HLCN061: An "Off-the-Shelf" Gene Engineered Human iPSC-Derived NK Cell Product for the Treatment of Solid Tumors

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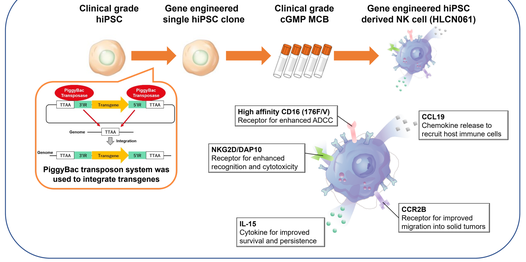
Healios

Background

NK cells are part of the innate immune system and contribute to the elimination of virus-infected cells, stressed cells, and tumor cells. Unlike T cells, NK cells do not require prior sensitization to target and do not cause GVHD in the allogeneic setting. Therefore, NK cells are considered as a good candidate for off-the-shelf products in adoptive cell therapy. Currently, peripheral blood or cord blood is used as the main source of NK cells for immunotherapy. However, these NK cells are heterogeneous, and their efficacy depends on the donor. In contrast, iPSC derived NK cells can be standardized for quality control. Moreover, iPSCs have the advantage of ease of gene modification to generate the desired cell. We developed HLCN061, which is an NK cells derived from genetically engineered human iPSC (hiPSC), expressing molecules that enhance anti-tumor activity, persistence, tumor homing, and host immune cell recruitment for the treatment of solid tumors.

HLCN061



① Clone selection

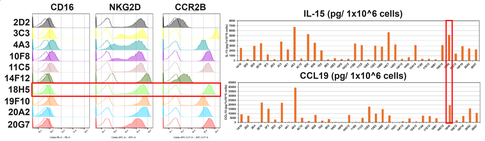


Table 1. NK cell differentiation capacity

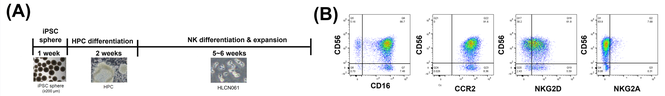
Clone	NK cell population	Yield
4A3	Low	Low
6G8	Low	Low
11C5	Medium	Low
18H5	High	High
19E12	Low	Low
19F10	High	Low

Table 2. Copy number of transgenes

Transgene	Copy number
IL-15	2
CCL19	3
CCR2	3
CD16 (F176V)	1
NKG2D	2
DAP10	2

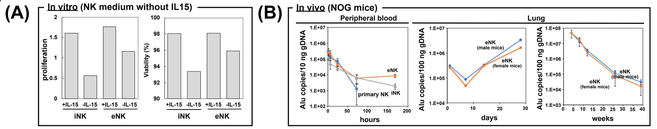
Clone 18H5 was selected as a starting material of HLCN061.
Clinical grade hiPSCs were electroporated with constructs containing the expression cassettes of *CD16 (F176V)*, *NKG2D*, *DAP10*, *CCR2B*, *IL-15*, *CCL19*, and *PiggyBac transposase* genes, controlled under human *EF1a* promoter. Single cell clones were isolated from the antibiotics-resistant population by limiting dilution. The isolated clones were evaluated for transgene expression (upper panel) and NK cell differentiation capacity (Table 1). Copy number of transgenes integrated in clone 18H5 are listed in Table 2.

② NK differentiation



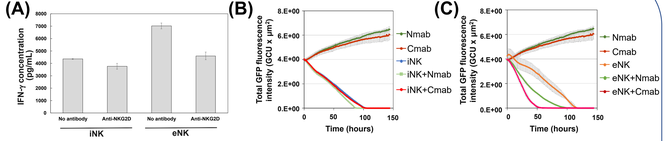
Gene engineered hiPSCs (clone 18H5) can be differentiated into NK cells.
(A) NK cell differentiation method. (B) Expression of NK markers and transgenes on clone 18H5 derived NK cells (HLCN061).

