Evaluation of antitumor efficacy in PDX model using humanized NOG mice

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Objective

In recent years, research and development of immunity checkpoint inhibitors have become active. HuNOG mice have a human immune system in part (humanized mice) and useful for preclinical immunotherapy research. In this study, we used a patient-derived xenograft (PDX) tumor-implanted model in huNOG mice to investigate the antitumor efficacy of an immune checkpoint inhibitor (anti-PD-1 antibody). In addition, to evaluate the high-plex spatial profiling, immunostaining and analysis using the GeoMx Digital Spatial Profiler were performed using the specimens obtained from huNOG mice.

Summary in Japanese

近年，免疫チェックポイント阻害薬の研究開発が活発化している．ヒト化マウス（huNOGマウス）は，部分的にヒトの免疫系を持っており，前臨床における免疫療法の研究に役立つ．今回，我々はヒト化マウスにPDX（patient–derived xenograft）腫瘍を移植したモデルを用いて，免疫チェックポイント阻害薬の抗腫瘍効果およびハイプレックス空間プロファイリングの評価法について検討した．

ヒト肺がん由来PDX腫瘍をhuNOGマウスに移植し，腫瘍体積によりコントロール群と抗ヒトPD-1抗体処置群に振り分けた．群分け後，5日毎に抗体を投与し，21日まで観察を行った．その間，腫瘍径を測定し，抗体の抗腫瘍効果を評価した．観察最終日に摘出した腫瘍を10%中性ホルマリン緩衝液に浸漬した後，パラフィン包埋ブロックを作製した．得られた標本を用いて，免疫染色およびGeoMx Digital Spatial Profilerを用いた解析を行った．

コントロール群と抗ヒトPD-1抗体処置群で腫瘍体積は変わらなかったが，免疫関連因子のクラスター解析により，腫瘍内の因子の変動が認められた．

Materials and Methods

【Animals】

NOG-EXL mice (NOG-hGM-CSF/hIL-3, male) obtained from Central Institute for Experimental Animals were used. The animals were inoculated human CD34+ cells after X-ray irradiation (1.5Gy).

【PDX】

PDX tumor were obtained from National Cancer Center Research Institute.
PDX code: LU-019-LSIM, Origin: lung cancer

【Reagents】

- KEYTRUDA Injection 100mg (Pembrolizumab: anti-PD-1 antibody), MSD
- Human IgG4 kappa Isotype Control (control antibody), Cat. No. C0004-3, MBL

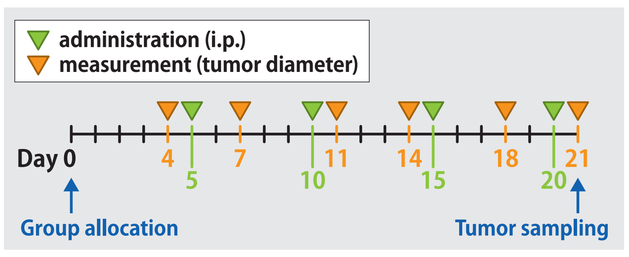
【Animal experiment】

Frozen PDX tumors were subcutaneously implanted into NOG mice. After the tumor grew, it was removed from the animal and implanted into huNOG mice.
Tumor diameter was measured using caliper and the volume was calculated by the following equation.

Tumor volume (mm^3) = 1/2×long diameter (mm)×short diameter (mm)×short diameter (mm)

Tumor-bearing animals were assigned homogeneously to each test group by the "stratified randomization method" based on the tumor volume.

【Administration schedule】



【Group configuration】

Group	Dose	N
Control	10 mg/kg	5
Anti-PD-1 antibody	10 mg/kg	5

【HE staining and IHC】

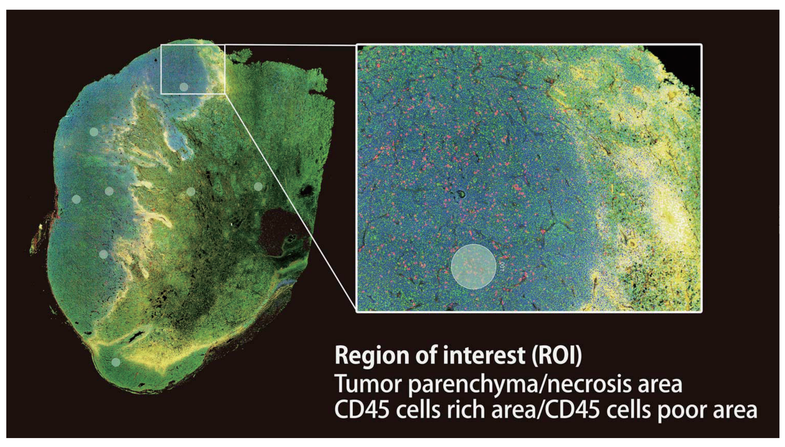
Paraffin-embedded blocks were created from tumor immersed in 10% formalin neutral buffer solution. Then, HE staining and IHC were performed.
Anti-PD-L1 antibody [SP142] -C-terminal, Cat. No. ab228462, Abcam plc.

