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Glypican-3 (GPC3) and NKp46 directed FLEX-NK™ cell engager antibody (CYT-303) distributes to tumors and shows dose-dependent tumor growth inhibition in a hepatocellular carcinoma (HCC) mouse model*

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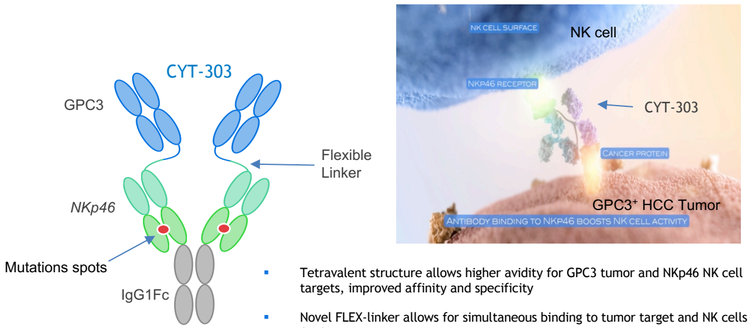
Abstract

BACKGROUND: GPC3 is an oncofetal antigen that is highly expressed in HCC while it is hardly expressed in adult normal tissues except placenta. CYT-303 is a multifunctional bispecific NK cell engager built on our Flex-NK™ scaffold, which engages NK cells through NKp46 and targets GPC3 expressed on tumor cells. Previously, we have reported that CYT-303 showed *in vitro* redirected Hep3B tumor cells cytolysis as well as *in vivo* Hep3B tumor growth inhibition with peripheral blood NK cells (PBNK) and our NK cells derived from iPSC (iNK), respectively. Here, we further characterized the *in vivo* CYT-303 dose-response for tumor growth inhibition and its influence on NK cell trafficking and distribution in the blood and the tumor. **METHODS:** CYT-303 pharmacokinetics were evaluated in PBNK injected NOG-hIL15 mice bearing subcutaneous Hep3B tumors (HCC). Anti-tumor efficacy and dose-response of CYT-303 was evaluated in PBNK and iNK injected NSG-IL15 mice bearing subcutaneous HCC tumors. PBNK circulation in blood was analyzed by flow cytometry and CYT-303 distribution in blood and tumor by PK immunoassay. Blood alpha fetoprotein (AFP) was measured by immunoassay. **RESULTS:** Similar CYT-303 PK profile was observed in HCC tumor bearing mice compared to non-tumor bearing mice. In the Hep3B tumor model in PBNK or iNK injected HCC tumor bearing mice, CYT-303 showed dose-dependent tumor growth inhibition compared to control hIgG1 treated mice. Consistent with CYT-303 dose-dependent tumor growth inhibition, dose-dependent increases in CYT-303 concentrations were observed in the tumor. Blood NK cell count in CYT-303 treated animals were significantly lower compared to IgG1 isotype control treated mice suggesting CYT-303 may facilitate NK cell trafficking from blood into the tumor. Blood alpha fetoprotein (AFP), a biomarker in HCC, decreased with CYT-303 tumor growth inhibition. **CONCLUSIONS:** Pharmacologically active CYT-303 doses were identified and CYT-303 distribution to the tumor was demonstrated suggesting NK trafficking to the tumor.

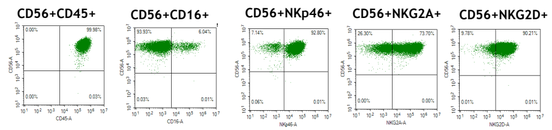
Figure 1: CYT-303 mediated NK cell activation in HCC



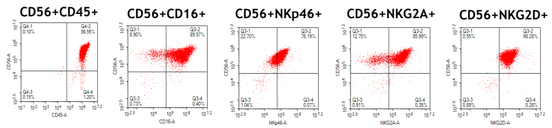
- Tetravalent structure allows higher avidity for GPC3 tumor and NKp46 NK cell targets, improved affinity and specificity
- Novel FLEX-linker allows for simultaneous binding to tumor target and NK cells facilitating immune synapse
- FLEX-NK™ construct enhances NK cell function against target cells

