Developing placental CD34⁺-derived natural killer cells with high affinity and cleavage resistant CD16 (CYNK-101) in combination with Avelumab for enhanced therapy against PD-L1⁺ solid tumors

Abstract #270

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INTRODUCTION

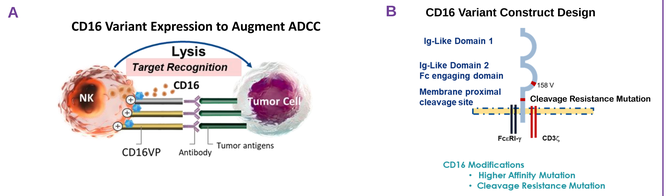
Celularity Inc. has developed a novel platform that enables production of genetically modified allogenic off-the-shelf ex-vivo expanded and cryopreserved human placental CD34⁺-derived natural killer (NK) cells named CYNK-101 for the treatment of various cancers.[1] NK cells are key mediators of antibody-dependent cellular cytotoxicity (ADCC) via the CD16 receptor, which recognizes the Fc region of an antibody bound to tumor target cells. NK cellular therapies can thus be targeted to tumor antigens when combined with tumor-specific antibodies.

CYNK-101 cells express a genetically modified version of the CD16 receptor. The 158Val/Val allele of CD16 was shown to have higher IgG binding affinity compared to the 158Phe/Phe form,[2] and is found in ~10-20% of the human population.[3,4] Furthermore, following NK cell activation, CD16 on the surface is protease cleaved, resulting in decreased ADCC. However, a mutation at this cleavage site can prevent this cleavage and would thus increase the ADCC activity of NK cells.[5] CYNK-101 described herein express this high IgG affinity and cleavage resistant variant of CD16 to increase ADCC.

The immune checkpoint protein PD-L1 was found to be overexpressed in 20-60% of non-small cell lung cancers (NSCLC), in 60% of triple negative breast cancers (TNBC), and in 15-45% of bladder cancers; often translating to a worse outcome for patients.[6-10] Avelumab is a human monoclonal antibody that binds to PD-L1 and prevents its interaction with its receptor PD-1. In this way Avelumab can block the PD-L1/PD-1 inhibitory immune pathway that tumor cells utilize for immune evasion. Avelumab also induces an ADCC immune cell response when bound to PD-L1 on the surface of tumor cells.[11-12]

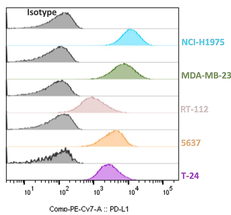
Here, we demonstrate improved and prolonged anti-tumor activity for CYNK-101, an NK cell immunotherapy product. CYNK-101 cells were functionally evaluated for their ADCC activity against PD-L1⁺ NSCLC, TNBC, and bladder cancer tumor cells in combination with Avelumab.

Figure 1. CYNK-101 Mediated ADCC



(A) Schematic representation of CYNK-101-mediated ADCC. (B) Engineered construct of CD16 with high-affinity and protease cleavage-resistant mutations that were expressed on placental CD34⁺ hematopoietic stem cell-derived CYNK-101 cells.

Figure 2. PD-L1 Expression on NSCLC, TNBC, and Bladder Cancer Cell Lines



Tumor Cell Line	PD-L1+ (%)	PD-L1 Mean Fluorescence Intensity (gMFI)
NCI-H1975	100.0	10635
MDA-MB-231	99.8	6545
RT-112	89.8	1071
5637	99.5	3298
T-24	99.8	2682

PD-L1 expression on NSCLC (NCI-H1975), TNBC (MD-MB-231), and bladder cancer (RT-112, 5637, T-24) tumor cell lines. Isotype-stained cells, shown in grey, were used as gating controls for each cell line. The percentage of tumor cells expressing PD-L1 and the geometric mean fluorescence intensity (gMFI) for each of the cell lines are indicated in the adjacent table.

