LSIM安全科学研究所　LSIメディエンスグループ

第49回日本毒性学会学術年会
ランチョンセミナー　L3-8

PDXを用いた創薬支援サービス

LSIメディエンス

LSIM安全科学研究所

株式会社LSIM安全科学研究所
熊本研究所　薬理研究部
榎　成憲

本日の内容

1. 抗がん剤の開発支援ツール
2. PDXモデル
   - PDXモデルとは
   - 細胞株からPDXへ
3. J-PDXライブラリー
   - ライブラリーの特長
   - がん種内訳
4. 抗がん剤の開発支援～LSSIの技術
   - J-PDXの増殖確認
   - PDX担癌マウス抗腫瘍試験
   - 標的確認（組織マイクロアレイ）
   - in vitro 3D培養法
   - PDCを用いたin vitro（2D）抗腫瘍試験
   - ヒト化マウスを用いた抗腫瘍試験
   - 利用可能なPDX

1．抗がん剤の開発支援ツール

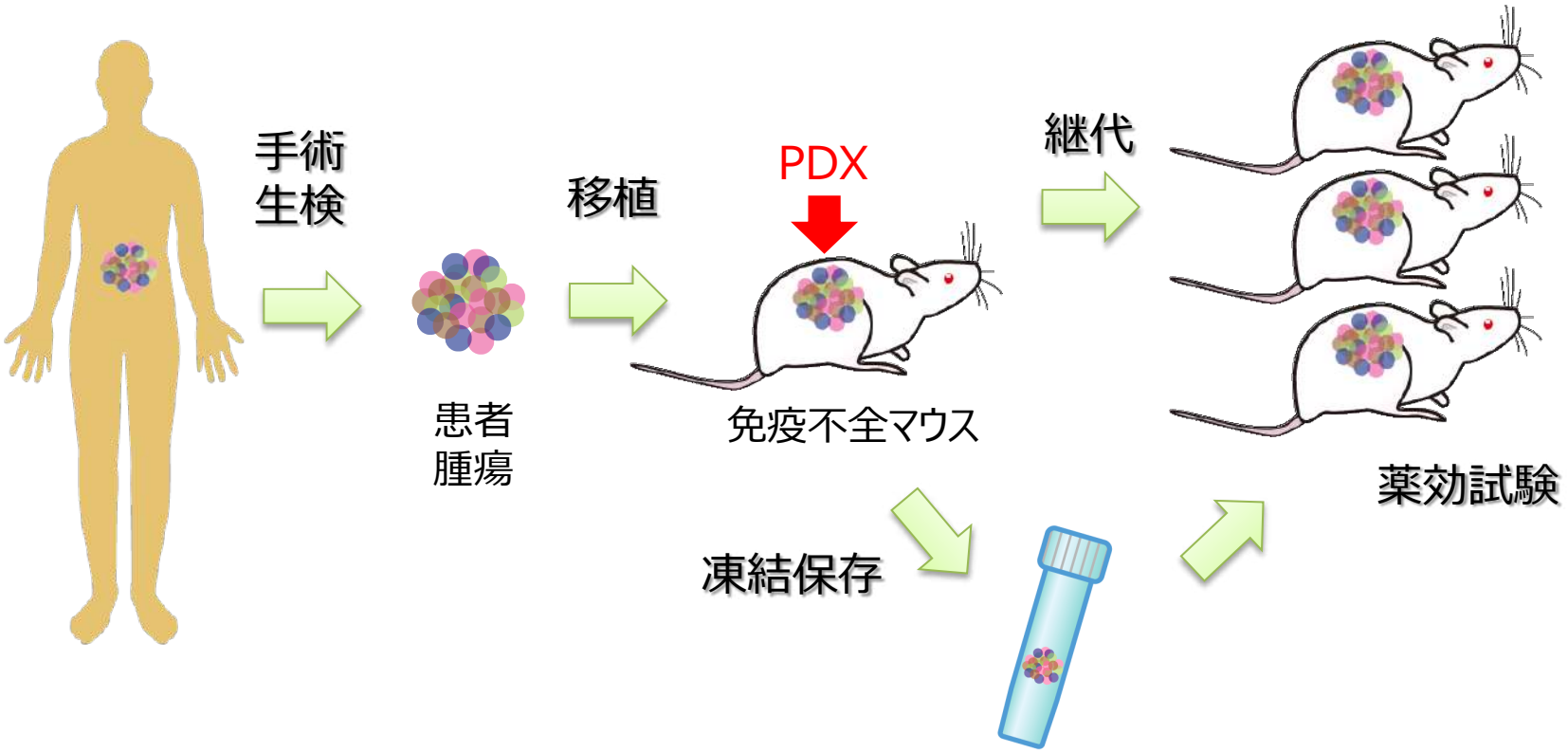
- 非臨床で薬効を認めた抗がん剤のうち米国FDAで承認された薬剤は5%程度と報告
- 培養細胞株による評価と臨床の結果が一致しない
- 培養細胞株を免疫不全マウスに移植したCDX（cell line-derived xenograft）モデルは、がんの多様性、複雑性が反映されない、臨床試験の結果予測には不向きなモデル

抗がん剤開発の成功確率を向上させるツールの導入が必要

- 患者由来の細胞・組織を用いた評価技術の活用が期待
- 患者腫瘍を免疫不全マウスに移植し、がんの微小環境を再現する**PDXモデル**（patient-derived xenograft:患者由来異種移植モデル）に着目

2．PDXモデル：PDXモデルとは

- 患者由来の腫瘍組織を免疫不全マウスに移植して作成されるモデル



PDXは患者のアバター

2．PDXモデル：細胞株からPDXへ

欧米の動向：PDXの利用が進む方向へ

2013年：EurOPDXの立ち上げ
アカデミア中心に18施設参加、
PDXコレクションと標準化の取り組み

2016年：米国・国立がん研究所が
細胞株パネル (NCI-60) から
PDXへの切り替えを宣言

2017年：NCI Patient-Derived
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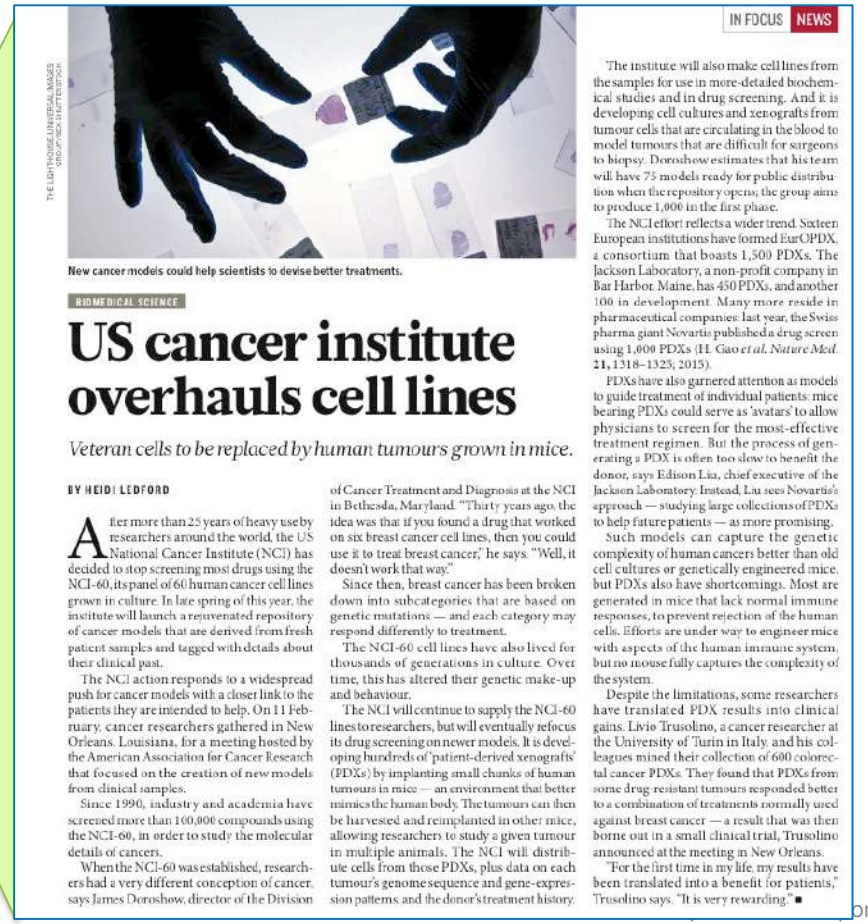
2018年：AMED CiCLE
J-PDXライブラリー整備事業

Nature *530:391,2016*



IN FOCUS NEWS

New cancer models could help scientists to devise better treatments.

