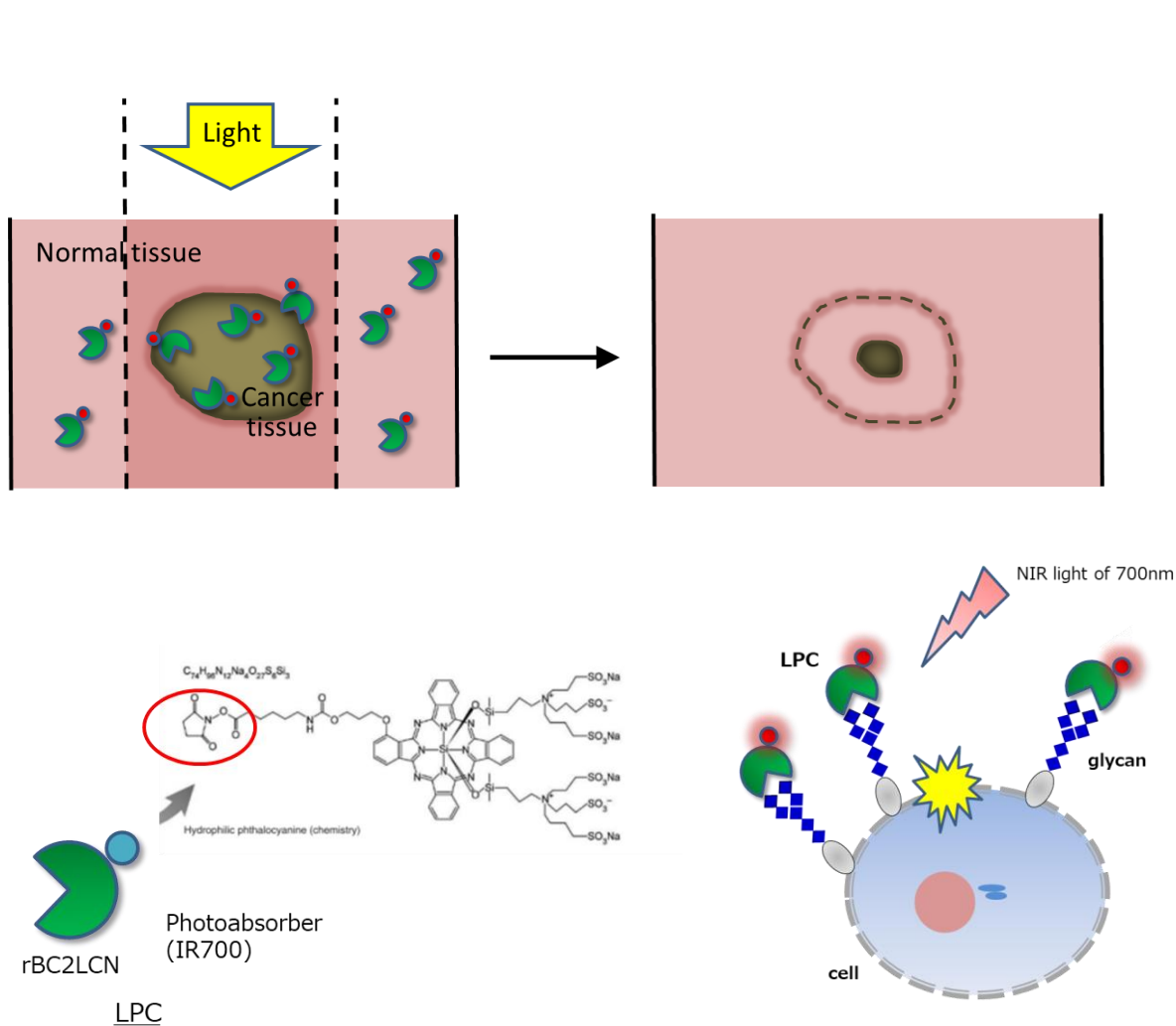
# rBC2LCNレクチン-IR700結合体の合成

Kuroda, Shimomura, Tateno et al., Int J Cancer. 2022

## SDS-PAGE



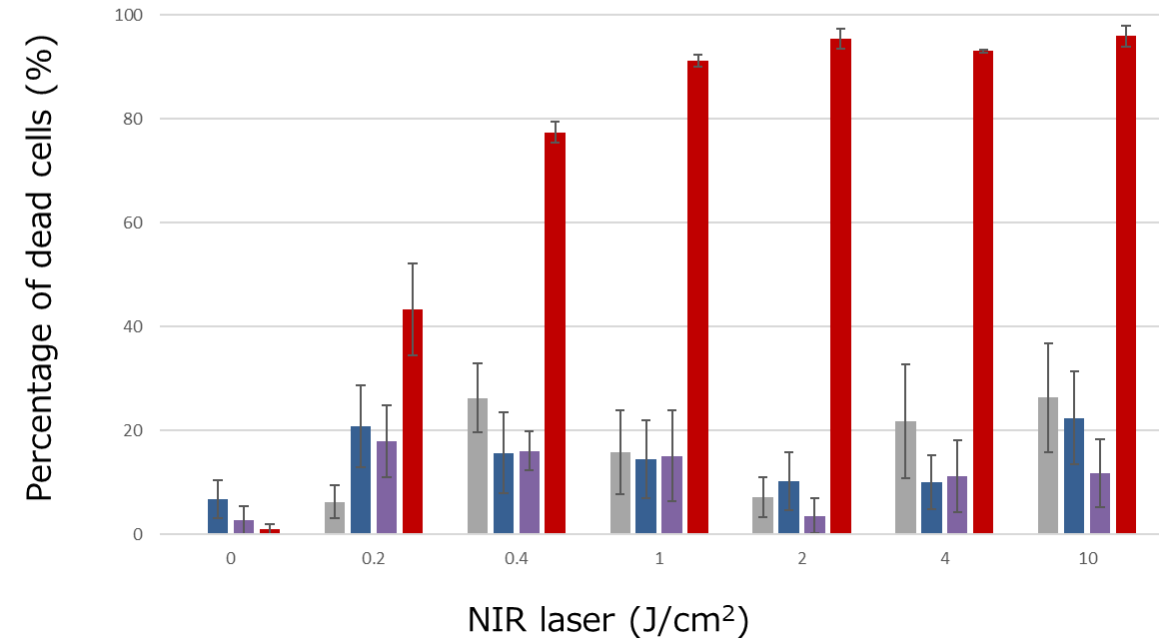