③ Survival and persistence



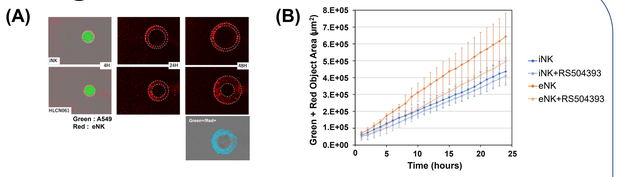
HLCN061 (eNK) exhibited prolonged survival both in vitro and in vivo.
(A) HLCN061(eNK) proliferated and showed higher viability compared to non-engineered iPSC derived NK (iNK) without IL-15 supplement. The cells were cultured in NK medium without IL-15 for 4 days. (B) HLCN061(eNK) demonstrated longer persistence than primary NK or iNK cells in peripheral blood (up to 7 days), and was detected in the lung until 39 weeks after transplantation in immune deficient mice. The eNKs were transplanted at day 0 and detected by qPCR with a human *Alu* sequence primer set at each time point.

④ In vitro cytotoxicity



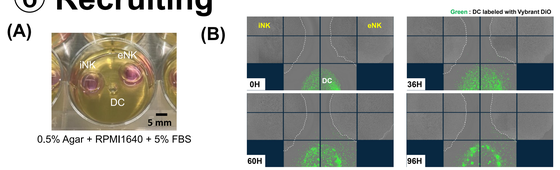
HLCN061 (eNK) exhibited superior anti-tumor activity compared to iNK in IFN-γ secretion (A) and ADCC (B).
(A) HLCN061(eNK) demonstrated NKG2D dependent anti-tumor activity. A549 cells were co-cultured with either iNK or eNK in the presence or absence of anti-NKG2D antibodies. The concentration of IFN-γ in the medium was measured by ELISA. The data are presented as mean ± standard deviation (n=3). (B and C) A549-GFP spheroids were co-cultured with iNK or eNK in the presence or absence of anti-EGFR antibodies (Cmab: Cetuximab, Nmab: Necitumumab). Green fluorescence intensity was measured with Incucyte. The data are presented as mean ± standard deviation (n=3).

⑤ Migration



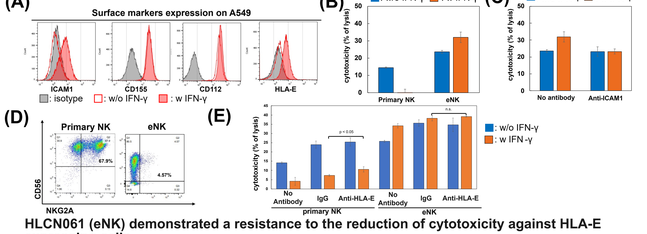
HLCN061 (eNK) migrated into A549-GFP spheroids via CCL2-CCR2B axis.
A549-GFP spheroids were co-cultured with iNK or eNK labeled with Vybrant DiD. The migration of iNK or eNK was evaluated by measuring the overlapped area of green and red fluorescence signals by timelapse imaging (A). To confirm the contribution of CCR2B in the migration of eNK, the CCR2B inhibitor, RS504394, was added to the assay. The overlapped area values at each time point were plotted (B). The data are presented as mean ± standard deviation (n=6).

⑥ Recruiting



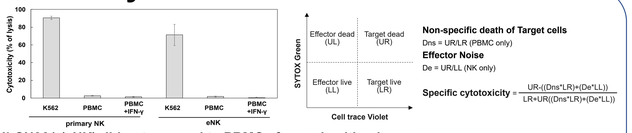
HLCN061 demonstrated recruitment of dendritic cells.
Dendritic cells (DCs) were obtained by differentiating PBMC derived CD14+ cells. The DCs were labeled with Vybrant DiO. The labeled DC, iNK and eNK were co-cultured separately in agar (A). The timelapse images were captured by Incucyte (B).