BIOMEDICAL SCIENCE

US cancer institute overhauls cell lines

Veteran cells to be replaced by human tumours grown in mice.

BY HEIDI LEDFORD

After more than 25 years of heavy use by researchers around the world, the US National Cancer Institute (NCI) has decided to stop screening most drugs using the NCI-60, its panel of 60 human cancer cell lines grown in culture. In late spring of this year, the institute will launch a rejuvenated repository of cancer models that are derived from fresh patient samples and tagged with details about their clinical past.

The NCI action responds to a widespread push for cancer models with a closer link to the patients they are intended to help. On 11 February, cancer researchers gathered in New Orleans, Louisiana, for a meeting hosted by the American Association for Cancer Research that focused on the creation of new models from clinical samples.

Since 1990, industry and academia have screened more than 100,000 compounds using the NCI-60, in order to study the molecular details of cancers.

When the NCI-60 was established, researchers had a very different conception of cancer, says James Doroshow, director of the Division of Cancer Treatment and Diagnosis at the NCI in Bethesda, Maryland. "Thirty years ago, the idea was that if you found a drug that worked on six breast cancer cell lines, then you could use it to treat breast cancer," he says. "Well, it doesn't work that way."

Since then, breast cancer has been broken down into subcategories that are based on genetic mutations — and each category may respond differently to treatment.

The NCI-60 cell lines have also lived for thousands of generations in culture. Over time, this has altered their genetic make-up and behaviour.

The NCI will continue to supply the NCI-60 lines to researchers, but will eventually refocus its drug screening on newer models. It is developing hundreds of 'patient-derived xenografts' (PDXs) by implanting small chunks of human tumours in mice — an environment that better mimics the human body. The tumours can then be harvested and reimplanted in other mice, allowing researchers to study a given tumour in multiple animals. The NCI will distribute cells from those PDXs, plus data on each tumour's genome sequence and gene-expression patterns, and the donor's treatment history.

The institute will also make cell lines from the samples for use in more-detailed biochemical studies and in drug screening. And it is developing cell cultures and xenografts from tumour cells that are circulating in the blood to model tumours that are difficult for surgeons to biopsy. Doroshow estimates that his team will have 75 models ready for public distribution when the repository opens; the group aims to produce 1,000 in the first phase.

The NCI effort reflects a wider trend. Sixteen European institutions have formed EurOPDX, a consortium that boasts 1,500 PDXs. The Jackson Laboratory, a non-profit company in Bar Harbor, Maine, has 450 PDXs, and another 100 in development. Many more reside in pharmaceutical companies: last year, the Swiss pharma giant Novartis published a drug screen using 1,000 PDXs (H. Gao *et al. Nature Med.* **21**, 1318–1325; 2015).

PDXs have also garnered attention as models to guide treatment of individual patients: mice bearing PDXs could serve as 'avatars' to allow physicians to screen for the most-effective treatment regimen. But the process of generating a PDX is often too slow to benefit the donor, says Edison Liu, chief executive of the Jackson Laboratory. Instead, Liu sees Novartis's approach — studying large collections of PDXs to help future patients — as more promising.

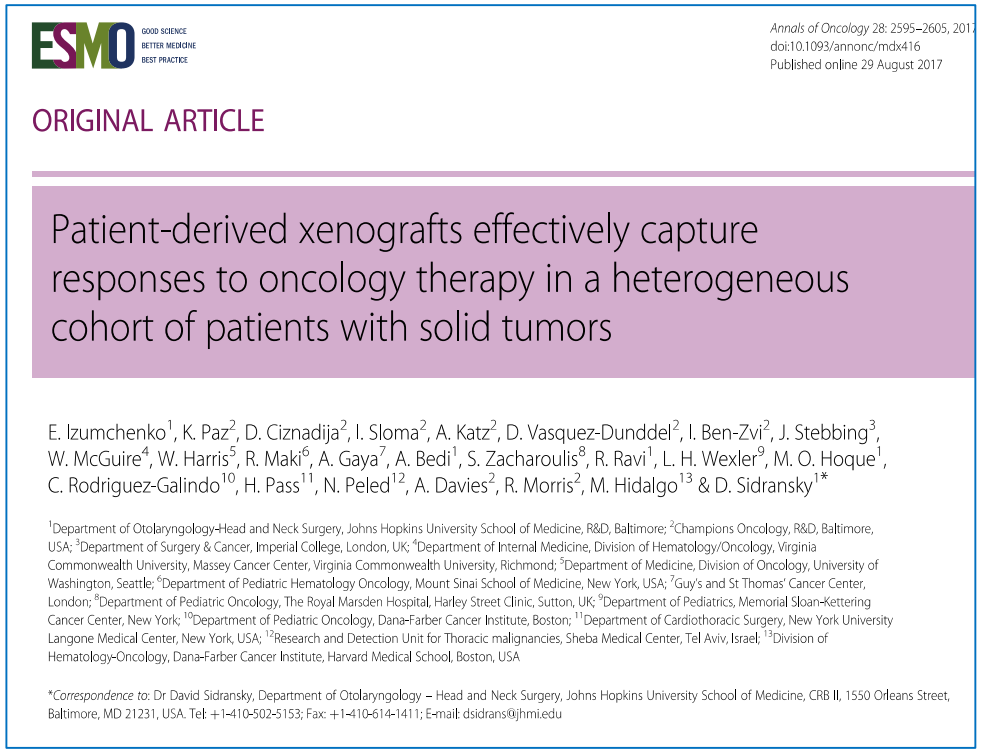
Such models can capture the genetic complexity of human cancers better than old cell cultures or genetically engineered mice, but PDXs also have shortcomings. Most are generated in mice that lack normal immune responses, to prevent rejection of the human cells. Efforts are under way to engineer mice with aspects of the human immune system, but no mouse fully captures the complexity of the system.

Despite the limitations, some researchers have translated PDX results into clinical gains. Livio Trusolino, a cancer researcher at the University of Turin in Italy, and his colleagues mined their collection of 600 colorectal cancer PDXs. They found that PDXs from some drug-resistant tumours responded better to a combination of treatments normally used against breast cancer — a result that was then borne out in a small clinical trial, Trusolino announced at the meeting in New Orleans.

"For the first time in my life, my results have been translated into a benefit for patients," Trusolino says. "It is very rewarding." ■

2．PDXモデル：細胞株からPDXへ

文献報告からPDXモデルの臨床予測性能は80%以上



ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

Annals of Oncology 28: 2595–2605, 2017
doi:10.1093/annonc/mdx416
Published online 29 August 2017

ORIGINAL ARTICLE

Patient-derived xenografts effectively capture responses to oncology therapy in a heterogeneous cohort of patients with solid tumors

E. Izumchenko[1], K. Paz[2], D. Ciznadija[2], I. Sloma[2], A. Katz[2], D. Vasquez-Dunddel[2], I. Ben-Zvi[2], J. Stebbing[3], W. McGuire[4], W. Harris[5], R. Maki[6], A. Gaya[7], A. Bedi[1], S. Zacharoulis[8], R. Ravi[1], L. H. Wexler[9], M. O. Hoque[1], C. Rodriguez-Galindo[10], H. Pass[11], N. Peled[12], A. Davies[2], R. Morris[2], M. Hidalgo[13] & D. Sidransky[1]*

[1]Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins University School of Medicine, R&D, Baltimore; [2]Champions Oncology, R&D, Baltimore, USA; [3]Department of Surgery & Cancer, Imperial College, London, UK; [4]Department of Internal Medicine, Division of Hematology/Oncology, Virginia Commonwealth University, Massey Cancer Center, Virginia Commonwealth University, Richmond; [5]Department of Medicine, Division of Oncology, University of Washington, Seattle; [6]Department of Pediatric Hematology Oncology, Mount Sinai School of Medicine, New York, USA; [7]Guy's and St Thomas' Cancer Center, London; [8]Department of Pediatric Oncology, The Royal Marsden Hospital, Harley Street Clinic, Sutton, UK; [9]Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York; [10]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston; [11]Department of Cardiothoracic Surgery, New York University Langone Medical Center, New York, USA; [12]Research and Detection Unit for Thoracic malignancies, Sheba Medical Center, Tel Aviv, Israel; [13]Division of Hematology-Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA

*Correspondence to: Dr David Sidransky, Department of Otolaryngology – Head and Neck Surgery, Johns Hopkins University School of Medicine, CRB II, 1550 Orleans Street, Baltimore, MD 21231, USA. Tel: +1-410-502-5153; Fax: +1-410-614-1411; E-mail: dsidrans@jhmi.edu

Annals of Oncology 28: 2595, 2017

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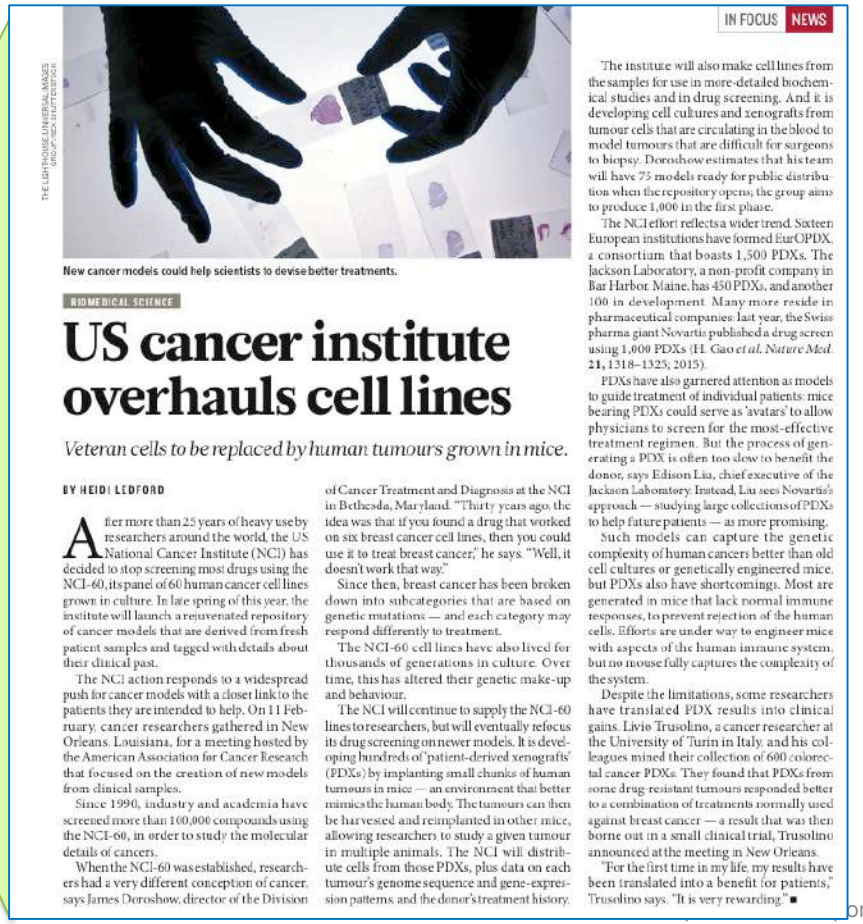

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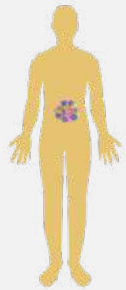
3．J-PDXライブラリー

LSIメディエンスがAMEDの医療研究開発革新基盤創成事業（CiCLE）で国立がん研究センターと協働で基盤整備した日本人PDX（J-PDX）ライブラリー

国立がん研究センター

病院・研究所

倫理審査
IC取得
検体採取



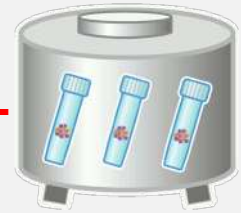
移植・生着

LSIメディエンス

ＬＳＩＭ安全科学研究所
熊本研究所 GLP施設

増殖・樹立

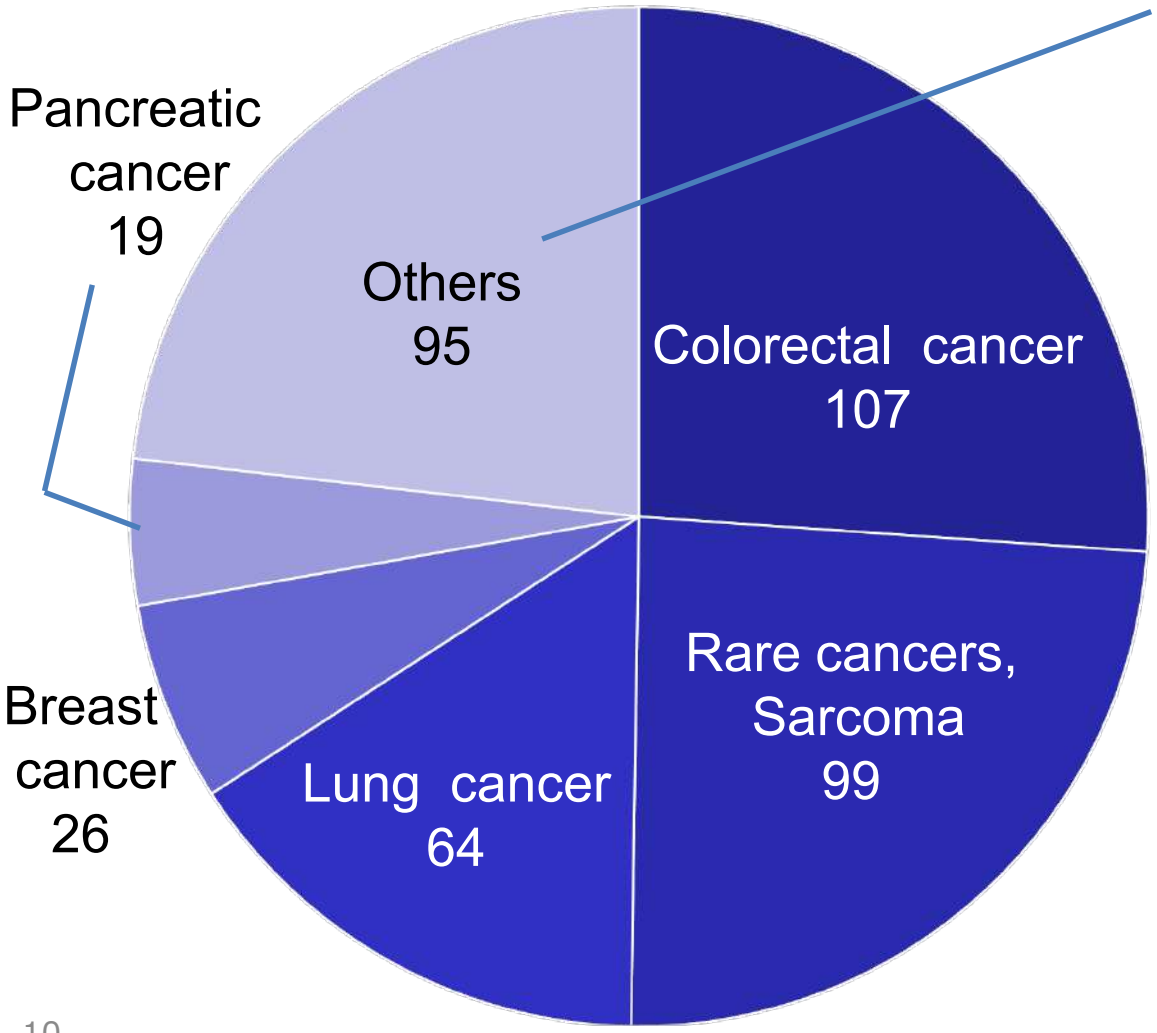
J-PDX
ライブラリー



3．J-PDX：ライブラリーの特長

1. 商用利用に関する患者同意が取得済み
2. 熊本研究所GLP施設で維持・管理
3. 治療歴等の臨床情報が付帯
4. 希少がんを含む多様ながん種のPDX
5. 進行期・再発期、薬剤耐性期のPDXが多数