# BC2レクチン-IR700による膵癌細胞株治療



*in vitro* NIR phototherapy



**Capan-1**



# 標的細胞への結合性評価

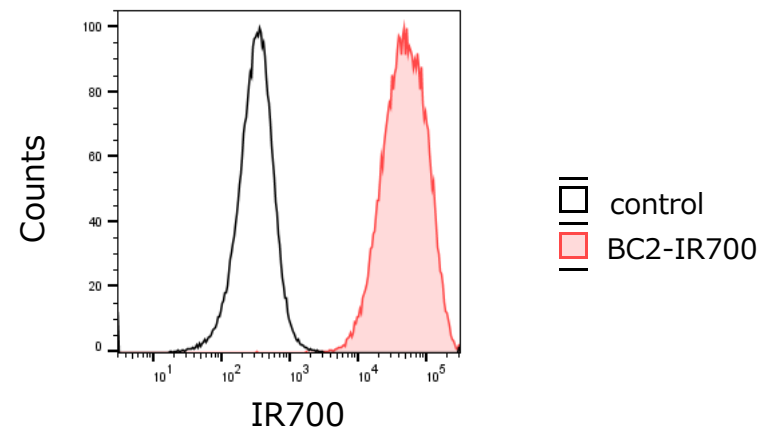
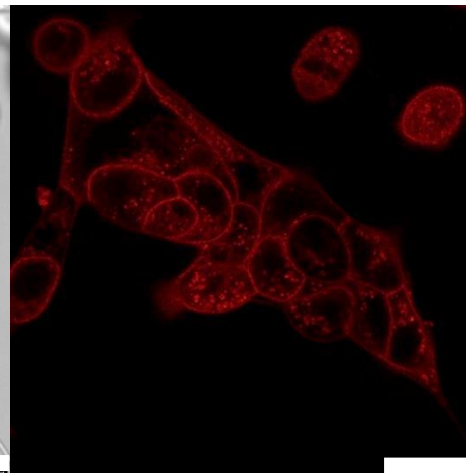
Kuroda, Shimomura, Tateno et al., Int J Cancer. 2022

