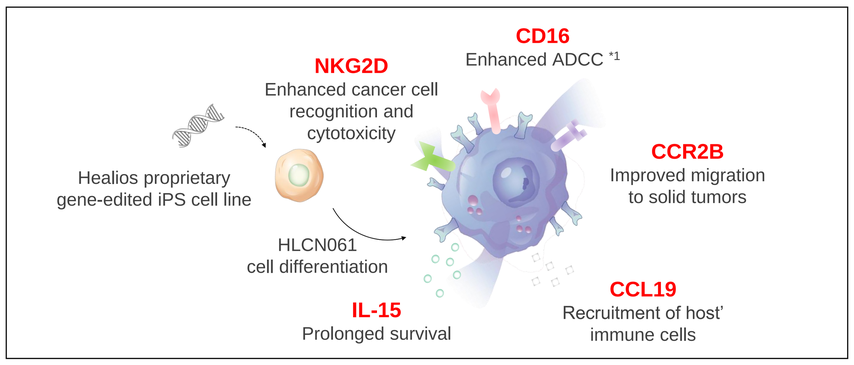
Purpose:
To propose an innovative and new therapy of HLCN061, gene-engineered iPSC-derived NK cells armed with NKG2D, IL-15, CD16, CCL19, and CCR2B molecules, for Lung cancer.

Method:
・The effect of HLCN061 on A549 human lung cancer cell line was observed using Incucyte® live-cell analysis system (*in vitro*).
・H1975 or A549 human lung cancer cell lines were implanted intravenously or subcutaneously into NOG mice, HLCN061 was administered intravenously or intratumorally, and Cmab (Cetuximab) and Nmab (necitumumab) were administered intraperitoneally.
・PDX (Lung cancer patient derived xenograft from J-PDX® library) was implanted subcutaneously into NOG mice, HLCN061 was administered intratumorally, and Cmab was administered intraperitoneally.



*1 ADCC: antibody-dependent cellular cytotoxicity

Fig. 1　HLCN061, gene-engineered iPSC-derived NK cells armed with NKG2D, IL-15, CD16, CCL19, and CCR2B molecules

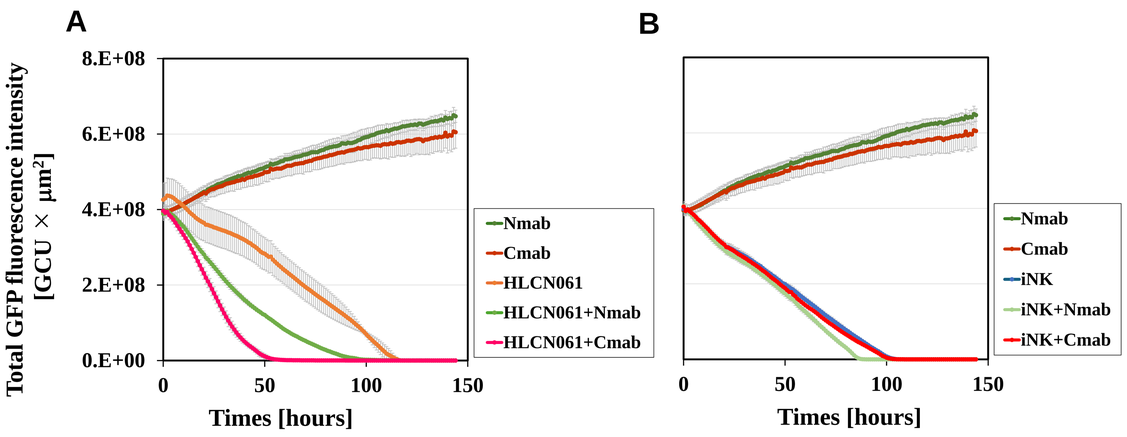


Fig. 2　ADCC activity of HLCN061 against A549 lung cancer spheroid in Incucyte® single spheroid assay for live-cell analysis.
The cytotoxicity of HLCN061 at 3:1 effector/target ratio was clearly enhanced by the addition of Nmab (10 μg/mL) or Cmab (2.5 μg/mL) , neither of which exhibit cytotoxicity against A549 cells on their own. In contrast, the cytotoxicity of iNK (iPSC derived NK) cells was not enhanced by either Nmab or Cmab. These results suggest that HLCN061, which was transfected with the CD16 gene, exhibits ADCC activity.

