PRECLINICAL CHARACTERIZATION OF CYT-100 IPSC DERIVED NK CELLS ALONE AND IN COMBINATION WITH CYT-303 NK CELL ENGAGER FOR HEPATOCELLULAR CARCINOMA (HCC)

Liang Lin*, Andrea Chambers, Marshall Chao Ma, Hao-Ming Chang, David Zou, Preeti Ashok, Elizabetta Burchi, Armin Rath, Stanley Frankel, Jean Kadouche, Daniel Tepper, Nejmi Dilmac, Antonio Arulanandam, Wei Li. Cytovia Therapeutics, Natick, MA, USA

Background 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 activation receptor on NK cells and Glypican-3 (GPC3) expressed in the tumor 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 compared CYT-100 expansion capacity potential to PBNKs (peripheral blood natural killer cells).

Methods CYT-100 immunophenotyping was conducted by flow cytometry using a panel of NK cell directed antibodies. Intracellular signaling following stimulation of CYT-100 with rIL-2 or rIL-15 was assessed using a phospho STAT-5 antibody by flow cytometry and cell growth monitored by counting cells using the flow cytometer or a cell counting kit (CCK8). Hep3B tumor spheroids were formed in special U-bottom adhesive plates and tumor spheroid killing assays were conducted using the Incucyte™ Live Cell Analysis System. Serial killing assays were conducted by repeatedly adding the same CYT-100 cells to fresh tumor cells following each round of tumor killing. The chromosome telomere length was assessed by a q-PCR assay.

Results CYT-100 immunophenotyping results showed > 95% expression of CD56/CD45, high expression of all activating receptors, except CD16, and some chemokine receptors including CXCR3, and minimal expression of most inhibiting receptors. Stimulation with rIL-2 or rIL-15 resulted in tyrosine phosphorylation of STAT-5 and increased cell growth over a period of 6 days, comparable to PBNKs, demonstrating the capacity of these cells to expand in the presence of cytokines. CYT-100 showed increased time-dependent killing of Hep3B tumor spheroids that peaked 2–3 days following of initiation of killing. This killing was enhanced in the presence of CYT-303 in a dose dependent manner. Furthermore, CYT-100 showed serial killing of Hep3B tumors and this was enhanced in combination with CYT-303. Chromosome telomere length analysis of CYT-100 showed greater telomere length compared to that of PBNKs, suggesting greater expansion capacity potential of these cells.

Conclusions The allogeneic IPSC derived NK cell product CYT-100 demonstrates cytotoxicity against hepatocellular carcinoma Hep3B cells, sensitivity to cytokine activation and expansion potential. The cytotoxicity of this iNK product is further enhanced by combination with the NK cell engager antibody CYT-303. These data support further development of the combination of iNK cells and NKEs as therapeutics for HCC.