**Capan-1**  
**(特異的糖鎖+)**

Phase contrast

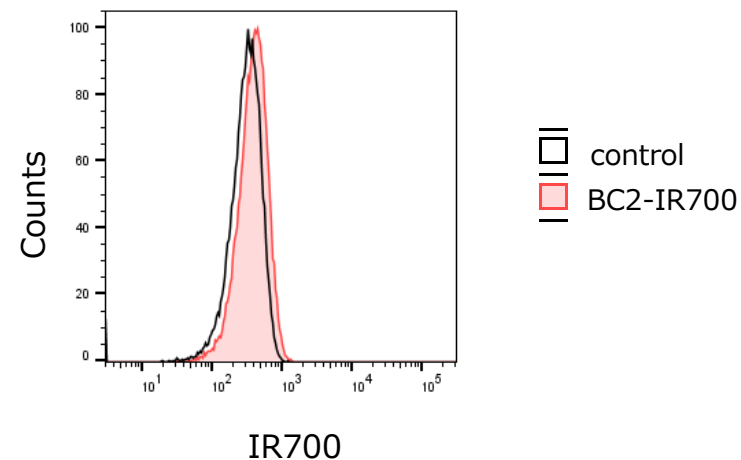
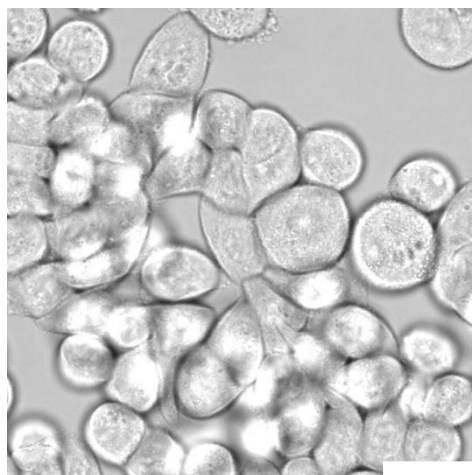


IR700



標的細胞に対する特異的結合性

**SUIT-2**  
**(特異的糖鎖-)**



Scale bars = 25μm

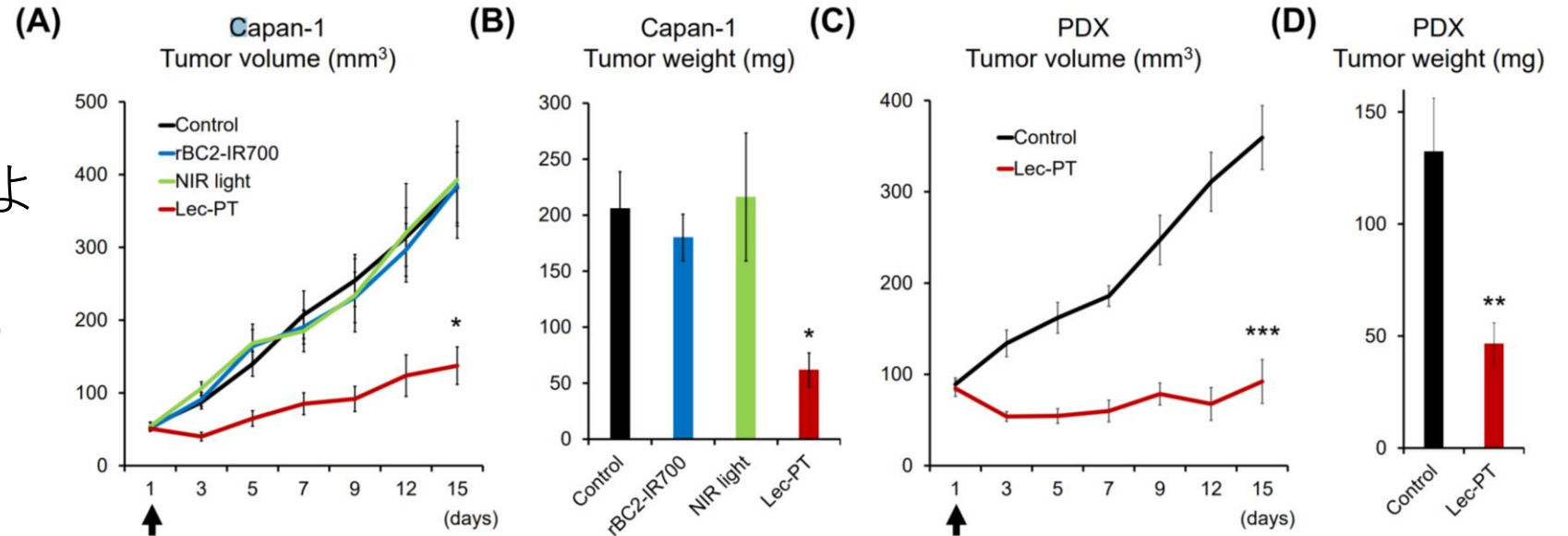
Dept. GI and HBP Surgery, Univ of Tsukuba