3．J-PDX：がん種内訳



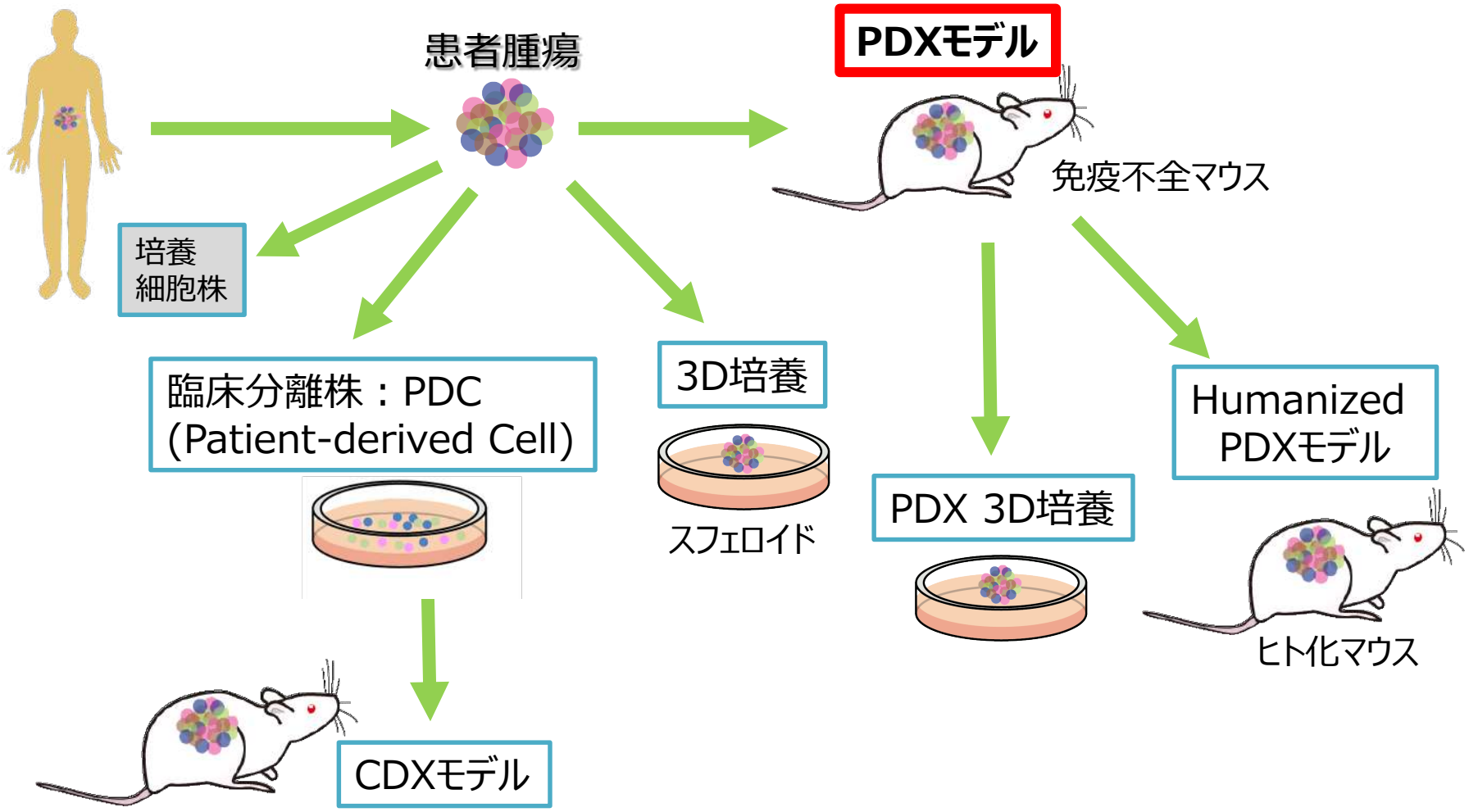
Biliary tract cancer	18	Gastric cancer	13
Brain tumor	15	Ovarian cancer	13
Endometrial cancer	15	Esophageal cancer	10

400株以上、 30種以上のがん種のPDXが生着
国立がんセンターから熊本研究所に移管
ライブラリー整備中

第80回日本癌学会学術総会（2021年）発表資料を改変

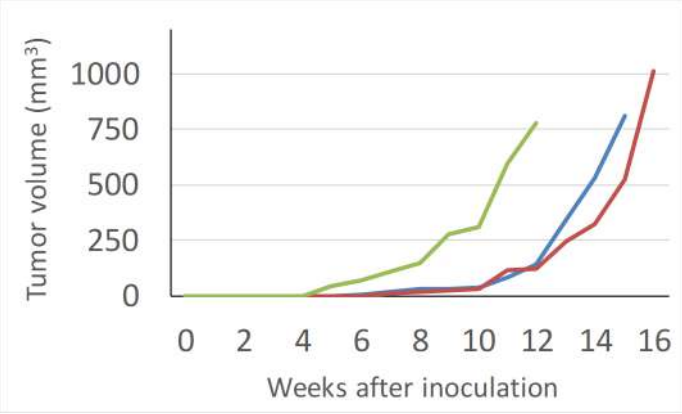
４．抗がん剤の開発支援～LSSIの技術

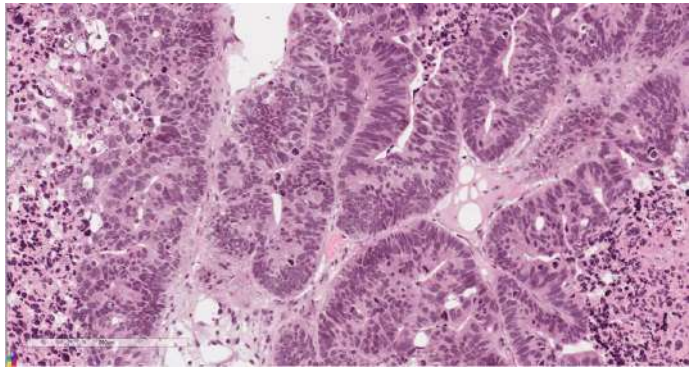
ヒト腫瘍由来の細胞・組織を用いた評価系



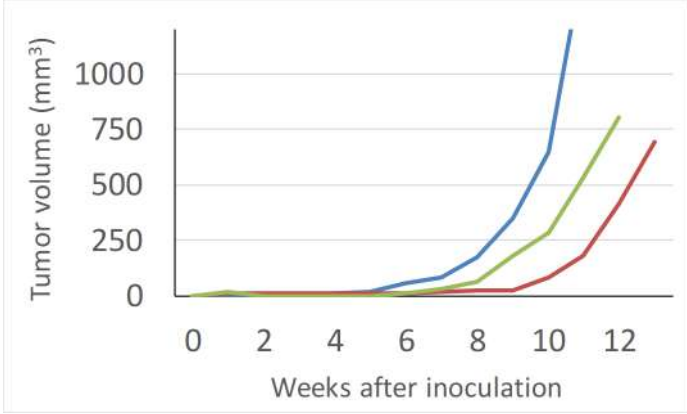
4．抗がん剤の開発支援：J-PDXの増殖確認

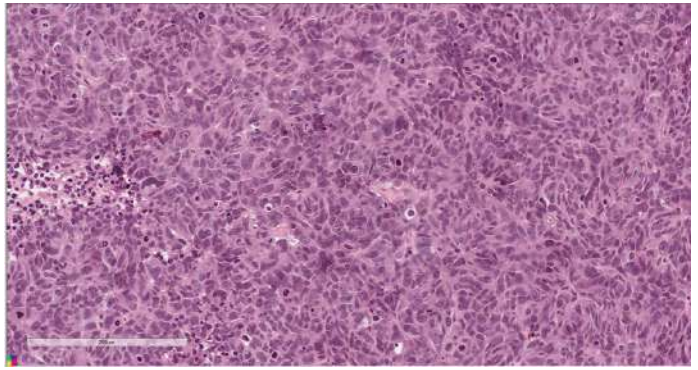
Cancer	Colorectal cancer
LSIM ID	CO-005-LSIM
Gene mutation	*KRAS, TP53*



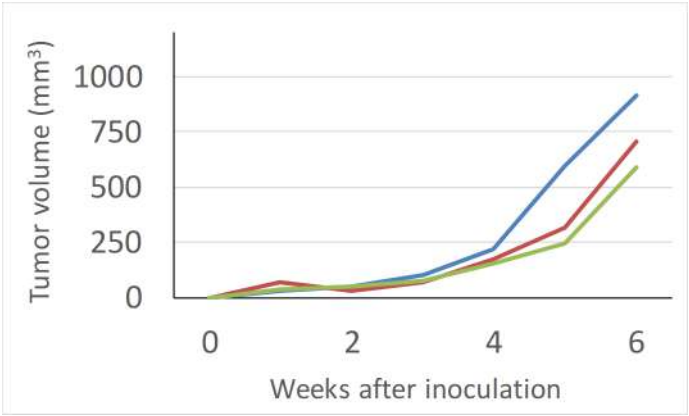


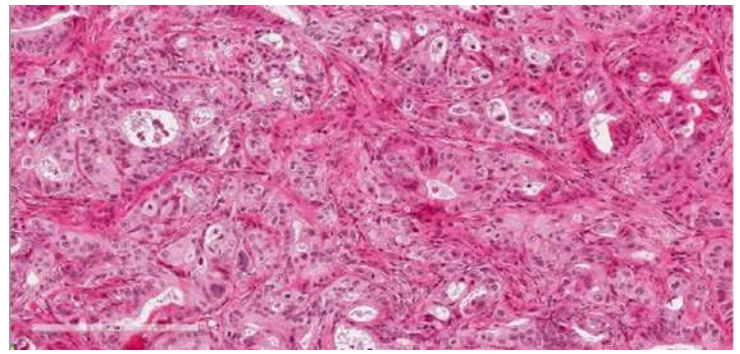
Cancer	Lung cancer
LSIM ID	LU-015-LSIM
Gene mutation	*C4B, EGFR, TNXB*





