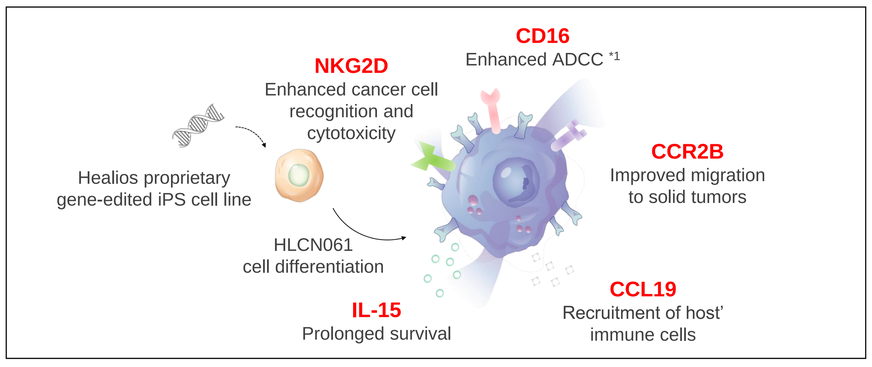
**Purpose:**
The antitumor effect of HLCN061, gene-engineered iPSC-derived NK cells armed with NKG2D, IL-15, CD16, CCL19, and CCR2B molecules, on peritoneal dissemination of gastric cancer.

**Method:**
・Expression levels of EGFR and HER2 in 44As3, NUGC-4 and N87 by FCM (Flow cytometry) analysis.
・44As3-Luc or N87 human gastric cancer cell lines were implanted intraperitoneally, and HLCN061 was administered intraperitoneally, and Cmab (Cetuximab) and Tmab (Trastuzumab) were administered intraperitoneally.
・NUGC-4 human gastric cancer was implanted intraperitoneally, HLCN061 was administered intraperitoneally at the time of ascites accumulation.




*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**

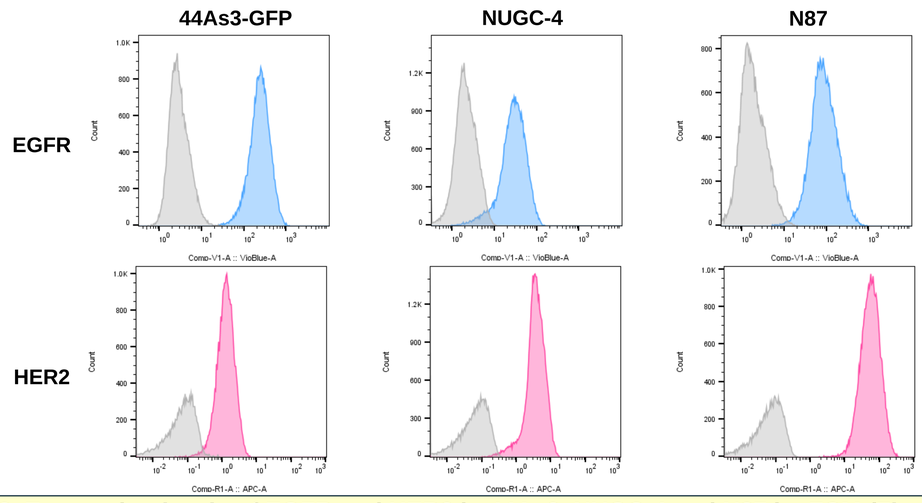



**Fig. 2 Expression levels of EGFR and HER2 in 44As3, NUGC-4 and N87 by FCM (Flow cytometry) analysis.**
EGFR expression is highest in 44As3 and HER2 expression is highest in N87.

**These results suggest that HLCN061, which was transfected with the CD16 gene, exhibits ADCC activity when 44As3 is combined with Cmab (Cetuximab: anti-human EGFR antibody) and N87 with Tmab (Trastuzumab: anti-human HER2 antibody).**