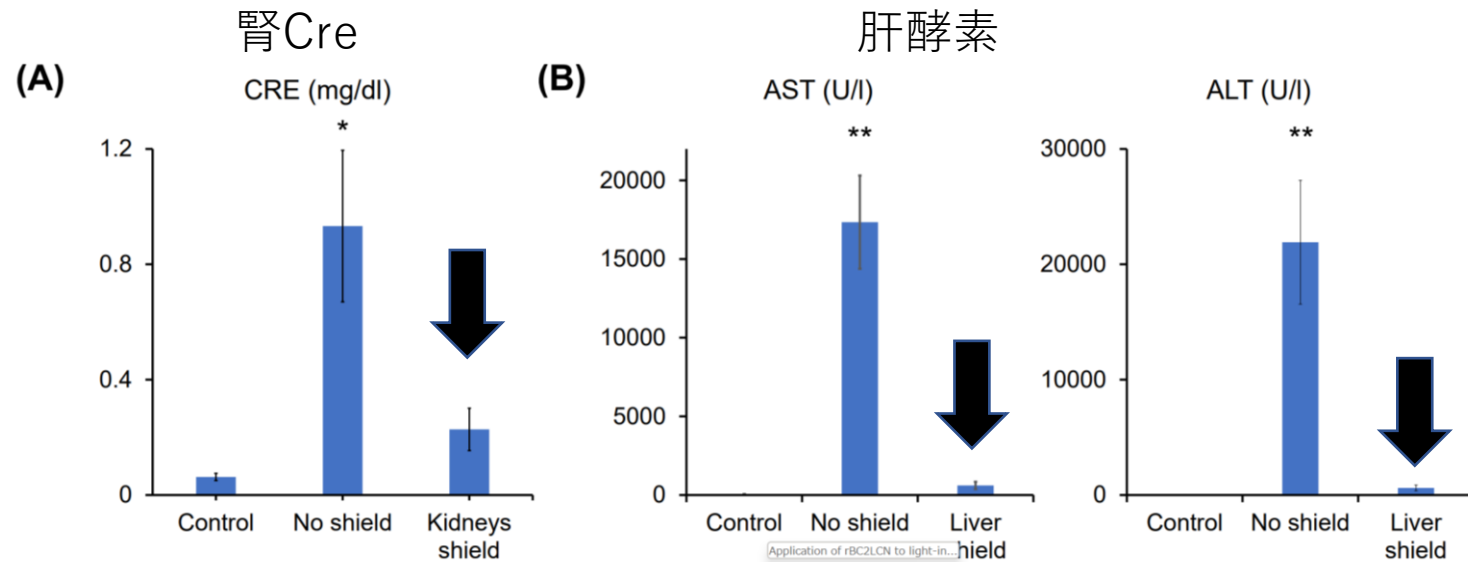


# BC2-IR700

投与 + 赤外線照射による抗腫瘍効果  
(膵癌マウスモデル)



