Pharmacokinetics and anti-tumor effects of HLCN061, iPS cell-derived gene-edited NK cells

○Yoichi Naritomi, Kumiko Goto, Yuka Sato, Rumiko Sho, Ryuta Takahashi, Fusako Nishigaki, Hironobu Kimura, Kouichi Tamura

HEALIOS K.K.

Purpose

We are currently developing HLCN061, gene engineered iPS cell-derived NK cells (eNK® cells) expressing NKG2D, IL-15, CD16 (F176V), CCL19, and CCR2B for the treatment of refractory solid tumors. In this study, we investigated the pharmacokinetic properties and anti-tumor effects of HLCN061.

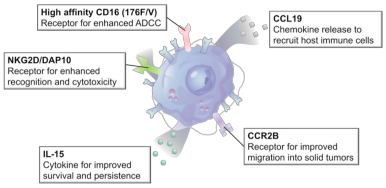


Fig. 1 HLCN061

Methods

【Experimental animals】 NOD. Cg-*Prkdc*^{scid}*Il2rg*^{tm1Sug}/ShiJic (NOG) mice and H1975-bearing mice were used. H1975-bearing mice, an orthotopic lung transplant model, were generated by implanting H1975-Luc, a human lung cancer cell line expressing luciferase gene, at a dose of 3.0 × 10^5 cells/mouse intravenously (iv) to NOG mice .

【Pharmacokinetics and biodistribution】 iPSC-derived NK cells (iNK cells), iNK expressing IL-15 (IL-15-iNK cells) and HLCN061 were administered iv to NOG mice at a dose of 1.0 × 10^6 or 5.0 × 10^6 cells/mouse. HLCN061 were also administered iv to H1975-bearing mice at a dose of 5.0 × 10^6 cells/mouse.

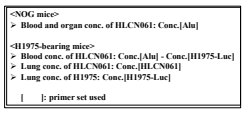
DNA was extracted from blood and organs collected at different time points after administration. The blood and organ concentrations of NK and H1975 per DNA content in the samples were determined by qPCR using a human-specific Alu, a HLCN061-specific and a H1975-Luc-specific primer sets (Scheme 1).

Estimation of concentrations with volume-based units

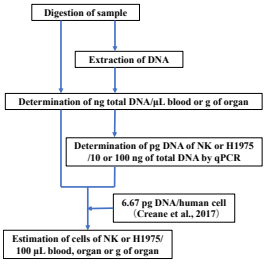
For blood, lung and liver samples in biodistribution experiments, the concentrations of HLCN061 per blood volume or organ weight were calculated considering the DNA concentration in each sample (Scheme 2).

【Anti-tumor effects】 After administrating iNK cells, IL-15-iNK cells and HLCN061 iv to the mice at a dose of 1.0 × 10^7 cells/mouse, tumor growth was determined by in vivo imaging system (IVIS).

Scheme 1 Quantification methods



Scheme 2
Procedure of estimating conc. with volume-based units



Results and Discussions

(1) Comparison of pharmacokinetics between iNK cells, IL-15-iNK cells and HLCN061

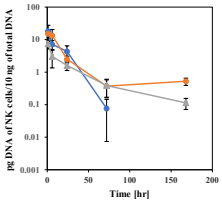


Table 1 Blood half-lives of iNK cells, IL-15-iNK cells and HLCN061 in NOG mice

	$t_{1/2\beta}$ (hr)
iNK cells	9.63
IL-15-iNK cells	77.0
HLCN061	38.5

Fig. 2 Blood concentrations of iNK cells, IL-15-iNK cells and HLCN061 in NOG mice

Mouse: Female Dose: 1.0 × 10^6 cells/mouse
Mean ± SD (n=3 or 4) ●: iNK cells, ●: IL-15-iNK cells, ▲: HLCN061
LLOQ: 0.12 pg DNA of NK cells/10ng of total DNA

The half-lives of IL-15-iNK cells and HLCN061 up to 7 days after administration were longer than that of iNK cells, suggesting improved persistence due to IL-15 expression.

(2) Pharmacokinetics and biodistribution of HLCN061

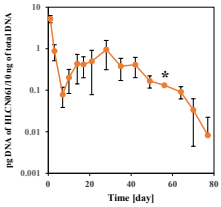


Fig. 3 Blood concentrations of HLCN061 up to day 77 after administration in NOG mice

Mouse: Female Dose: 5.0 × 10^6 cells/mouse
Mean ± SD (n=3) *n=1
LLOQ: 0.024 pg DNA of HLCN061/10 ng of total DNA

The blood concentration of HLCN061 was lowest at 7 days after administration, and then increased until 14~28 days. Subsequently, the blood concentration became decreased.