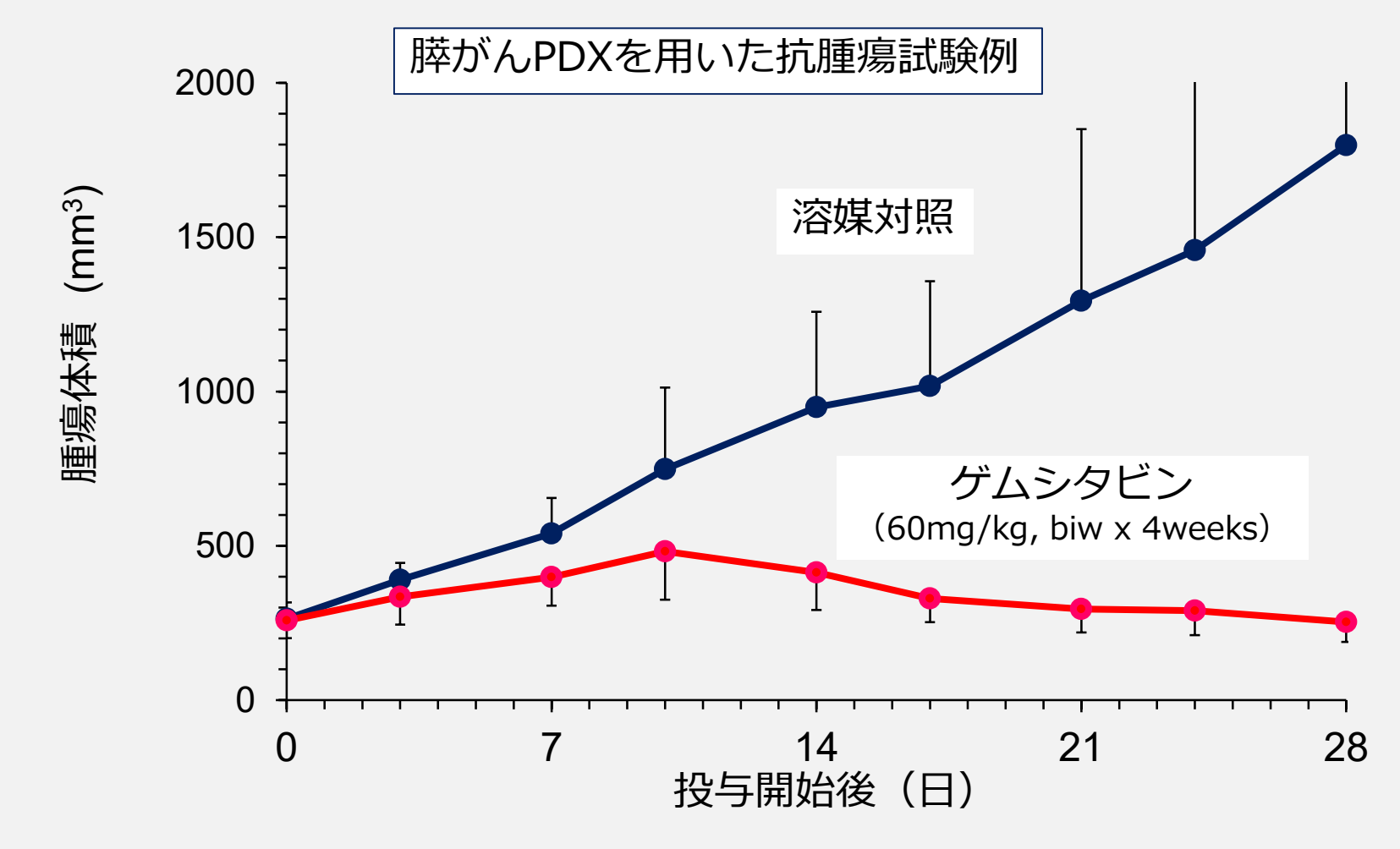
Cancer	Pancreatic cancer
LSIM ID	PA-004-LSIM
Gene mutation	*KRAS, POLH*





