1320 NON-CLINICAL CHARACTERIZATION OF CYT-303 FLEX-NK™ ENGAGER ANTIBODY SUPPORTS CLINICAL EVALUATION

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Background 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^{TM} 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 Hep3B tumor serial killing assays. CYT-303 pharmacokinetics and safety in non-human primates were also evaluated.

Methods CYT-303 Fc effector function against Hep3B tumors was evaluated for antibody dependent cellular phagocytosis (ADCP) using human macrophages differentiated from purified monocytes isolated from peripheral blood and for complement dependent cytotoxicity (CDC) in the presence of rabbit complement. Hep3B tumor spheroids were established in special U-bottom adhesive plates and tumor spheroid killing assays were conducted with PBNKs and CYT-303 using the Incucyte ^{TM} Live Cell Analysis System. Serial killing assays were conducted by repeatedly adding the same PBNK cells to fresh tumor cells and CYT-303 following each round of tumor killing. 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.

Results CYT-303 showed dose dependent ADCP by human macrophages against Hep3B tumors that was maximal at 0.4 ug/ml. CYT-303 also showed maximal CDC against Hep3B tumors at 0.4 – 2 ug/ml concentrations. CYT-303 in the presence of freshly isolated PBNKs showed increased time dependent killing of Hep3B tumor spheroids that peaked at 2-3 days following initiation of killing. This killing was enhanced in the presence of CYT-303 in a dose dependent manner. Furthermore, PBNK serial killing of Hep3B tumors was also enhanced by CYT-303. In the CYT-303 single dose range finding pharmacokinetics study in cynomolgus monkeys the C_{max} and $AUC_{0\text{-}168h}$ values increased with dose and increases were approximately dose-proportional. CYT-303, half-lives ($T_{1/2}$) ranged from 39 to 47.6 hrs and exposures persisted up to 1-week. No evidence for any cytokine release was observed. In the 4-week repeat dose toxicity study no CYT-303 related toxicities were observed, enabling CYT-303 clinical development.

Conclusions CYT-303 demonstrated potent ADCP and CDC against Hep3B tumors as well as Hep3B tumor spheroid and serial killing activities in the presence of PBNKs. Preclinical pharmacokinetics and safety study results in cynomolgus monkeys support CYT-303 clinical development.

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