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Preclinical characterization of CYT-100 iPSC derived NK cells alone and in combination with CYT-303 NK cell engager for hepatocellular carcinoma (HCC)

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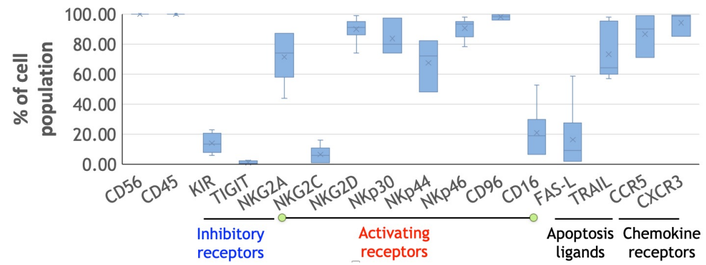
Introduction

CYT-100 is a first-generation iPSC derived NK cell product in development for use in combination with NK cell engager antibody (NKE) CYT-303 targeted against NKp46 activating receptor on NK cells and Glypican-3 (GPC3) expressed in the tumor cells for treatment of hepatocellular carcinoma (HCC). The combination of CYT-100 and CYT-303 is expected to activate endogenous NK cells and provide additional functional NK cells in the HCC microenvironment. Here we describe preclinical characterization of CYT-100 alone or in combination with CYT-303. In addition, we compare CYT-100 expansion capacity potential to PBNKs (peripheral blood natural killer cells).

Results

Immunophenotypic characterization of CYT-100 iNK cells

CYT-100 immunophenotyping was conducted by flow cytometry using a panel of NK cell directed antibodies.



CYT-100 immunophenotyping showed > 95% expression of CD56 + CD45+ cells indicative of a highly pure fully differentiated iNK population. CYT-100 showed increased expression of activation receptors and minimal expression of inhibitory receptors consistent with its increased effector function against tumors.

CYT-100 iNK cells are more stem like compared to PBNK cells

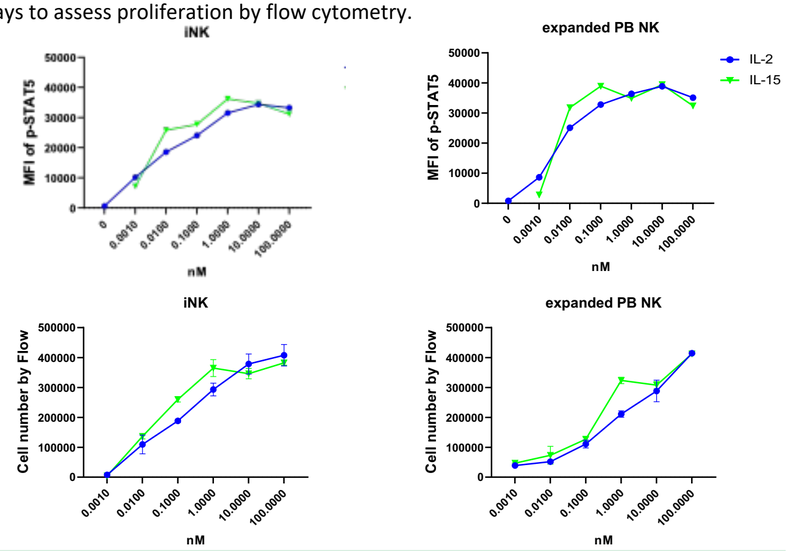
The chromosome telomere length was assessed by a q-PCR assay(ScienCell kit). Higher telomere lengths were associated with stem like iPSCs and iNKs and lower telomere lengths associated with mature PBNKs.

Sample	Average telomere length Per Cell	Average telomere length Per Chromosome	NK telomere length Ratio to iPSC
iPSC	1205.61 ± 88.22 kb	13.11 ± 0.96 kb	—
CYT-100 - Stage 2 iNK	874.46 ± 63.99 kb	9.50 ± 0.69 kb	0.73
CYT-100 - Stage 3 iNK	850.52 ± 62.23 kb	9.25 ± 0.68 kb	0.71
PBNK	508.08 ± 37.25	5.53 ± 0.40	0.42

CYT-100 iNKs are more stem like compared to PBNKs suggesting the potential for these cells to show greater expansion and persistence in-vivo.