第80回日本癌学会学術総会（2021年）発表資料を改変

４．抗がん剤の開発支援：PDX担がんマウス抗腫瘍試験



第92回日本薬理学会年会（2019年）発表資料を改変

４．抗がん剤の開発支援：標的確認（組織マイクロアレイ）

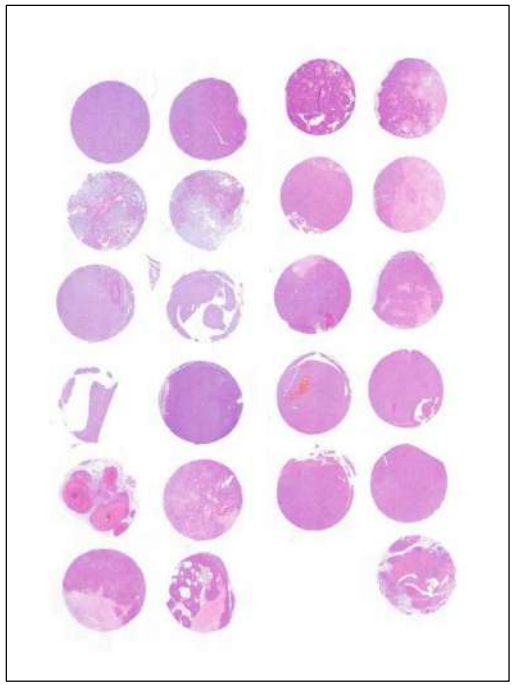
候補PDXの選択

がん種
臨床情報
遺伝子変異
遺伝子発現

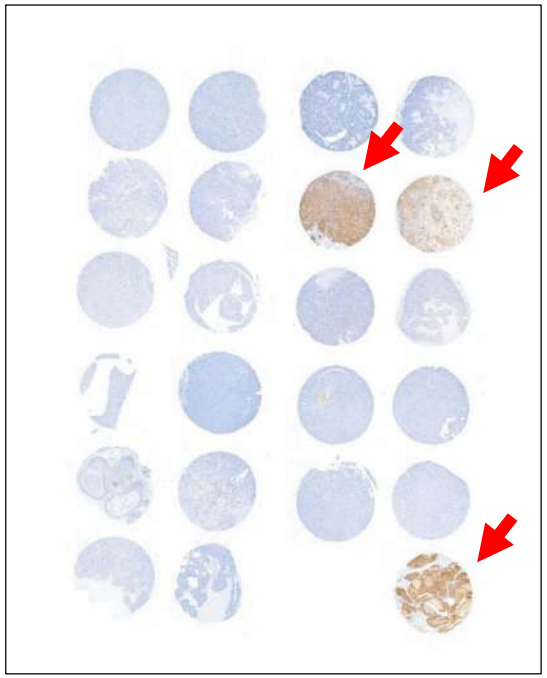
組織マイクロアレイ

乳がんPDX

HE染色



免疫染色（HER2）



：陽性像

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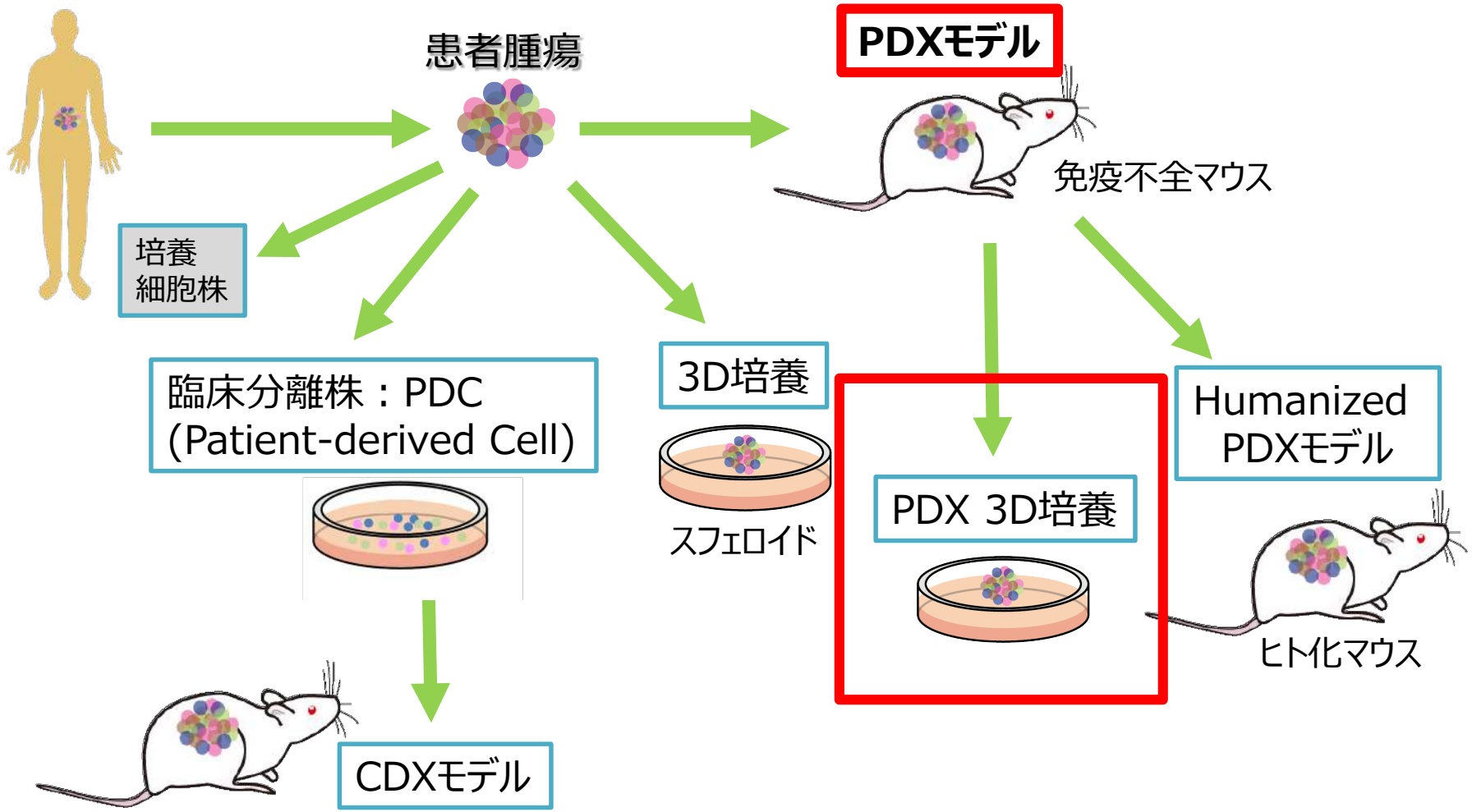
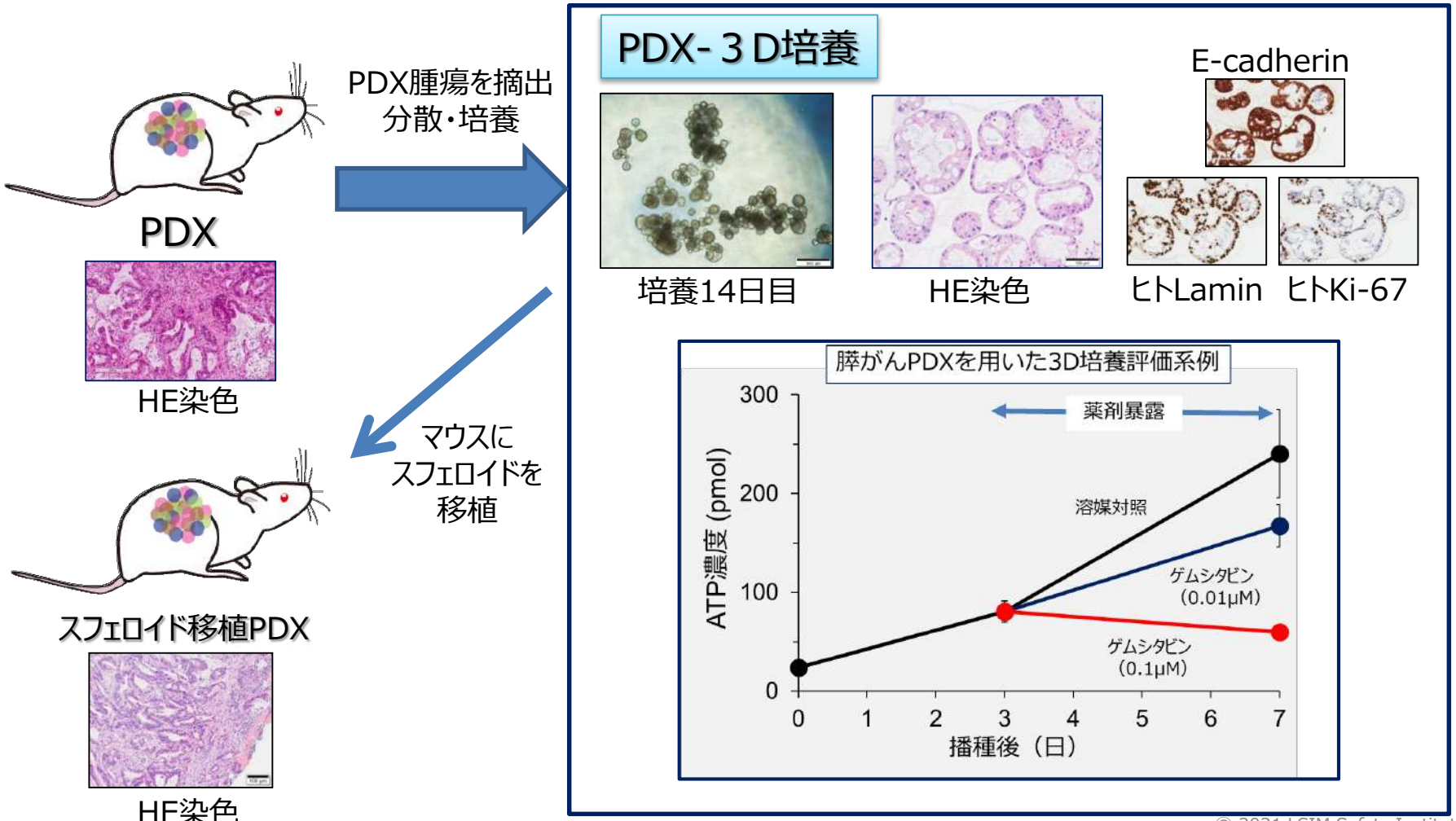
ヒト腫瘍由来の細胞・組織を用いた評価系



