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Non-clinical characterization of CYT-303 FLEX-NK™ engager antibody supports clinical evaluation

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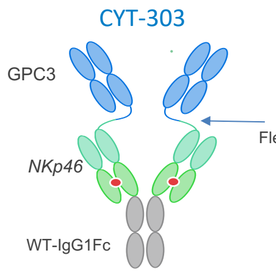
Introduction

CYT-303 is a multifunctional bispecific NK engager (NKE) targeting NK cell activating receptor NKp46 and tumor antigen Glypican-3 (GPC3) expressed in HCC (hepatocellular carcinoma). Cytovia's proprietary FLEX-NK™ platform utilizes a novel FLEX-linker and human IgG1 back bone to allow for simultaneous binding to targeted cancer cells and NK cells. We evaluated additional CYT-303 Fc effector functions and the impact of CYT-303 when added to peripheral blood NK cells (PBNK) in Hep3B tumor spheroid cytolysis and tumor serial killing assays. CYT-303 pharmacokinetics and safety in non-human primates were also evaluated.

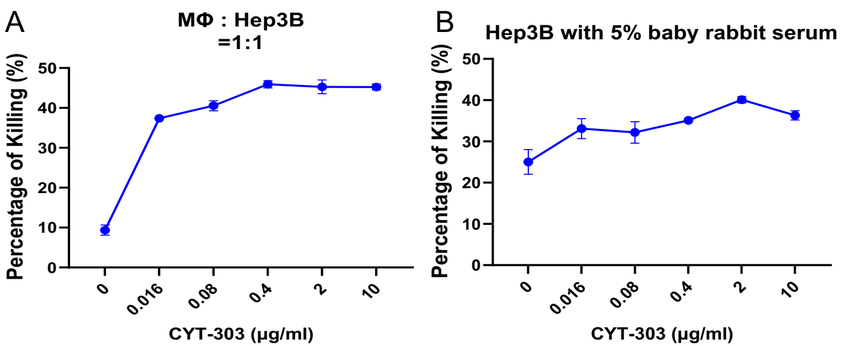


- Tetravalent structure allows higher avidity for GPC3 tumor and NKp46 NK cell targets & improved affinity and specificity
- Novel FLEX-linker allows for simultaneous binding to tumor target and NK cells
- FLEX-NK™ construct enhances NK Cell function against target cells.

Results

CYT-303 induces macrophage mediated antibody dependent cellular phagocytosis and complement dependent cytotoxicity of HCC tumors

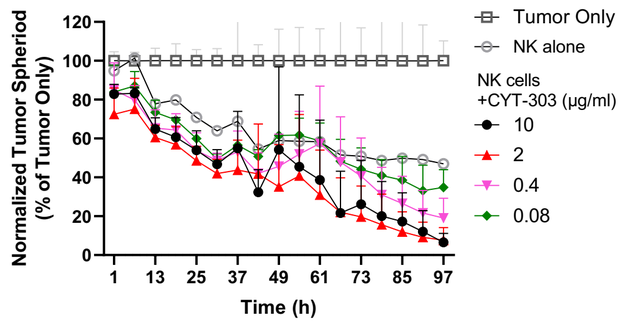
CYT-303 Fc effector function against Hep3B tumors was evaluated in antibody dependent cellular phagocytosis (ADCP) and complement dependent cytotoxicity (CDC) assays 4 hrs and analyzed by flow cytometry using cell viability dye (A & B respectively). ADCP was assessed using human macrophages differentiated from peripheral blood purified monocytes following culture with M-CSF for 5 days (A). Complement dependent cytotoxicity (CDC) was assed in the presence of rabbit serum containing complement (B).



CYT-303 Fc can activate macrophage phagocytosis of HCC tumors via CD16 and demonstrates an additional effector cell type targeted by CYT-303 (A); CYT-303 Fc can fix complement and activate the membrane attack complex to kill HCC tumors (B).

