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Human Placental Hematopoietic Stem Cell Derived Natural Killer Cells (CYNK-001) Mediate Protection Against Influenza A Viral Infection

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Background: Influenza A virus (IAV) infections are associated with a high healthcare burden around the world and there is an urgent need to develop more effective therapies. Natural killer (NK) cells provide the first line of innate defense against IAV by killing infected epithelial cells, by producing antiviral cytokines and affecting adaptive immunity. Preclinical studies have demonstrated that NK cells play a pivotal role in reducing IAV-induced pulmonary infection; however, little is known about the therapeutic potential of adoptively transferred NK cells for IAV infections. Celularity Inc. is developing human placental hematopoietic stem cell-derived allogeneic, off-the-shelf NK cell therapy (CYNK-001) for the treatment of viral infections, including coronavirus disease of 2019. Here, we report the evaluation of antiviral activities of CYNK-001 against IAV infection.

Methods: *In vitro* antiviral activities of CYNK-001 were evaluated using human alveolar epithelial cell line A549, infected with IAV strain A/PR/8/34 (H1N1) at variable multiplicity of infection (MOI). The expression of ligands for NK cell receptors was analyzed on infected A549 cells using Fc-coupled recombinant proteins. CYNK-001 was added to A549 cells 16 hours post infection. CYNK-001 degranulation was measured after 4 hours of coculture, and CYNK-001 cytotoxicity against IAV-infected A549 was measured real-time using impedance-based xCELLigence platform. *In vivo* antiviral and immunomodulatory activities of CYNK-001 were assessed in A/PR/8/34 (H1N1)-induced severe acute lung injury mouse model. Mice were intranasally infected with 2500 PFU IAV. PBS or 1×10^7 CYNK-001 cells were intravenously administered twice at 1 and 3 days post infection (dpi). At 6 dpi, lungs were collected for the evaluation of viral load by qPCR, lung injury and immune cell profiling by histology. Bronchoalveolar lavage fluid (BALF) was collected at 6 dpi for cytokine analysis by multiplex assays, total protein concentration by ELISA and immune cell profiling by flow cytometry.

Results: *In vitro*, IAV infection corresponded with dose-dependent expression of ligands to NK cell-activating receptors, including NKP44, NKP46 and NKG2D. CYNK-001 cells exhibited increased IFN γ , TNF α and GM-CSF production, and elevated level of degranulation upon coculture with IAV-infected A549 cells. Cytokines in culture supernatant and CD107a expression in CYNK-001 cells were upregulated in a virus dose-dependent manner. Consistent with this finding, CYNK-001 cytotoxicity against IAV-infected A549 cells increased from 35% at 0 MOI to 50%, 60% and 75% at 0.001, 0.01 and 0.1 MOI, respectively. These data indicate that CYNK-001 cells recognize virally infected cells, resulting in specific cytotoxic elimination of the source of infection. *In vivo*, treatment of IAV-infected mice with CYNK-001 reduced weight loss and increased their likelihood of survival. PBS control group developed a severe disease and 37.5% mortality was observed as early as day 4. In the group treated with CYNK-001, disease onset was delayed by 2 days. qPCR analysis of viral RNA showed that CYNK-001-treated mice had lower viral load in the lung than vehicle-treated mice, demonstrating antiviral function of CYNK-001 *in vivo*. CYNK-001-treated mice had reduced lung injury as assessed by lower total protein concentration in BALF. Moreover, CYNK-001 reduced BALF murine cytokines and chemokines, including IFN γ ($p < 0.001$), IL-6, TNF α , MCP-1 ($p < 0.05$), CXCL2 and CXCL9. Lastly, immunohistochemical analysis of the lung showed that CYNK-001-treated mice had an altered immune response to IAV with higher number of CD68⁺ macrophages and CD8⁺ T cells at 6 dpi.

Conclusions: Our *in vitro* and *in vivo* data show the promising antiviral activities of CYNK-001 against IAV infection. In a severe IAV infection mouse model, CYNK-001 treatment demonstrates lower mortality rate, lower weight loss, lower lung viral load and reduced lung injury along with reduced inflammation. These results support our hypothesis that the adoptive transfer of CYNK-001 could reduce the burden of viral infection through the elimination of infected epithelial cells, coordinate a more effective immune response, and result in a clinical benefit in patients with severe viral infection.

Disclosures

He: *Celularity Inc.*: Current Employment. **Mahlaköiv:** *Celularity Inc.*: Current Employment. **Gleason:** *Celularity Inc.*: Current Employment, Current equity holder in private company. **Van Der Touw:** *Celularity Inc.*: Current Employment. **Kang:** *Celularity Inc.*: Current Employment. **Hariri:** *Celularity Inc.*: Current Employment, Current equity holder in private company. **Zhang:** *Celularity Inc.*: Current Employment, Current equity holder in private company.

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