HLCN061 shows strong cytotoxicity due to enhancement of ADCC activity in *in vitro*

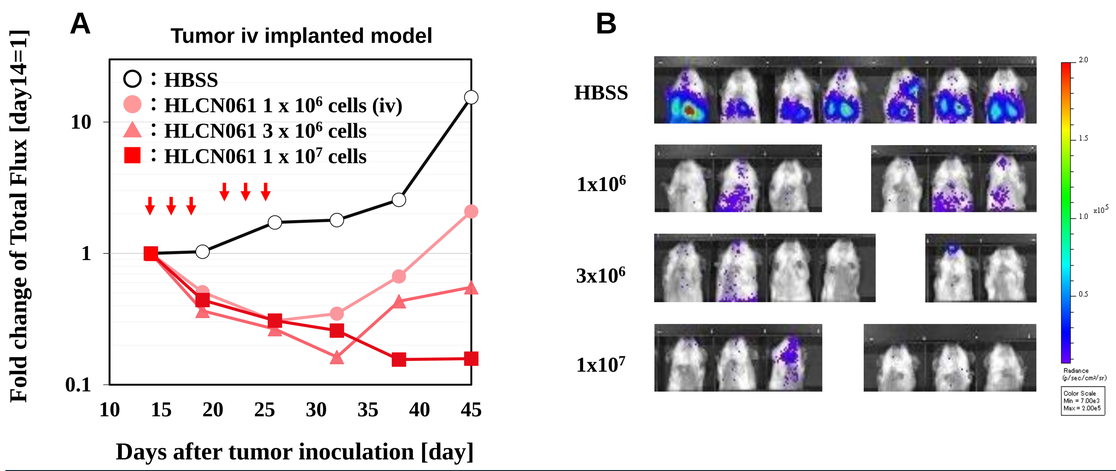


Fig. 3 Anti-tumor effects of HLCN061 in orthotopic models transplanted with human lung cancer cell line H1975
In human lung cancer bearing model mice that intravenously inoculated H1975, intravenously administered HLCN061 showed a clear tumor growth inhibitory effect, and at high dose, this effect continued even after administration had ended.

HLCN061 shows significant tumor growth inhibition in orthotopic lung cancer model, [Tumor (iv) - HLCN061 (iv) model]

The tumor growth was evaluated using in vivo imaging system (IVIS). The data showed as fold change of total Flux (A). The individual Bioluminescent monitoring of HLCN061 injected into H1975 transplanted mice on day 32 (B). n=7, ↓: HLCN061 administration

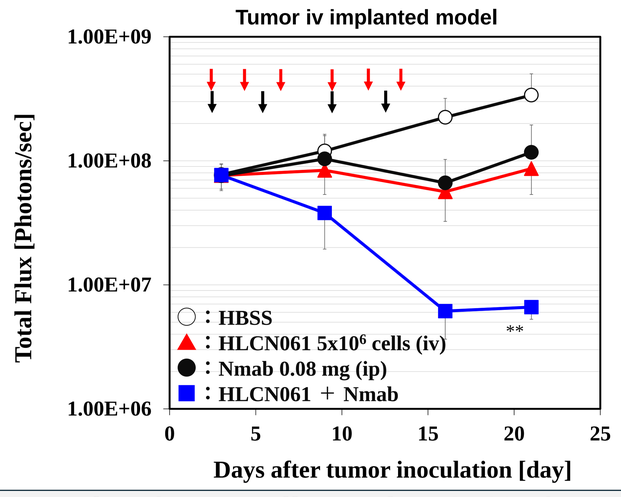


Fig. 4 Enhancement of anti-tumor effects of HLCN061 by Necitumumab-Mediated ADCC in A549 lung tumor orthotopic graft bearing mice
In human lung cancer bearing model mice that intravenously inoculated A549, the effects of HLCN061 was clearly enhanced by ADCC activity induced by intraperitoneally administered Nmab.

HLCN061 with enhanced ADCC activity induces potent anti-tumor effects in *in vivo* [Tumor (iv) - HLCN061 (iv) model]

n=5, mean ± S.D. , ↓: HLCN061, ↓: Nmab administrarton, **; p<0.01 vs Control (HBSS), Dunnett's multiple comparison test.