赤外線遮蔽による肝、腎障害の予防



# 膵癌研究 進行中のアプローチ

## 癌遺伝子

KRAS阻害薬  
遺伝子治療  
cfDNA, ctDNA, miRNA

## 間質CAF

間質阻害薬  
間質透過性ペプチド・・・

# 膵癌

## がん免疫

抗PD 1 抗体  
がん免疫療法

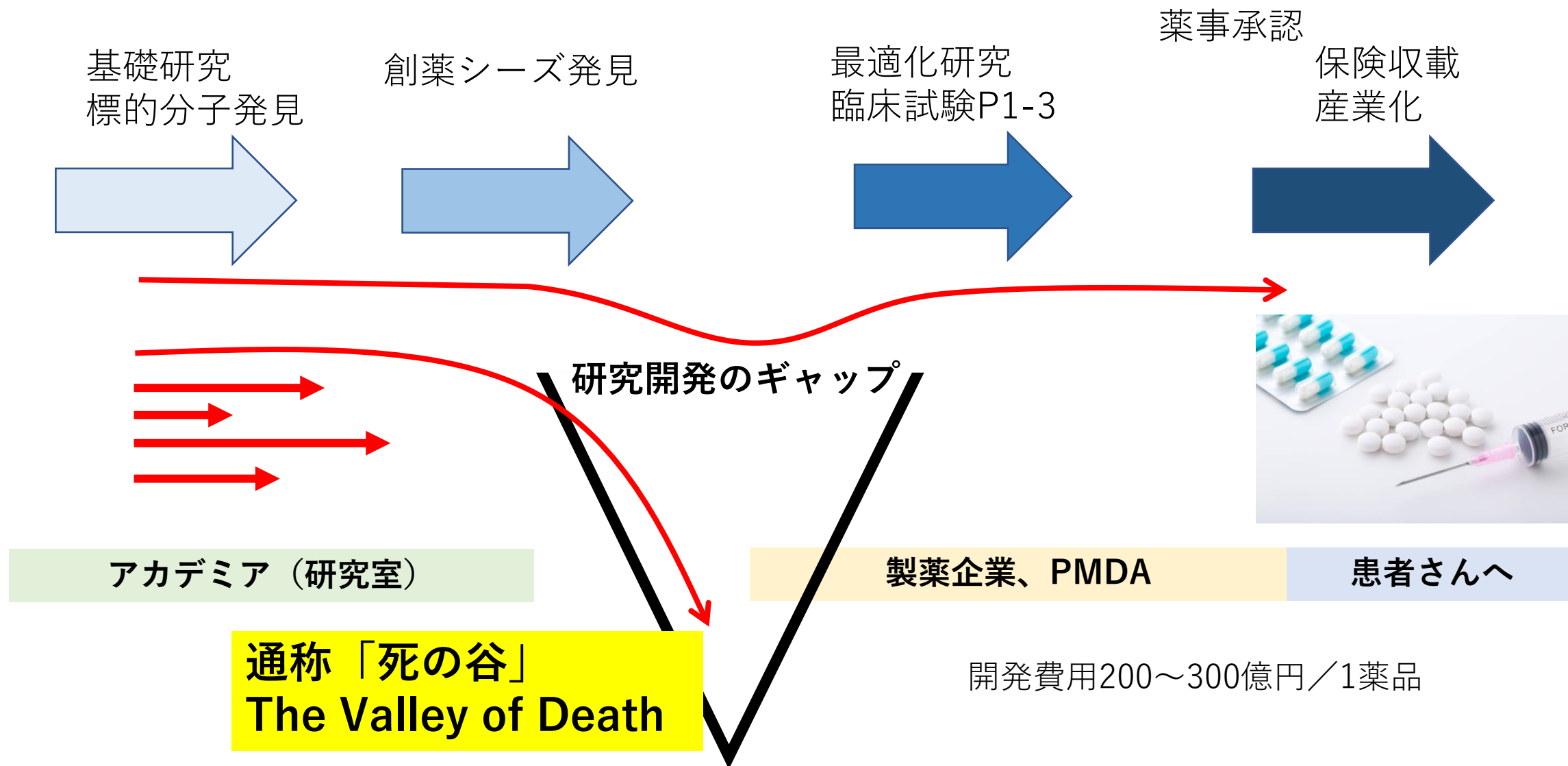
## 糖鎖

糖鎖標的治療  
診断薬開発  
腫瘍マーカー

## 薬剤耐性

# 基礎研究のゴール

→ 創薬、診断法、新しい治療法



# Take Home Message

- 膵癌は早期発見が重要
- レクチン-薬剤融合薬は高い治療効果があったが、膵癌に応用するにはまだハードルがある
- 膵癌の予後改善に向けて、力を合わせた研究開発が必要