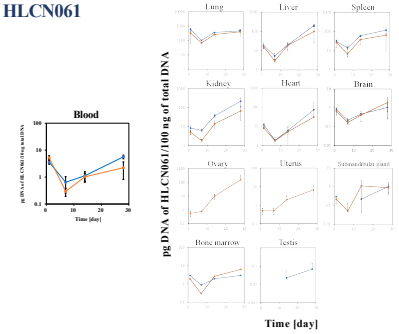


Fig. 4 Concentrations of HLCN061 in blood and various tissue organs of NOG mice

Dose: 5.0 × 10^6 cells/mouse Mean ± SD (n=3)
Bone marrow (pooled) *n=2 ●: Male, ●: Female
LLOQ: 0.12 pg DNA of HLCN061/10 ng of total DNA (blood), 0.61 pg DNA of HLCN061/100 ng of total DNA (heart), 0.12 pg DNA of HLCN061/100 ng of total DNA (other organs)

HLCN061 was mainly distributed in the lung, liver, spleen, and kidney, and its organ concentrations showed a similar trend to that in blood.

(3) Anti-tumor effects of NK cells and pharmacokinetics of HLCN061 in H1975-bearing mice

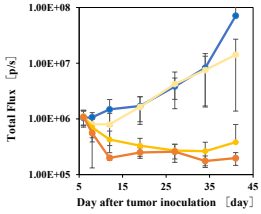


Fig. 6 Anti-tumor effects of iNK cells, IL-15-iNK cells and HLCN061

Mouse: female Dose: 1.0 × 10^7 cells/mouse at day 7, 9 and 11 after H1975 implantation
Mean ± SD (n=6)
●: Control, ●: iNK cells, ●: IL-15-iNK cells, ●: HLCN061

IL-15-iNK cells and HLCN061 inhibited tumor growth clearly compared to iNK cells, suggesting that the difference in pharmacokinetics of the NK cells may affect their anti-tumor effects.

Blood and lung concentrations of HLCN061 and H1975

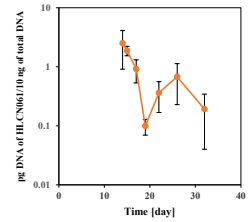


Fig. 7 Blood concentrations of HLCN061

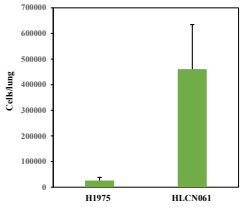
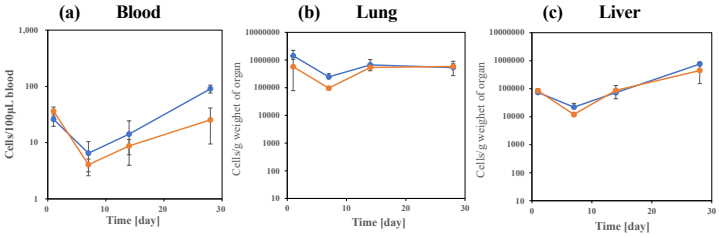


Fig. 8 Lung concentrations of HLCN061 and H1975 at day 32 after H1975 implantation

Mouse: Female Dose: 5.0 × 10^6 cells/mouse at day 13, 15 and 17 after H1975 implantation
Mean ± SD (n=3-5)

The concentrations of HLCN061 and H1975 were determined separately.
The concentration of HLCN061 in the lung was approximately 17.5-fold higher than that of H1975 at day 32 after H1975 implantation .

Estimation of blood, lung and liver concentrations of HLCN061 with volume-based units



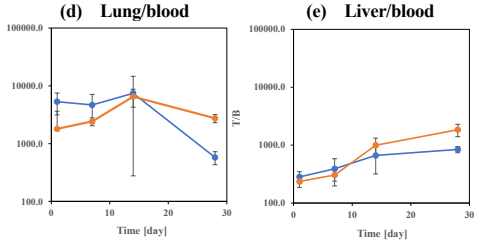


Fig. 5 Concentrations of HLCN061 and the tissue/blood ratios (T/B)

(a): Cells/100μL blood
(b)(c): Cells/g weight of organ
(d)(e): Tissue (cells/mg)/blood (cells/μL)
Dose: 5.0 × 10^6 cells/mouse
Mean ± SD (n=3)
●: Male, ●: Female

The concentrations of HLCN061 per organ weight were higher in the lung than in the liver at day 1-14 after administration, whereas the concentrations in the lung and the liver were comparable at day 28 after administration.

Conclusions

In this study, we clarified the pharmacokinetic properties and anti-tumor effects of HLCN061 in NOG and H1975-bearing mice.
Based on the results, we are investigating pharmacokinetics/pharmacodynamics (PK/PD) for HLCN061.

38th JSSX Annual Meeting
COI disclosure information

Authors : Yoichi Naritomi, Kumiko Goto, Yuka Sato, Rumiko Sho, Ryuta Takahashi, Fusako Nishigaki, Hironobu Kimura, Kouichi Tamura

We have the following financial relationships to disclose for our presentation contents.
- Employees : Yoichi Naritomi, Kumiko Goto, Yuka Sato, Rumiko Sho, Ryuta Takahashi, Fusako Nishigaki, Hironobu Kim (HEALIOS K.K.)
- Chief Scientific Officer: Kouichi Tamura (HEALIOS K.K.)