CYT-100 and PBNKs show comparable signaling and proliferation in response to rIL-2 and IL-15

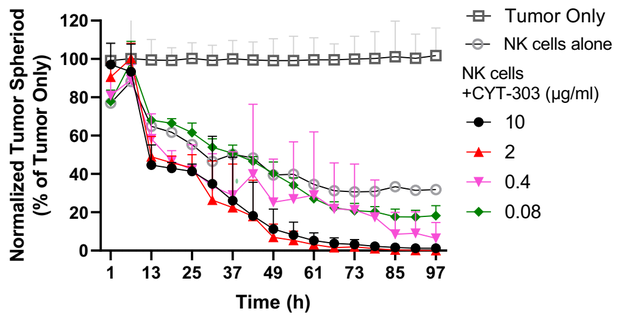
CYT-100 or PBNKs were stimulated with r-IL2 or IL-15 at the above indicated concentrations for either 15 minutes to assess STAT-5 phosphorylation or for 6 days to assess proliferation by flow cytometry.



CYT-100 and PBNKs showed similar dose dependent increases in STAT-5 tyrosine phosphorylation and proliferation in response to r-IL2 and IL-15.

CYT-100 shows time-dependent killing of Hep3B tumor cells that is enhanced by CYT-303 in a dose-dependent way

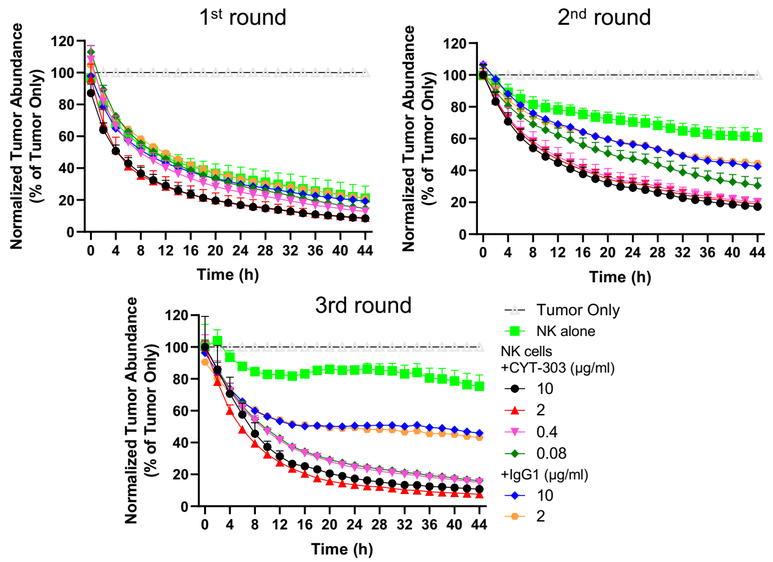
Hep3B-GFP tumors (10e3 cells/well) were cultured for 2 days in ultra low attachment U-bottom plates to form tumor spheroids. NK cells (10e3 cells/well) were added to tumor spheroids either alone or in combination with CYT-303 at different concentrations.



CYT-100 showed cytolysis of Hep3B tumor spheroids indicating the potential of these cells to invade and kill solid tumors. CYT-100 in combination with CYT-303 showed enhanced dose-dependent cytolysis of Hep3B tumor spheroids.

CYT-303 enhances CYT-100 serial killing of HCC tumors and reversed dysfunction of CYT-100 in later rounds of serial killing

Serial killing of Hep3B-GFP tumors was evaluated with either CYT-100 alone or in combination with CYT-303 using fixed E/T ratios (1:2) at each round of killing in 3 technical replicates. Tumor lysis was monitored by reduction of GFP positive tumors using the Incucyte.



CYT-100 alone showed gradual reduction in serial killing suggesting dysfunction of these cells over time. CYT-303 in combination with CYT-100 reversed this dysfunction and enhanced serial killing of Hep3B tumors in a dose-dependent manner.

Conclusions

- The allogeneic IPSC derived NK cell product CYT-100 demonstrated desirable immunophenotypic characteristics, sensitivity to cytokine activation and proliferation, and stem like features that may allow for more expansion and *in vivo* persistence than PB NK cells.
- HCC tumor spheroid killing by CYT-100 was enhanced by the GPC3 NK cell engager antibody CYT-303 showing the potential of this combination to invade and kill solid tumors.
- CYT-303 enhanced CYT-100 serial killing of HCC tumors and reversed dysfunction of these cells in later rounds of serial killing.
- These data support further development of the combination of CYT-100 iNK cells and CYT-303 as therapeutics for HCC.

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