References

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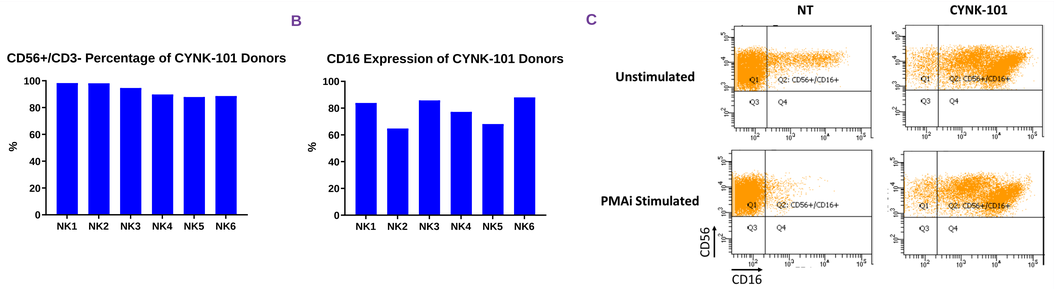
Disclosure

IR: GF: EH: HR: NG: XG: RH: LK: Celularity Inc. Employment

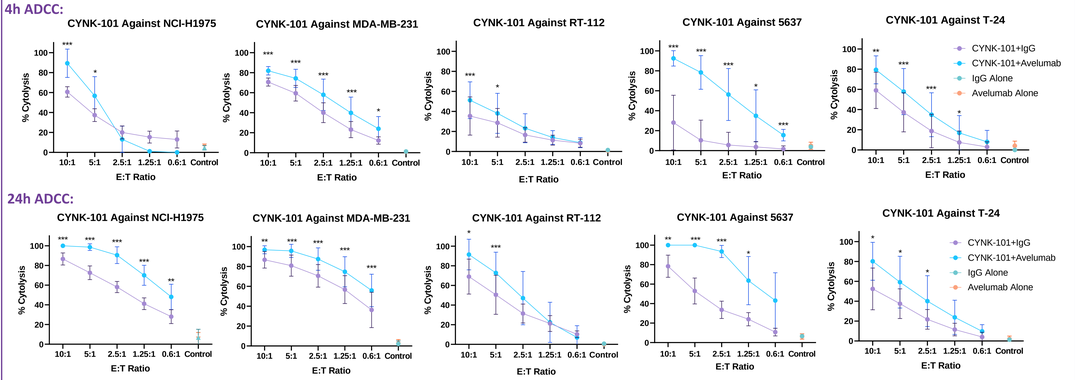
RESULTS

Figure 3. High Expression and Cleavage Resistance of CD16 on CYNK-101



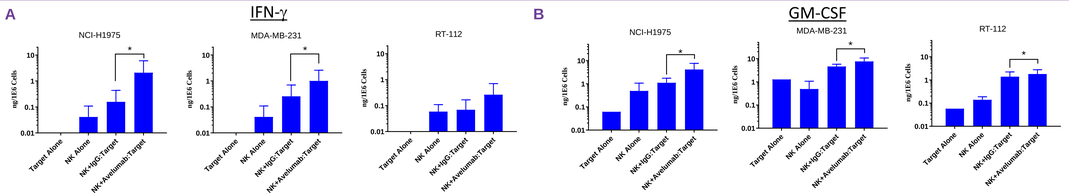
(A) Percentage of CD56+/CD3- cells in CYNK-101 donors post thaw, NK percentage was 92.9% ± 4.4% (mean ± SD, n=6 donors). (B) CD16 expression on CD56+/CD3- CYNK-101 cells post thaw, 78.0% ± 10.3% (mean ± SD, n=6 donors). (C) Representative flow cytometry diagrams showing CD16 loss following cleavage on non-transduced (NT) cells but cleavage resistance on CYNK-101 following 2h activation by PMA/ionomycin (PMAi).

Figure 4. CYNK-101 Cells in Combination with Avelumab Have Increased ADCC Against NSCLC, TNBC, and Bladder Cancer Cell Lines



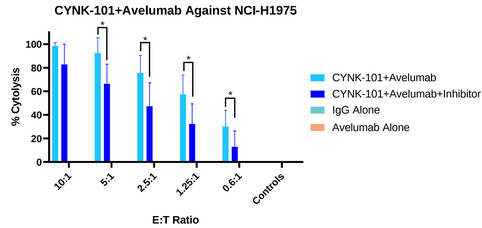
4h and 24h ADCC activity (mean ± SD) of CYNK-101 cells (n=6 donors) in combination with Avelumab compared to the control IgG antibody against NSCLC cell line NCI-H1975, TNBC cell line MDA-MB-231, and bladder cancer cell lines RT-112, 5637, and T-24 at the indicated effector-to-target (E:T) ratios. Cytotoxicity from Avelumab or IgG alone is included for reference. * indicates significantly higher activity of CYNK-101 with Avelumab compared to that of IgG (***p<0.005, **p<0.01, and *p<0.05).

