DEVELOPING PLACENTAL CD34+-DERIVED NATURAL KILLER CELLS WITH HIGH AFFINITY CLEAVAGE RESISTANT CD16 (CYNK-101) AND CETUXIMAB FOR ENHANCED THERAPY OF EGFR+ NON-SMALL CELL LUNG AND HEAD AND NECK CANCERS

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Background Natural killer (NK) cells are key mediators of antibody dependent cellular cytotoxicity (ADCC) via the CD16 Fc receptor. NK cellular therapies can effectively be targeted to tumor antigens when combined with tumor specific antibodies. Celularity Inc. is developing human placental CD34+-derived, cryopreserved, off-the-shelf, allogenic NK cells (CYNK-101) with high IgG binding affinity proteinase cleavage resistant CD16 variant (CD16VP) for cancer treatment. We hypothesize that expressing CD16VP augments anti-tumor ADCC activity. Here, we report the results of evaluating CYNK-101 in combination with Cetuximab, an anti-EGFR antibody, against EGFR+ non-small cell lung cancer (NSCLC) and head and neck squamous cell carcinomas (HNSCC).

Methods Human placental CD34+ cells were transduced with lentivirus expressing CD16VP and expanded in the presence of cytokines to generate CYNK-101 cells. The anti-tumor activity of CYNK-101 in combination with Cetuximab was assessed against EGFR+ NSCLC and HNSCC cell lines. The PI3K kinase inhibitor Wortmannin was used to investigate the molecular mechanisms underlying cytotoxicity of CYNK-101.

Results In vitro ADCC activity of CYNK-101 against NSCLC and HNSCC targets was assessed in combination with Cetuximab. At 4h at the effector to target (E:T) ratio of 2.5:1, CYNK-101 displayed increased cytotoxicity against NSCLC lines SK-MES-1 and NCI-H226 at $78.7 \pm 11.4\%$ and $58.3 \pm 10.0\%$ with Cetuximab vs. $44.9 \pm 9.2\%$ and $41.7 \pm 5.0\%$ with IgG control, respectively (n=5 donors, $p < 0.01$). For HNSCC targets the cytotoxicity with Cetuximab compared to IgG control was $57.0 \pm 14.1\%$ vs. $23.3 \pm 6.2\%$ for CAL-27, $69.6 \pm 13.7\%$ vs. $34.0 \pm 12.5\%$ for FaDu, $41.6 \pm 22.6\%$ vs. $22.9 \pm 14.9\%$ for A-253, and $25.8 \pm 29.1\%$ vs. $4.2 \pm 8.0\%$ for SCC-25 (n=6 donors, $p < 0.005$ for first 3 targets). CYNK-101 (n=6 donors) in the presence of Cetuximab secreted higher levels of GM-CSF ($p < 0.01$), IFN- γ ($p < 0.05$), and TNF- α ($p < 0.05$) when co-cultured with the HNSCC lines A-253, FaDu, and SCC-25; and higher GM-CSF and IFN- γ levels ($p < 0.01$) when co-cultured with NCI-H226 compared to that of IgG control. The enhanced cytotoxicity of CYNK-101 was PI3K pathway-dependent as at an E:T ratio of 2.5:1 Wortmannin significantly decreased the cytotoxicity for NCI-H226 from $49.2 \pm 18.1\%$ to $27.7 \pm 18.1\%$ ($p < 0.005$) and for CAL-27 from $41.6 \pm 20.7\%$ to $19.4 \pm 13.0\%$ ($p < 0.05$) (n=4 donors).

Conclusions Our results demonstrate that CYNK-101 have enhanced Cetuximab-mediated ADCC activity against EGFR+ NSCLC and HNSCC tumor cell lines. Further development of the combinational therapy for these solid tumor indications is warranted.

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