# CYT-303 FLEX-NK™ engager dose response efficacy mechanisms in HCC tumor model and safety in cynomolgus monkey toxicology studies support clinical evaluation in hepato-cellular carcinoma

#5667

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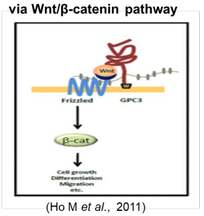
## Background

**BACKGROUND:** CYT-303 is a multifunctional bispecific NK engager (NKE) targeting NK cell activating receptor NKp46 and oncofetal tumor antigen Glypican-3 (GPC3) expressed in HCC (Hepatocellular carcinoma). GPC3 signaling via Wnt/beta catenin in HCC is oncogenic, proliferative, and immunosuppressive promoting tumorigenesis. Moreover, HCC patients who are refractory to checkpoint inhibitor Atezolizumab + Bevacizumab first line immunotherapy express a high GPC3, AFP, and β-catenin signature (Zhu A *et al.*, 2023), suggesting modulation of GPC3 is a good approach for combination therapy with checkpoint blockade or exogenous iPSC derived NK (iNK) cell therapy. Cytovia's proprietary FLEX-NK™ platform utilizes a novel FLEX-linker and human IgG1 back bone to allow for simultaneous binding to target cancer cells and NK cell effectors and enhances pharmacokinetics. We evaluated CYT-303 dose response efficacy and mechanistic pharmacology studies in humanized HCC tumor models, including NK cell trafficking from blood to the tumor and associated biomarker changes. We also conducted pre-clinical safety assessment studies in cynomolgus monkeys to support human clinical studies in HCC patients. These results provide mechanistic insights and enable monotherapy and combinational CYT-303 clinical studies in HCC patients.

### Wnt/β-catenin oncogenic signaling and immunosuppressive HCC tumor microenvironment

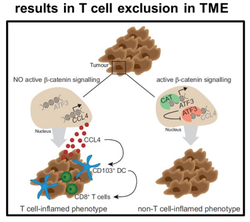
GPC3 induces HCC proliferation via Wnt/β-catenin pathway




(Ho M *et al.*, 2011)

- High tumor GPC3 levels are known to be associated with increased Wnt/β-catenin oncogenic signaling resulting in HCC tumor proliferation

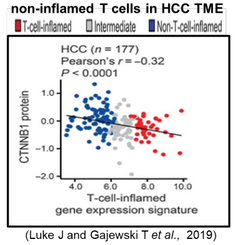
Wnt/β-Catenin activation results in T cell exclusion in TME




(Spranger T and Gajewski T *et al.*, 2015)

- Wnt/β-catenin signaling activates ATF-3 transcription factor that represses chemokine (CCL4) production and DC migration to tumor resulting in T cell exclusion in TME

High β-catenin associated with non-inflamed T cells in HCC TME



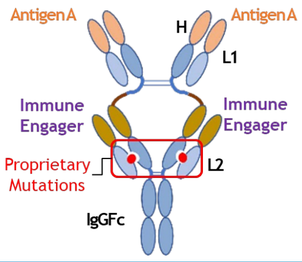

(Luke J and Gajewski T *et al.*, 2019)

- Increased β-catenin levels show reduced T cell inflamed signatures in HCC and other solid tumors (TCGA data)

**METHODS:** CYT-303 dose response mechanistic pharmacology studies were conducted in NSG-hIL-15 mice bearing Hep3B tumors. Flow cytometry was used to assess NK cells in blood and tumor. Immunoassays were used to detect blood AFP biomarker and CYT-303 blood levels. A 4-week CYT-303 repeat dose GLP toxicology study in cynomolgus monkeys at 6, 20, and 60 mg/kg doses was conducted following weekly intravenous infusions followed by a 6-week recovery period. CYT-303 toxicokinetic and anti-drug antibody (ADA) assessments were conducted using validated immunoassays.