Figure 2: Immunophenotypic profile of iNK cells and PBNK cells

iNK Cells



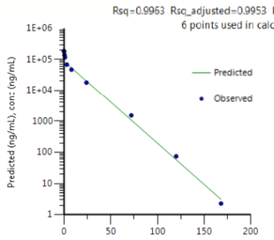
PBNK Cells



iNKs and PBNKs expanded similarly with feeder cells were harvested and immunophenotyped using above indicated directly conjugated antibodies and isotype control antibodies. Mean fluorescence intensity (MFI) and percent positive cell staining with each antibody are shown.

Figure 3: CYT-303 pharmacokinetic profile in HCC tumor bearing mice

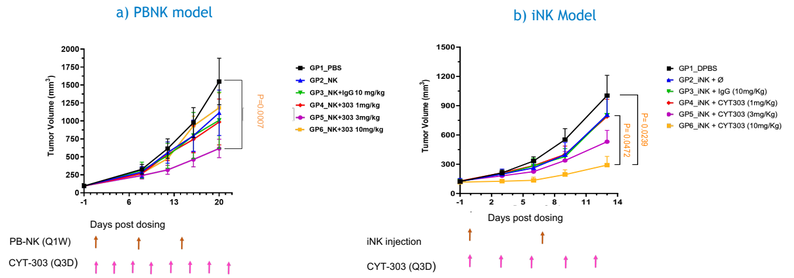
CYT-303 NCA: 3h-168h (6 points)



NCA analysis WNL		CYT303 estimate	
Parameter	Unit	Balb/c	Tumor-bearing mice
AUC(0-t)	*ng*h/mL*	2529406	1654265
AUC(0-inf)	*ng*h/mL*	2529884	1654303
DNAUC	*h*kg*ng/mL/mg*	252988	165430
T1/2	*h*	14	11.4
MRT	*h*	28	14.8
CL	*mL/h/kg*	4.0	6.0
VSS	*mL/kg*	111	89.5

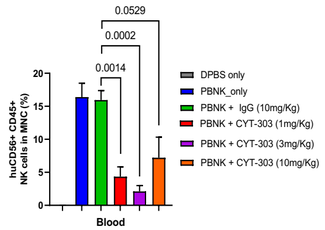
A single 10 mg/kg CYT-303 intravenous bolus dose was administered to PBNK injected NOG-hIL15 mice bearing small Hep3B tumors; Blood was collected at the indicated times, measured using a CYT-303 target capture and detect PK immunoassay, and analyzed using WinNonlin non-compartmental analysis.

Figure 4: CYT-303 combination with PBNK and iNK cells show dose-dependent tumor growth inhibition in HCC tumor bearing mice



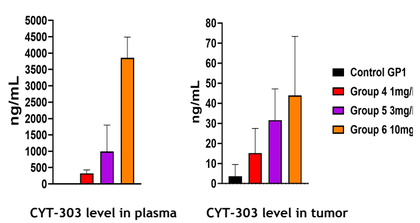
CYT-303 or control IgG1 was administered (q3d) by intraperitoneal injections at the above indicated doses to NSG-hIL15 mice bearing Hep3B subcutaneous tumors that were a) intratumorally injected on day 0 with 5.0 × 10^6 PBNK cells followed by IV injections on days 7 and 14 with 10 x 10^6 cells or b) intratumorally injected on day 0 with 2.5 × 10^6 iNK cells followed by IV injection on day 7 with 10 x 10^6 cells. Group mean tumor volumes and p values determined by 2-way ANOVA are shown.