４．抗がん剤の開発支援：in vitro 3D培養法

PDX腫瘍のin vitro三次元培養法（特許出願）



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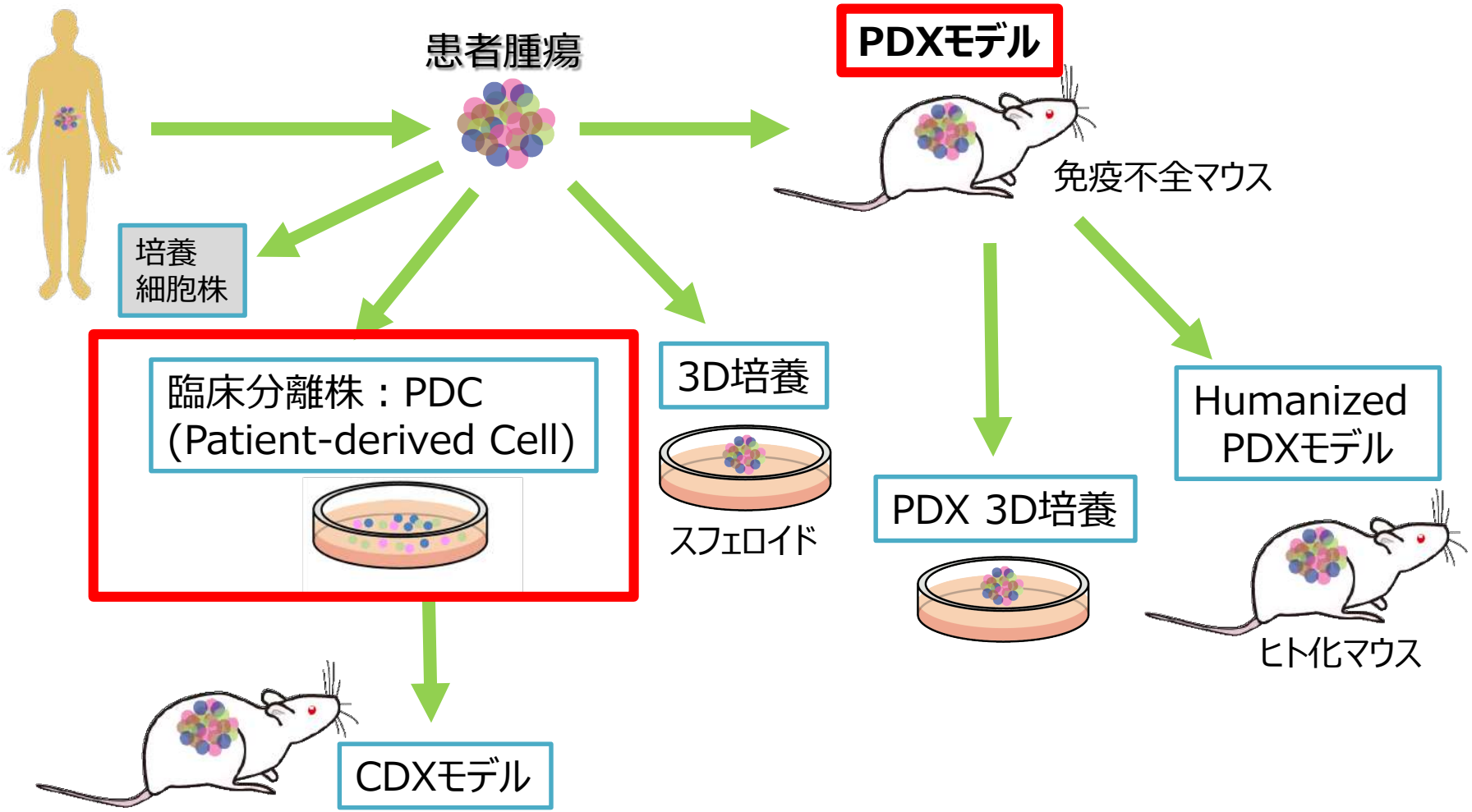
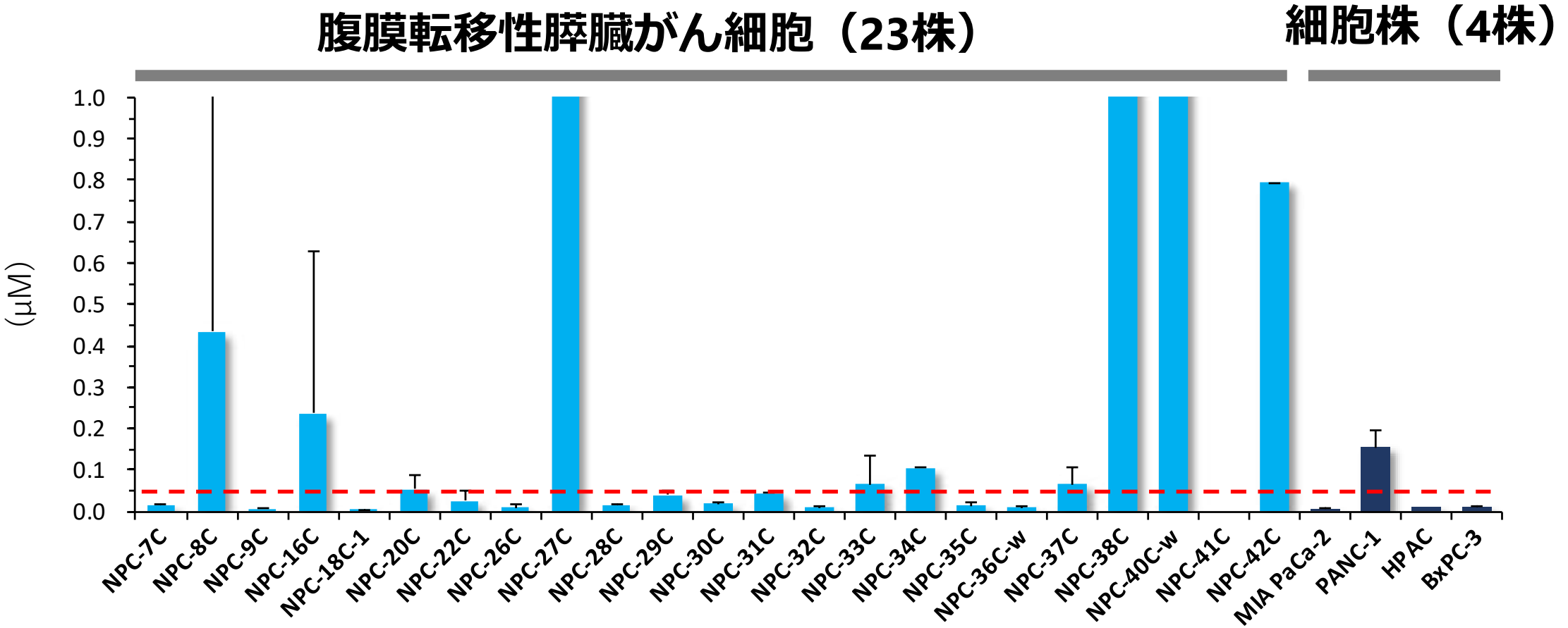


4．抗がん剤の開発支援：PDCを用いたin vitro (2D) 抗腫瘍試験

IC_{50} in cell growth inhibitory activity of Gemcitabine to PMPCC and PCCs

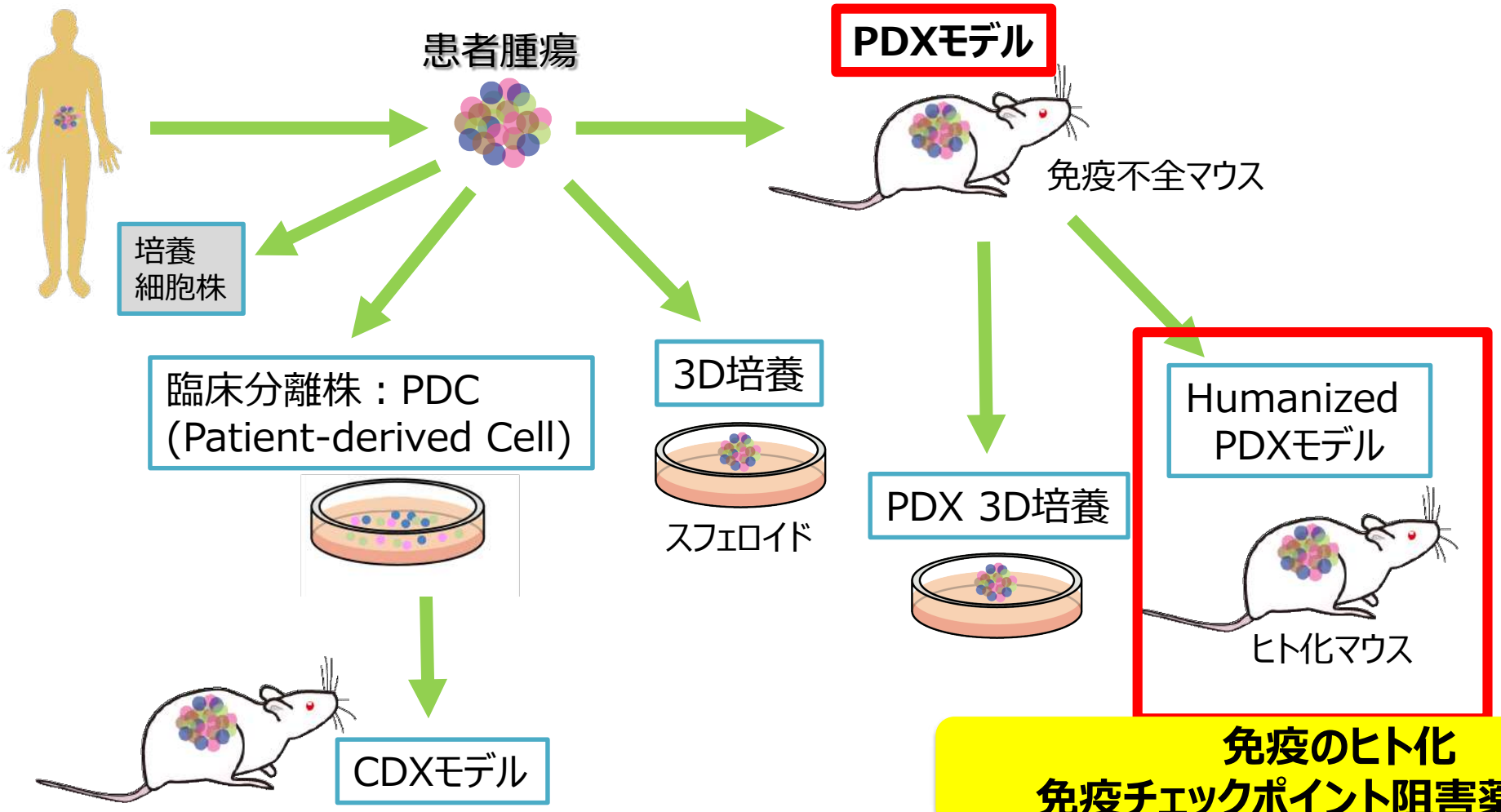


Data represent the mean ± standard deviations. Bars without standard deviation represent duplicate data or singlicate data.