Figure 5. CYNK-101 in Combination with Avelumab Have Increased Cytokine Secretion Against NSCLC, TNBC, and Bladder Cancer Cell Lines



Level of the inflammatory cytokines IFN-γ (A) and GM-CSF (B) in ng/1E6 cells from culture supernatants following 24h co-culture at a 1:1 E:T ratio of CYNK-101 cells in combination with Avelumab or IgG control against the NSCLC cell line NCI-H1975, the TNBC cell line MDA-MB-231, or the bladder cancer cell line RT-112 (mean ± SD, n=6 donors). The cytokine secretion from target cells without CYNK-101 cells is also included for reference, as well as that from CYNK-101 cells alone. * indicates significantly higher secretion of the indicated cytokine from CYNK-101 cells when in combination with Avelumab compared to IgG control (*p<0.05).

Figure 6. PI3K Pathway Inhibition Decreased CYNK-101 in Combination with Avelumab ADCC



24h cytolysis of untreated or 1h 0.1 μM Wortmannin (phosphatidylinositol 3-kinase (PI3K) inhibitor) pretreated CYNK-101 cells in combination with Avelumab against the NSCLC cell line NCI-H1975 at the indicated E:T ratios (mean ± SD, n=3 donors) * indicates significantly decreased cytolysis with the PI3K inhibitor pretreated CYNK-101 compared to untreated CYNK-101 at that E:T ratio (*p<0.05).

MATERIALS AND METHODS

- Cell culture: Human placental CD34⁺ cells were transduced with a lentivirus vector expressing the CD16 variant and cultured in the presence of cytokines to generate CYNK-101 cells.
- Cell characterization: Upon completion of cell expansion and differentiation, CYNK-101 cells were frozen. All subsequent characterization and functional analyses were performed on thawed product. CYNK-101 cells were evaluated for NK surface markers CD56, CD3, and CD16 by flow cytometry immediately post thaw.
- Evaluation of CYNK-101 anti-tumor activity in combination with Avelumab: The anti-tumor activity of CYNK-101 cells against PD-L1-positive NSCLC cell line NCI-H1975, TNBC cell line MDA-MB-231, and bladder cancer cell lines RT-112, 5637, and T-24 were assessed in combination with 0.1 μg/ml Avelumab or the IgG control antibody at various E:T ratios. ADCC activity was measured by real-time xCELLigence assay (ACEA Biosciences) and cytokine secretion by Luminex xMAP multiplex assay (Millipore Sigma). The PI3K inhibitor Wortmannin[13] was used to study the molecular mechanisms underlying cytotoxicity of CYNK-101 cells against the NSCLC tumor cell line.
- Statistical Analysis: Statistical analysis was performed using GraphPad Prism and Excel programs. Data are presented as mean ± SD. Paired 2-tailed Student's t-tests were used for comparing 2 groups.

SUMMARY

- Celularity has developed genetically modified human placental CD34⁺-derived cryopreserved off-the-shelf allogenic NK cells (CYNK-101) with a high IgG binding affinity and proteinase resistant CD16 variant for cancer treatment.
  - >92% NK (CD56+/CD3-) purity was achieved by the process
  - 65%~88% CD16 expression was maintained post thaw, and the CD16 was resistant to being cleaved from the surface following NK cell activation
- CYNK-101 cells demonstrated enhanced Avelumab-mediated ADCC activity against PD-L1⁺ NSCLC, TNBC, and bladder cancer tumor cell lines.
  - CYNK-101, when in combination with Avelumab, secreted higher levels of IFN-γ and GM-CSF against the NSCLC cell line NCI-H1975 and TNBC cell line MDA-MB-231, and increased GM-CSF against the bladder cancer cell line RT-112.
  - The PI3K pathway plays a role in the Avelumab-mediated increased cytotoxicity of CYNK-101 as its inhibition with Wortmannin decreased the ADCC.
- CYNK-101 cells provide a combination immunotherapy option by harnessing the anti-tumor activities of both targeted biologics and innate cytotoxicity of NK cells. Further development of CYNK-101 in combination with the therapeutic antibody for these solid tumor indications is warranted.