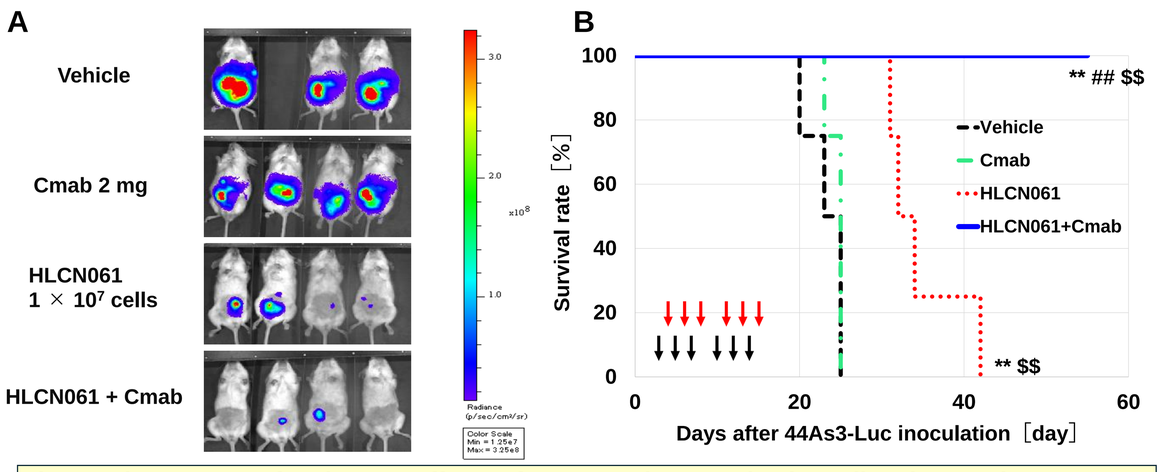



**Fig. 3 Enhancement of anti-tumor effects of HLCN061 by Cetuximab-Mediated ADCC in 44As3-Luc signet-ring cell gastric cancer cell intraperitoneally bearing mice.**
The tumor growth was evaluated using in vivo imaging system (IVIS). The individual Bioluminescent monitoring of HLCN061 injected into 44As3-Luc inoculated mice on day 20 (A). Prolonged survival time by administered HLCN061 and Cetuximab in 44As3-Luc tumor bearing mice (B).

**HLCN061 showed significantly antitumor effects and prolongation of survival rate in a gastric cancer peritoneal dissemination model. Moreover, the effects were significantly enhanced in combination with Cetuximab.**

n=4, ↓: HLCN061 administration, ↓: Cmab administration, **: p<0.01 vs Vehicle, ##: p<0.01 vs HLCN061, $$: p<0.01 vs Cmab, Log-rank test.

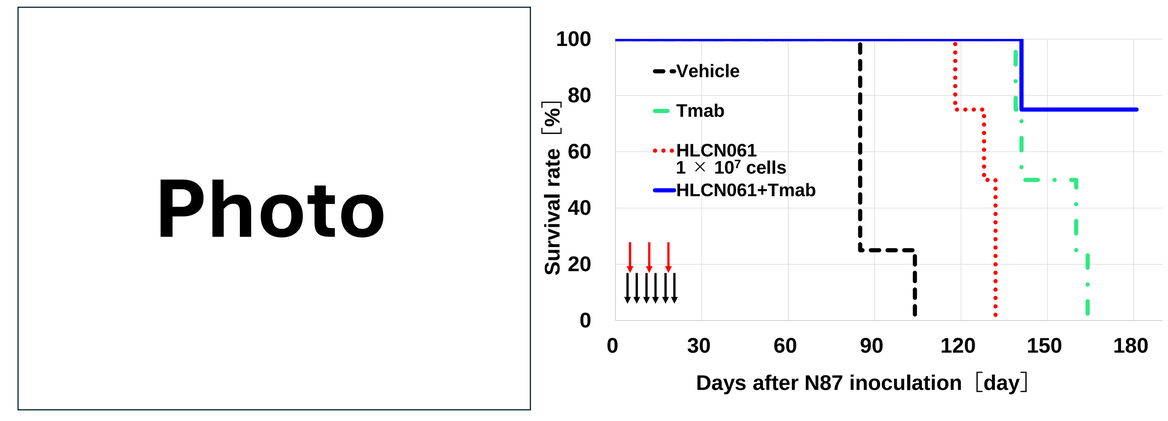
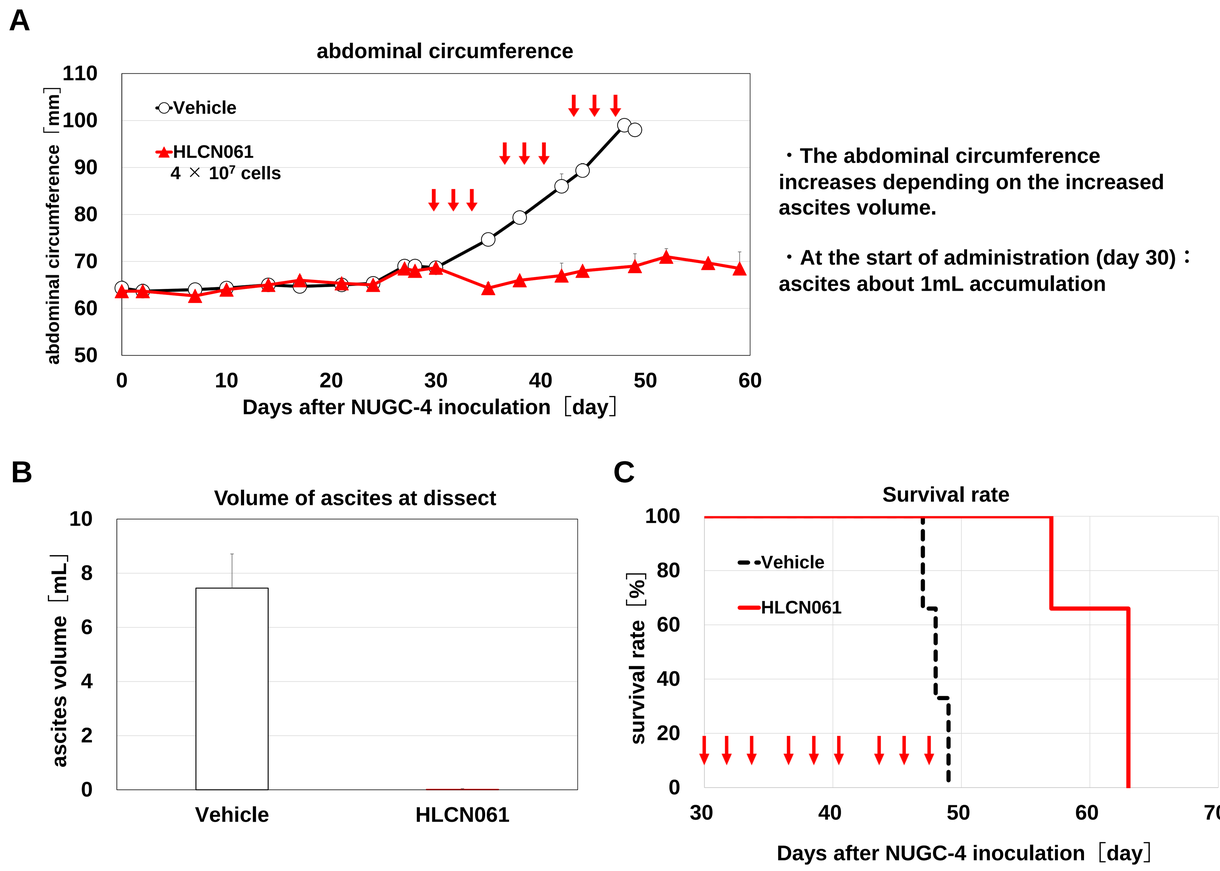