【High-plex spatial profiling】

An arbitrary region was selected from the FFPE sections and the protein expression level in that region was evaluated using GeoMx Digital Spatial Profiler (NanoString Technologies).

● GeoMx Immune Cell Profiling

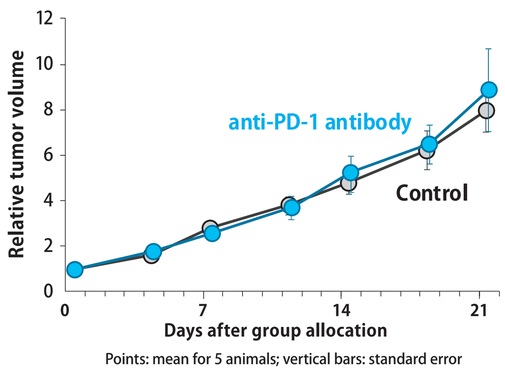
- **Targets:** PD-1, CD68, HLA-DR, Ki-67, Beta-2-microglobulin, CD11c, CD220, CD3, CD4, CD45,CD56, CD8, CTLA4, GZMB, PD-L1, PanCK, SMA, Fibronectin
- **Controls:** Rb IgG, Ms IgG1, Ms IgG2a, Histone H3, S6, GAPDH



Green: PanCK
Red: CD45
Blue: cell nucleus

Results

Effect of anti-PD-1 antibody on tumor growth in PDX-bearing huNOG mice



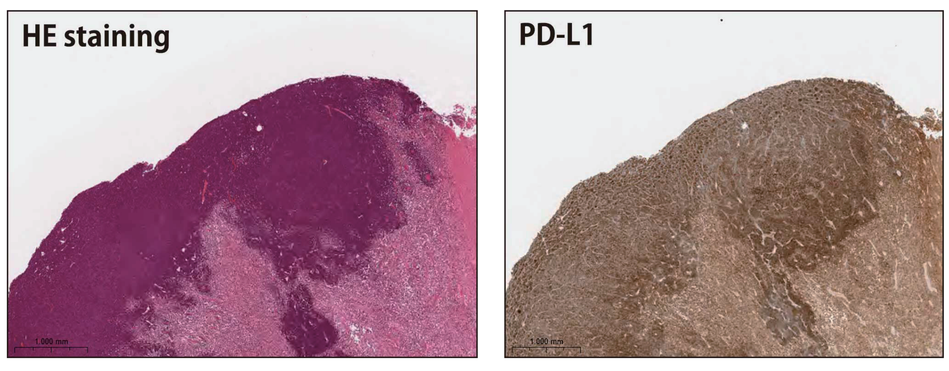
Points: mean for 5 animals; vertical bars: standard error

Group	Animal No.	Chimeric rate (%) 18w	27w
Control	1	49.9	36.5
	2	40.0	25.1
	3	55.6	56.5
	4	48.8	28.0
	5	40.0	26.5
Anti-PD-1 antibody	11	40.5	61.4
	12	39.8	13.3
	13	55.0	57.3
	14	47.2	-
	15	43.2	29.9

Chimeric rate (%) = hCD45 cells/hCD45 cells + mCD45 cells
18w: 11 weeks after cell inoculation
The analysis was performed by CLEA Japan, Inc.
27w: the end point of experiments
The analysis was performed by LSIM Safety Institute Corporation.

No difference in tumor volume was observed. Although there were fluctuations between animals, the chimeric rate was maintained even at the end of the experiment.

Histopathological examination



It was confirmed that the PDX tumor used in this study (LU-019-LSIM, lung cancer) highly expressed PD-L1.

Protein expression of immune-related factors in tumors

Factor	Function
GZMB	Cytotoxicity, T cell activation
CD68	M2 Macrophage, Myeloid
PD-L1	Checkpoint, Myeloid activation
CD56	NK cells
Beta-2-microglobulin	Antigen presentation
SMA	Stroma
HLA-DR	Antigen presentation, MHC class II
CD11c	Dendritic cells, Myeloid

The expression level of some factors in tumors were changed between the control group and the antibody-treated group.

Conclusion

Because immune function plays an important role in experiments on immune checkpoint inhibitors, syngeneic model (mouse tumors are transplanted into mice with normal immunity) are often used. However, from the perspective of extrapolation to humans, it is desirable to conduct evaluations using human tumors. In this study, we evaluated the antitumor effects of immune checkpoint inhibitor using PDX tumor-implanted model in huNOG mice. As a result, no difference in tumor volume was observed under this study condition. New humanized mouse models have been developed one after another in recent years, and model selection requires further consideration.

In examining the evaluation method of high-plex spatial profiling, we were able to detect changes in immune-related factors within tumors between the control group and the antibody-treated group.

This study made it possible to evaluate antitumor drugs from multiple angles, not just tumor size.