CYT-303 enhances time-dependent PBNK cytolysis of HCC tumor spheroids in a dose-dependent way

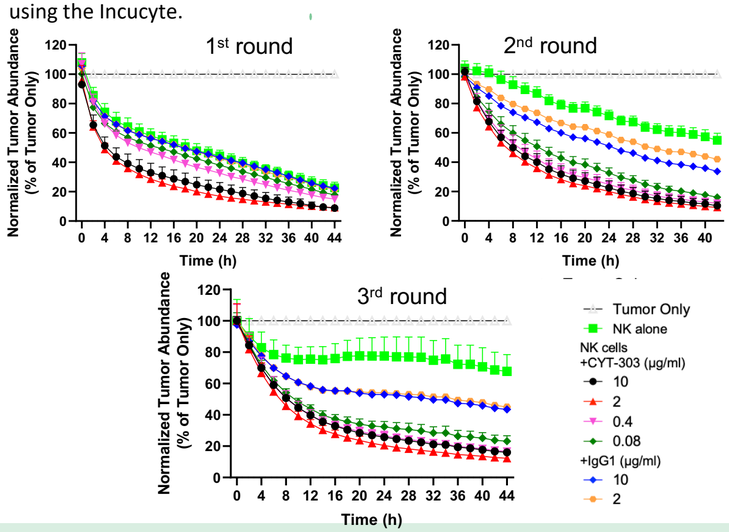
Hep3B-GFP tumors (10e3 cells/well) were cultured for 2 days in ultra low attachment U-bottom plates to form tumor spheroids. Then a fixed number of NK cells (10e3 cells/well) were added to tumor spheroids either alone or in combination with CYT-303 at different concentrations.



CYT-303 enhanced PBNK cytolysis of Hep3B tumor spheroids over a period 4 days in a dose dependent manner.

CYT-303 enhances PBNK serial killing of HCC tumors

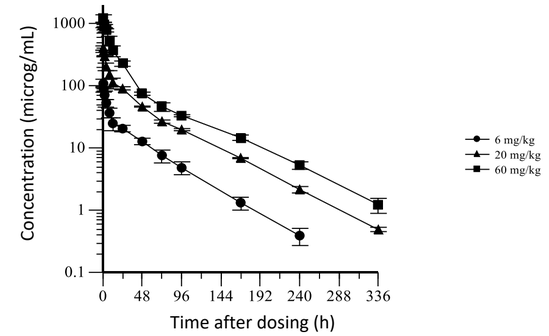
Serial killing of Hep3B-GFP tumors was evaluated with either PBNKs cells alone or in combination with CYT-303 using fixed E/T (1:2) ratios at each round of killing in 3 technical replicates. Tumor lysis was monitored by reduction of GFP positive tumors using the Incucyte.



PBNK cells alone showed gradual reduction in serial killing of Hep3B tumors in subsequent 2nd and 3rd rounds of killing suggesting dysfunction of these cells over time. CYT-303 reversed dysfunction of PBNKs and enhanced serial killing of Hep3B tumors in a dose dependent manner.

CYT-303 single dose pharmacokinetics and tolerability in cynomolgus monkeys

CYT-303 single dose range finding pharmacokinetics and safety and 4-week repeat dose safety studies were conducted in cynomolgus monkeys by intravenous infusion dosing at 6, 20 and 60 mg/kg doses.



Pharmacokinetic parameters of CYT-303 are shown in the following table.

Dose (mg/kg)	C_{max} (μg/mL)	$AUC_{0\text{-}168h}$ (μg·h/mL)	T_{max} (h)	$T_{1/2}$ (h)	Vdss (mL/kg)
6	109 ±16.9	1910 ±286	0.500	39.0 ±1.48	136 ±13.5
20	408 ±26.9	7740 ±302	0.500	44.3 ±0.651	118 ±8.39
60	1240 ±175	19800 ±1530	0.500	47.6 ±3.11	115 ±20.6

Data represent the mean ±SD of three animals.

CYT-303 was well tolerated and did not show any evidence for cytokine release or any toxicity. CYT-303 showed dose proportionate increases in C_{max} and AUC's and a T ½ of 39 hrs at the 6 mg/kg dose.

Conclusions

- Evaluation of additional mechanisms and effector cell types targeted by CYT-303 showed activation of macrophages by antibody dependent cellular phagocytosis (ADCP) and complement dependent cytotoxicity (CDC) of HCC tumors.
- CYT-303 enhanced PBNK cytolysis of HCC tumor spheroids in a dose-dependent manner.
- CYT-303 enhanced PBNK serial killing of HCC tumors and reversed dysfunction of these cells in later rounds of serial killing.
- CYT-303 single dose tolerability and pharmacokinetic study in cynomolgus monkeys showed dose dependent increases in C_{max} and AUCs and a $T_{1/2}$ of 39 hrs at 6 mg/kg.
- The above results with CYT-303 invoking additional effector cell types and anti-tumor mechanisms against HCC together with the pharmacokinetic profile of CYT-303 in cynomolgus monkeys supports clinical investigation of CYT-303 in HCC.

Cytovia Therapeutics