PDC：国立がん研究センター・佐々木　博己先生よりご提供
第79回日本癌学会学術総会（2020年）発表資料を改変

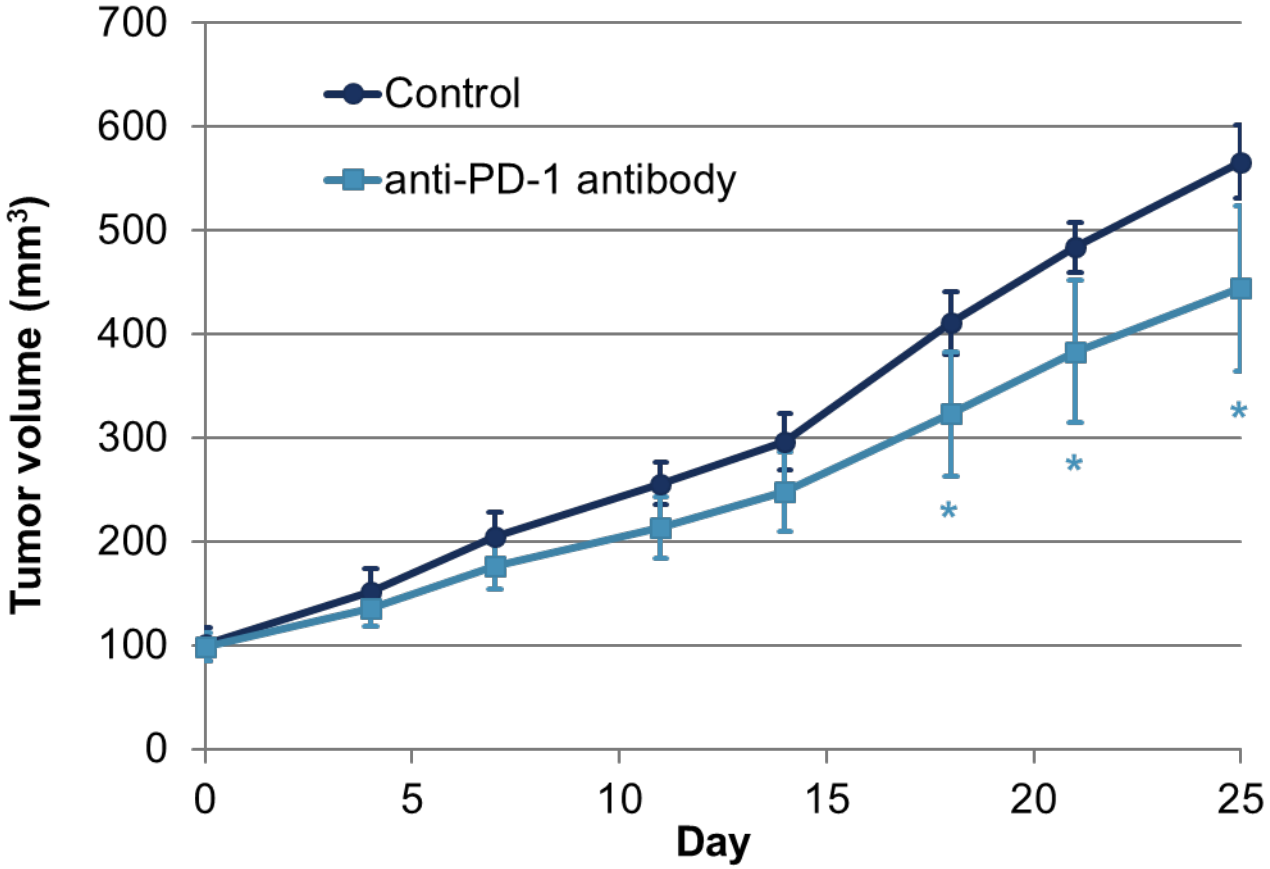
４．抗がん剤の開発支援～LSSIの技術

ヒト腫瘍由来の細胞・組織を用いた評価系

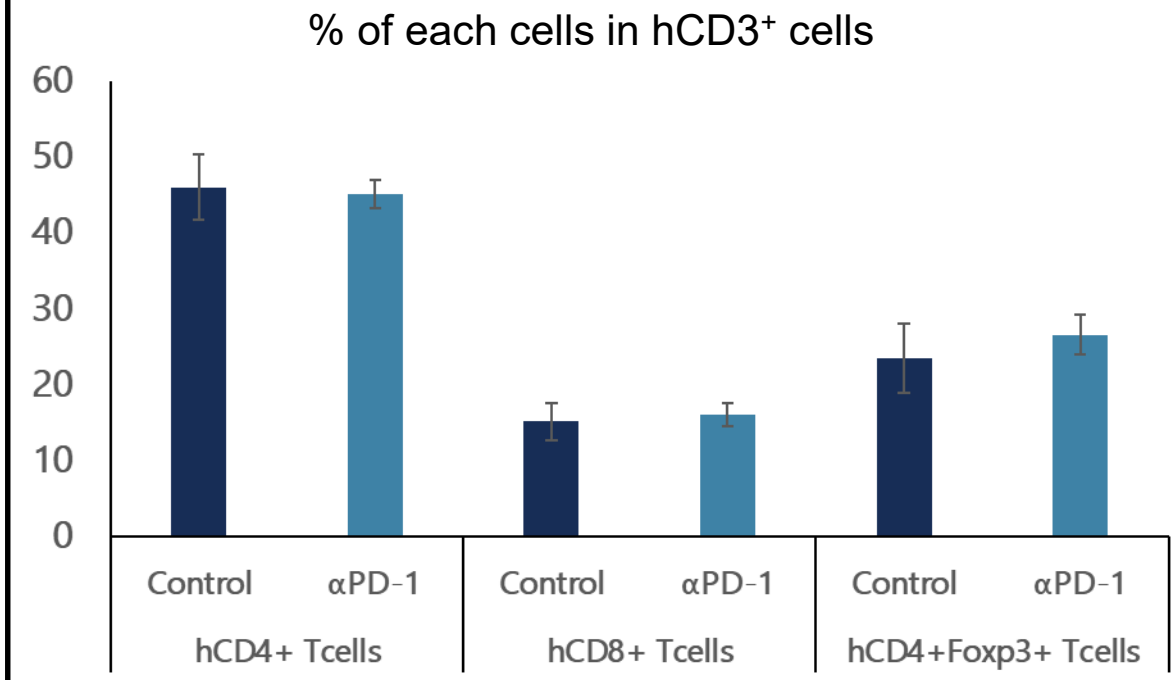


4．抗がん剤の開発支援：ヒト化マウスを用いた抗腫瘍試験

Anti-tumor effect of Pembrolizumab in MDA-MB-231 bearing-huNOG mice



Points, mean for 6 animals; vertical bars, S.E.
* p<0.05 vs the control group (Student's t test).



Columns, mean for 6 animals; vertical bars, S.E.

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４．抗がん剤の開発支援：利用可能なPDX

ライブラリー	樹立マウス	付帯情報
国立がん研究センター （J-PDXライブラリー）	NOGマウス	臨床情報 遺伝子変異* 発現情報*
実験動物中央研究所 （CIEA-PDX®）	ヌードマウス	お問い合わせください
埼玉医科大学（婦人科がん）	NOGマウス	お問い合わせください

*遺伝子変異（Whole exome）、発現解析（RNAseq）

ご清聴ありがとうございました