## Results

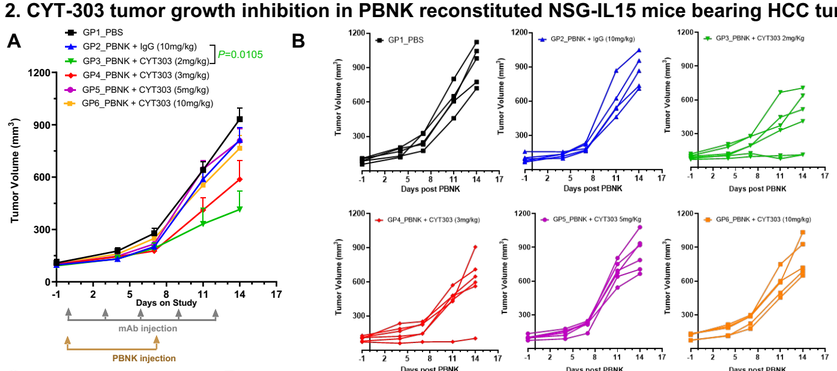
### 1. Proprietary BsAb Technology Leading to Novel Multifunctional Flex Format

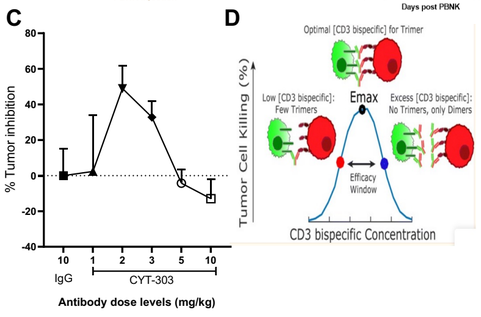



- Flexible linker allowing simultaneous binding to effector and target tumor cells
- Full IgG with Fc allows for a half-life longer than other bi-specifics supporting at least weekly administration
- Proprietary mutation ensuring proper pairing of light and heavy chains
- Tetravalent structure for increased affinity and avidity
- Up to 2 years stability

## Results

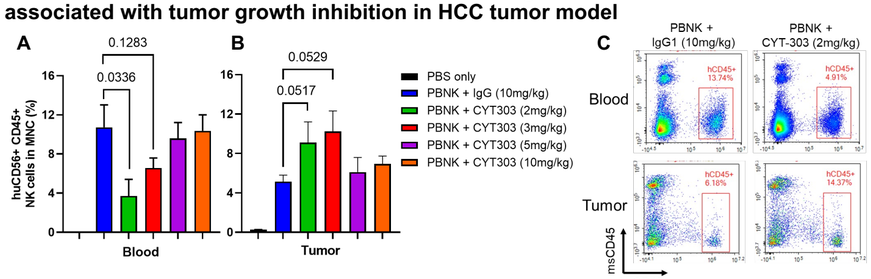
### 2. CYT-303 tumor growth inhibition in PBNK reconstituted NSG-IL15 mice bearing HCC tumors




- CYT-303 showed dose dependent tumor growth inhibition with maximum efficacy at 2-3 mg/kg, typical of immune synapse related dose responses. Representative data from 3 independent studies are shown.
- NSG-IL15 mice bearing Hep3B tumors received 5 x 10^6 PBNK cells on day 0 and 10 x 10^6 on day 7 and additionally received either control IgG1 or mentioned doses of CYT-303 antibody q3d by intraperitoneal injections. (**A**) Mean tumor volumes across the groups (**B**) Individual animal tumor growth curves across the groups (**C**) Percentage of tumor inhibition compared to IgG control group (**D**) Illustration of MOA for bell shape activities for engagers.

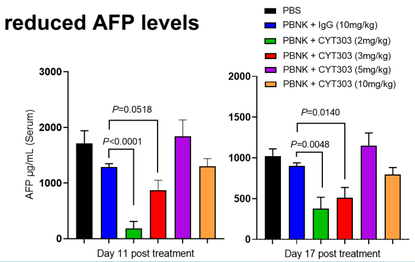
### 3. CYT-303 treatment facilitates PBNK cell trafficking from blood to tumor and is associated with tumor growth inhibition in HCC tumor model



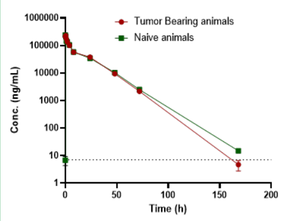

CYT-303 efficacious doses of 2 and 3 mg/kg showed reductions in blood NK cells and corresponding increases in the tumor, suggesting facilitation of NK cell trafficking from blood to tumor and retention in tumor following GPC3 engagement. Bar diagram showing the percentage of PBNK cells in blood (**A**) tumor (**B**). Representative FACS plots in Blood and Tumor on day 14 post treatment (**C**).

### 4. Tumor growth inhibition is associated with reduced AFP levels

Alpha Fetoprotein levels in plasma at Day 11 (**A**) and Day 17 (**B**) were evaluated by ELISA. This is consistent with above HCC tumor growth inhibition data. Animals receiving PBNK cells in combination with CYT-303 showed reduced AFP levels compared to hIgG1 isotype control treated animals.