**Fig. 4 Enhancement of anti-tumor effects of HLCN061 by Trastuzumab-Mediated ADCC in N87 gastric cancer cell intraperitoneally bearing mice.**
Disappearance of intra-abdominal tumor, including solid tumor in the HLCN061+Tmab group (A). Prolonged survival time by administered HLCN061 and Tmab in N87 tumor bearing mice (B).

**HLCN061 showed clearly antitumor effects and prolongation of survival rate in a gastric cancer peritoneal dissemination model, the effects were significantly enhanced in combination with Tmab. Moreover, all intraperitoneal tumors, including solid tumors, had disappeared in the HLCN061 and Tmab combination group at day 181 observation.**

n=4, ↓: HLCN061 administration, ↓: Tmab administration




・**The abdominal circumference increases depending on the increased ascites volume.**

・**At the start of administration (day 30)：ascites about 1mL accumulation**




**Fig. 5 Therapeutic antitumor effect of HLCN061 administered after malignant ascites accumulation in mice intraperitoneally implanted with the human gastric adenocarcinoma cell line NUGC-4.**
After ascites accumulation, HLCN061 administration resulted in a decrease in abdominal circumference and no recurrence (A, D). HLCN061 showed significant removal and retention inhibition of malignant ascites in a gastric cancer peritoneal dissemination model, and accumulated ascites disappeared immediately (B, E). Prolonged survival time (C) and Inhibited mass growth in the mesentery (F) by administered HLCN061 in NUGC-4 tumor bearing mice.

**HLCN061 showed significantly effects of removal ascites, all ascites disappeared soon in a gastric cancer peritoneal dissemination model. Moreover, HLCN61 also clear prolongation of survival rate. On the other hand, it also showed clear inhibitory effects on tumor growth in the abdominal cavity.**

n=3, ↓: HLCN061 administration

**Conclusion:**
**HLCN061 showed significant antitumor effect against gastric cancer suggest that it holds promise the treatment of peritoneal dissemination of gastric cancer.**


**COI Disclosure Information**

**Anti-tumor effect of gene-engineered iPS cell-derived NK cells (HLCN061) on peritoneal dissemination of gastric cancer**
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**The Lead Presenter/Principal Researcher, Yuka Sato, has no financial relationships to disclose.**