Figure 5: CYT-303 treatment showed reductions in blood PBNK cells suggesting trafficking to tumors



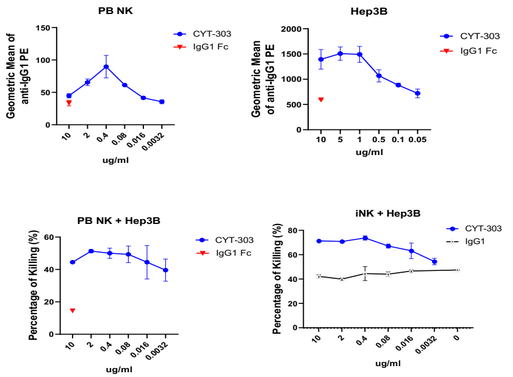
PBNK circulation in blood was analyzed by flow cytometry. Blood NK cell count in CYT-303 treated animals were significantly lower compared to IgG1 isotype control treated mice suggesting CYT-303 may facilitate NK cell trafficking from blood into the tumor.

Figure 6: CYT-303 levels in plasma & tumor provide a correlation between efficacious concentrations in blood and tumor



CYT-303 plasma and intratumoral levels were measured 24 hrs post last CYT-303 dose in the NSG-hIL15 Hep3B tumor model using PK immunoassay.

Figure 7: CYT-303 binding and cytotoxicity dose-responses with PBNK and iNK cells are consistent with the dose-responses observed in the HCC tumor models



The bell shaped *in-vitro* dose-responses observed *in vitro* for CYT-303 binding to PBNK and Hep3B tumors and CYT-303driven PBNK cytolysis of Hep3B are consistent with the bell-shaped dose-response observed with CYT-303 in the PBNK injected HCC tumor model.

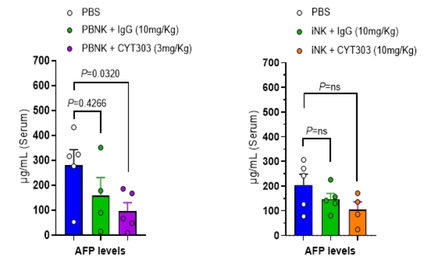
The linear dose-response observed *in vitro for CYT-303* driven iNKs cytolysis of Hep3B tumors are consistent with the dose-dependent linear responses observed with CYT-303 in the iNK cell injected HCC tumor model.

The lower PBNK binding and Hep3B cytolysis at high CYT-303 doses is likely driven by monovalent binding due to excess antibody at that dose resulting in lower avidity binding and signaling of the antibody resulting in a bell-shaped dose-response *in-vitro* and *in-vivo* – Prozone effect.

Figure 8: Alpha Fetoprotein level reductions following CYT-303 treatment in HCC tumor models

PBNK model
Blood AFP levels at end of study (day 24)

iNK model
Blood AFP levels at end of study (day 20)



CYT-303 at pharmacologically active doses showed reductions in alpha fetoprotein (AFP) biomarker compared to control treated animals in both PBNK and iNK injected models.

AFP reductions with CYT-303 in the PBNK model were statistically significant compared to control PBS treated mice.

AFP immunoassay was performed according manufacturers instructions (Invitrogen).

Conclusions

- CYT-303 showed dose-dependent HCC tumor growth inhibition in PBNK and iNK cell injected HCC tumor models.
- Dose-dependent increases in CYT-303 concentrations in tumor and blood were observed in the PBNK injected HCC tumor model showing the potential for CYT-303 to penetrate solid tumors.
- CYT-303 treated animals showed significant decreases in blood PBNKs suggesting CYT-303 may facilitate trafficking of these cells from blood to the tumor.
- Bell shaped dose-response observed with CYT-303 monotherapy in the PBNK injected HCC tumor model is consistent with CYT-303 *in vitro* studies showing similar dose-responses for PBNK and HCC tumor binding and tumor cytolysis.
- The linear dose-response observed with CYT-303 combination therapy with iNK cells in the HCC tumor model is consistent with the *in vitro* linear dose-response observed with iNK combination for cytolysis of HCC tumors.
- CYT-303 treatment resulted in reductions in blood AFP levels in both the PBNK and iNK injected HCC tumor models showing the utility of this biomarker for CYT-303 clinical studies.
- These CYT-303 preclinical proof-of-concept studies support clinical development of CYT-303 in HCC.

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