⑦ Feature of HLCN061



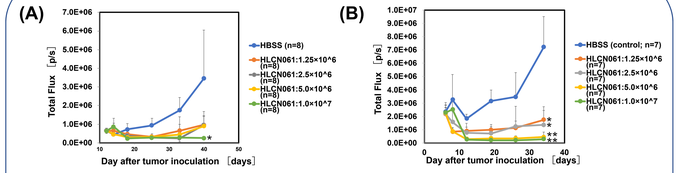
HLCN061 (eNK) demonstrated a resistance to the reduction of cytotoxicity against HLA-E expressing cells.
(A) IFN-γ treatment up-regulated the expression of ICAM1 and HLA-E on A549 cells. (B) IFN-γ treatment of target cells reduced cytotoxicity of primary NK cells, but not eNK. The data are presented as mean ± standard deviation (n=3). (C) ICAM1 expression contributed to the enhancement of eNK cytotoxicity against A549 treated with IFN-γ. The data are presented as mean ± standard deviation (n=3). (D) NKG2A was not expressed on eNK. (E) There was limited contribution of HLA-E/NKG2A axis to the reduction of cytotoxicity in primary NK. The data are presented as mean ± standard deviation (n=3). The p-values were calculated using Student's t-test.

⑧ Safety of HLCN061



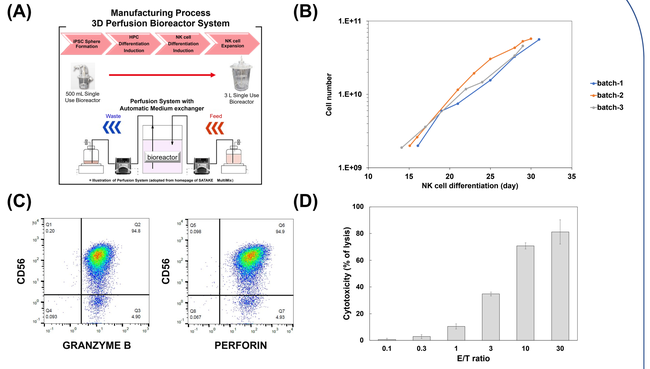
HLCN061 (eNK) did not respond to PBMCs from a healthy donor.
PBMCs were labeled with CellTrace Violet and treated with or without IFN-γ. The PBMCs were co-cultured with primary NK cells or eNK for 4 hrs. Dead cells were stained by CYTOX Green. The specific cytotoxicity was calculated using the formula indicated above. K562 cells were used as a positive control. The data are presented as mean ± standard deviation (n=3).

⑨ In vivo cytotoxicity



HLCN061 (eNK) exhibited dose-dependent anti-tumor activities in an orthotopic lung cancer mouse model.
Orthotopic lung cancer mice were generated by intravenously injecting H1975-luc (A) or HCC827-luc (B) on day 0. On day 13 for H1975 or day 7 for HCC827 transplanted mice, HLCN061 was administered at the indicated dose (cells/mouse) three times per week for two weeks. Luciferase activity was recorded at each time point using In Vivo Imaging System. The data are presented as mean ± standard deviation; *p < 0.05, **p < 0.01 (Steel's multiple compare test between HBSS and HLCN061 treated samples at day 34 or 40).

⑩ Manufacturing



Manufacturing of HLCN061 (eNK) using 3D perfusion bioreactor system.
(A) The manufacture process of HLCN061 using 3D perfusion bioreactor system with automatic medium exchanger. (B) Cell numbers were monitored at each time point during the manufacture process of three batches. This process can manufacture fifty billion cells of HLCN061 per 3 L bioreactor on average. (C) The manufactured HLCN061 expressed major NK cell markers. (D) The manufactured HLCN061 demonstrated cytotoxicity against A549 cells. A549 cells were co-cultured with eNK at indicated E/T ratio and cytotoxicity was measured by LDH assay. The data are presented as mean ± standard deviation (n=3).

Conclusion

- We established a gene engineered hiPSC clone expressing IL-15, NKG2D/DAP10, CD16 (F176V), CCR2B and CCL19.
- The gene engineered hiPSC derived NK cells (HLCN061) demonstrated enhanced persistence, cytotoxicity, migration, and dendritic cell recruitment compared to non-engineered cells.
- The HLCN061 demonstrated resistance to dysfunction of NK cell caused by IFN-γ treated cancer cells.
- The HLCN061 did not show any response to normal PBMCs.
- The HLCN061 exhibited anti-tumor activities in the orthotopic lung cancer model mice.
- We developed a manufacture process using 3D perfusion bioreactor system that can manufacture 100 billion cells with two 3 L bioreactors.