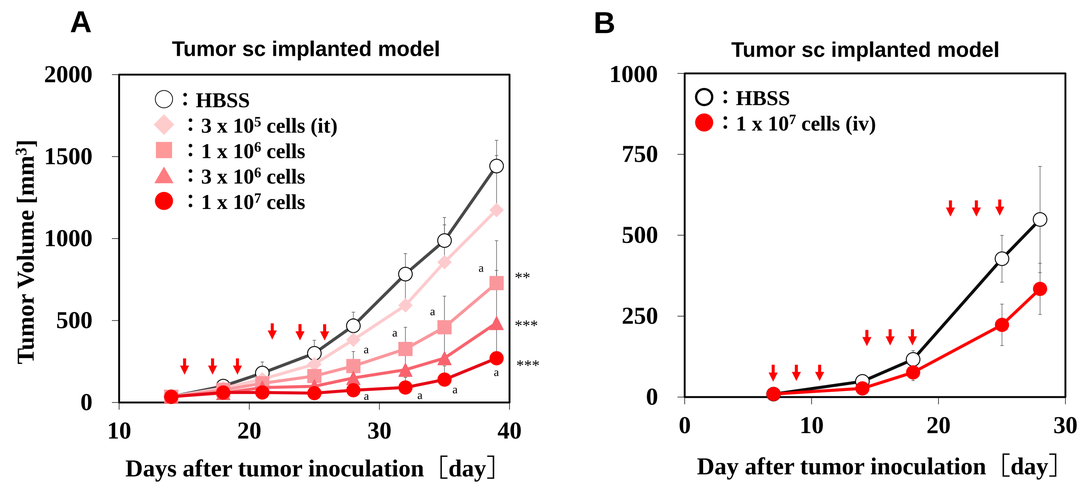


Fig. 5 The comparison of anti-tumor effects of HLCN061 between intratumoral and intravenous treatment on H1975 subcutaneously inoculated mice
Intratumoral (it) administration of HLCN061 in H1975 subcutaneous (sc) inoculated model mice significantly inhibited tumor growth (A), similar to the effects observed with intravenous (iv) administration in the orthotopic engraftment model (Fig. 3). However, iv administration of HLCN061 did not produce the same inhibitory effect (B). Based on these results, HLCN061 is being evaluated for intratumoral administration in Patient-derived xenograft model mice, as shown in Fig.6.

[Tumor (sc) - HLCN061 (it) model] showed strong antitumor effects similar to [Tumor (iv) - HLCN061 (iv) model], but not [Tumor (sc) - HLCN061 (iv) model].

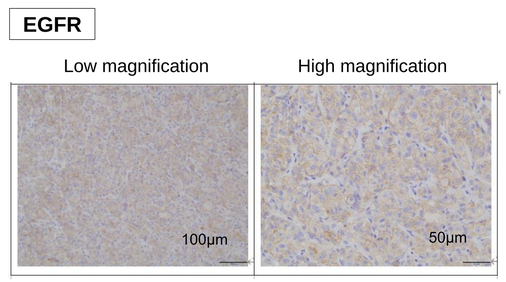
Tumor volume (mm³) = W (mm) x L (mm) x H (mm) x π/6, n=5 (a; n=4), mean ± S.D., ↓: HLCN061 administrarion, **; p<0.01, ***; p<0.001 vs Control (HBSS), Dunnett's multiple comparison test.

A

Characterization of J-PDX_E0050

J-PDX_E0050	
Age	50s
Sex	Male
Cancer	Lung Cancer
Type of Cancer	Adenocarcinoma
Primary site	Lung
Sample collection site	Pleural effusion
Chemotherapy	Yes
Radiotherapy	Yes
EGFR expression	++

B



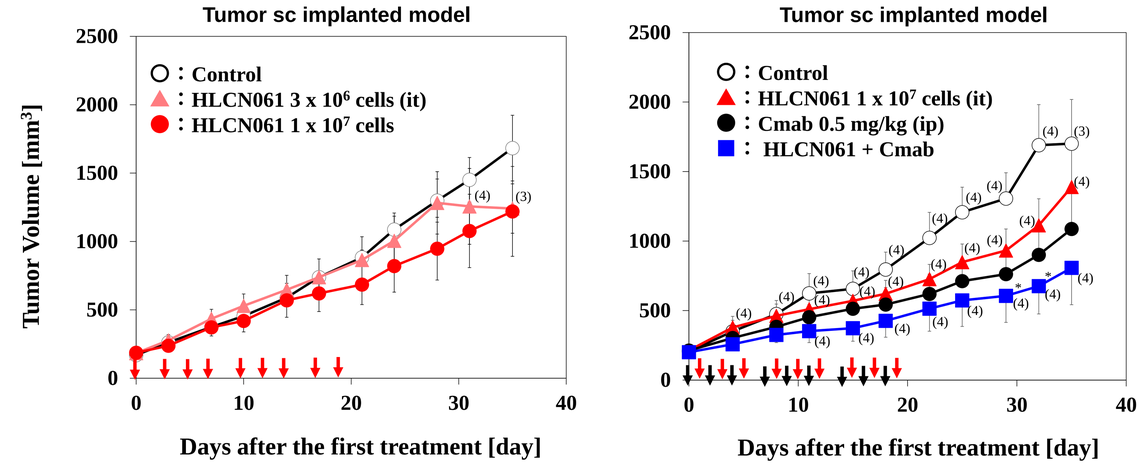


Fig. 6 The antitumor effects of HLCN061 on Patient-derived xenograft (PDX) model mice
J-PDX_E0050 highly expresses EGFR (A, B). HLCN061 was evaluated by intratumoral administration in subcutaneous transplanted PDX (Lung cancer patient derived xenograft, J-PDX_E0050) model mice, which shows clear tumor growth inhibition in monotherapy (C) and combination therapy with Cmab treated intraperitoneally (D).

HLCN061 showed clear antitumor effects in combination with Cmab in PDX model

Tumor volume (mm³) = W (mm) x W (mm) x L (mm) x 1/2, n=5, mean ± S.D., ↓: HLCN061, ↓: Cmab administration

Collaborative research with the National Cancer Center Japan

Conclusion:
HLCN061, particularly when combined with antibody agents, represents a promising novel therapeutic option with the potential to exhibit substantial antitumor effects in patients with Lung cancer.

COI Disclosure Information

An innovative treatment for Lung Cancer using gene-engineered iPS cell-derived NK cells (HLCN061)
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The Lead Presenter/Principal Researcher, Fusako Nishigaki, has no financial relationships to disclose.