### 5: CYT-303 Pharmacokinetics in naïve and tumor bearing mice

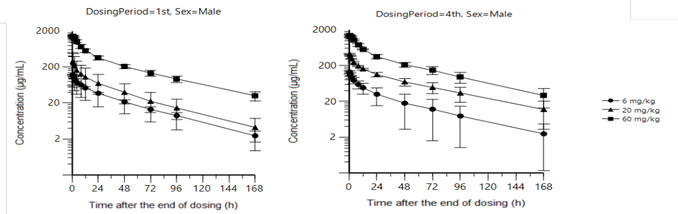



| | Tumor Bearing | Naïve | Unit |
|---|---|---|---|
| Slope | -0.026921402 | -0.0240896 | |
| Intercept | 5.217761397 | 5.17744587 | |
| $K_e$ | 0.0619 | 0.0554 | $h^{-1}$ |
| $T_{1/2}$ | 11.18 | 12.49 | $h^{-1}$ |
| $C_0$ | 165105.45 | 150468.60 | ng/mL |
| Vd | 60.57 | 66.46 | L |
| Clearance | 3.76 | 3.69 | L/h |
| AuC 0 to t | 6851.88 | 6244.45 | ng*h/mL |
| AuC 1 to t | 2455915.84 | 2426994.86 | ng*h/mL |
| AuC t to infinity | 75.81 | 269.77 | ng*h/mL |
| AuC TOTAL | 2462843.53 | 2433509.09 | ng*h/mL |

- A trend towards target mediated clearance was observed in Hep3B tumor bearing NSG-hIL-15 mice, compared to naïve mice
- CYT-303 was administered as an IV bolus to naïve and Hep3B tumor bearing NSG-hIL-15 mice which received two doses of PBNK cells on Day 0 and Day 7.

### 6. CYT-303 safety assessment in the 4-week toxicology study in cynomolgus monkey

| Group | Test and Control Articles | Dose Level (mg/kg) | Dose Volume (mL/kg) | Concentration (mg/mL) | Necropsy | Number of Animals (Animal No.) Males | Females |
|---|---|---|---|---|---|---|---|
| 1 | Control* | - | 5 | - | Terminal | 3 | 3 |
| | | | | | Recovery | 2 | 2 |
| 2 | CYT-303 | 6 | 5 | 1.2 | Terminal | 3 | 3 |
| 3 | CYT-303 | 20 | 5 | 4 | Terminal | 3 | 3 |
| 4 | CYT-303 | 60 | 5 | 12 | Terminal | 3 | 3 |
| | | | | | Recovery | 2 | 2 |




- No CYT-303 related cytokine release or toxicities were observed in the study, based on clinical observations, clinical chemistry, hematology, and anatomic pathology.
- CYT-303 dose dependent increases in $C_{max}$ and AUC were observed following the first and last dose with no observed evidence of accumulation. CYT-303 exposures were maintained throughout the 4-week duration of the study.
- Anti-drug antibodies (ADA) occurred in 1 out of 22 animals (6mg/kg group) and, as expected, were associated with reduced CYT-303 levels.
- *NOAEL in the study was the highest dose administered in the study = 60 mg/kg*

## Conclusions

- CYT-303 showed dose dependent HCC tumor growth inhibition in NSG-hIL-15 mice (2 and 3 mg/kg) that were associated with decreased blood PBNK levels and higher numbers in tumors. This suggests CYT-303 facilitates trafficking of these cells from blood to tumor and retention in the tumor following GPC3 engagement.
- CYT-303 mediated tumor growth inhibition was associated with reduction of HCC blood biomarker AFP levels, showing the utility of this biomarker for CYT-303 clinical studies.
- CYT-303 pharmacokinetic analysis in tumor bearing mice showed a trend towards target mediated clearance compared to non-tumor bearing mice.
- CYT-303 safety assessment in monkey did not show any evidence of cytokine release or toxicity.
- These pre-clinical proof-of-concept studies show the feasibility of CYT-303 to convert the highly proliferative and immunosuppressive HCC biomarker signature to a GPC3 low, AFP low, and β-catenin low signature that's associated with clinical responses to first line Atezolizumab + Bevacizumab checkpoint immunotherapy.
- These studies justify monotherapy and combination studies of CYT-303 with checkpoint blockade or exogenous iPSC derived NK cell therapies to overcome the highly immunosuppressive HCC tumor microenvironment.