Development of an IL-2 independent NK cell line expressing high-affinity Fc-receptor to augment monoclonal antibody therapy

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Objectives
- Develop a universal "off-the-shelf" high affinity NK cell therapy product (haNKTM) that augments the efficacy of ADCC-mediated monoclonal antibody treatments in all patients.

Introduction
- NK cells mediated Antibody Dependent Cell-mediated Cytotoxicity (ADCC) is considered a major mechanism of action of several IgG monoclonal antibodies (mAbs) used in cancer therapy1.
- Efficacy of these mAbs is limited by the fact that only 12% of the normal human population is homozygous for expression of the high affinity variant of FcγRIIIa (CD16-158V) that provides optimal ADCC2,3, and overall NK cell function may be compromised in many patients.
- The combination of mAbs with NK-92 modified to express CD16-158V has superior antitumor effect compared to mAbs infusions alone, as confirmed in SCID mouse/human xenotransplant models.
- Adoptive cell therapy using the NK-92 cell line has been shown to be safe in phase I clinical trials.
- NK-92 cells require IL-2 to maintain optimal cytotoxic activity but infusion of IL-2 can cause significant adverse effects in patients.
- The addition of an intracellular retention sequence to IL-2 sequesters the cytokine inside the cell, yet maintains autocrine growth while restricting secretion.

Results

Figure 1

haNKTM (high affinity NK) cells: Stable high expression of CD16-158V on NK-92 cells using p(CD16).

Figure 2

haNKTM cells mediate efficient ADCC towards solid and liquid tumor in vitro

Figure 3

Irradiation and cryopreservation on NK-92 and haNKTM cells

Figure 4

haNKTM cells: an "off-the-shelf" cellular therapy product

References

Material & Methods

Cells and media: K562, DoHH2, SKOV-3 and MIM1.R cells were cultured in RPMI 1640 medium + 10% FBS. NK-92 cells were grown in X-VivoTM 10 medium + 5% Human Serum, supplemented or not with human recombinant IL-2 at 500 IU/mL. NK-92 cells were subjected to an irradiation dose of 1000 cGy. NK-92 cells were frozen to 80°C using a CoolCell® device (Biosen) and then transferred to a LN2 cryotank.

Electroporation: The pNeuK1 plasmid was transfected de novo and electroporated into NK-92 cells using a Neon® electroporator.

Cytotoxicity and ADCC assays: For cytototoxicity assay, cells were mixed in various effector-to-target (E:T) ratios and co-incubated for 4h at 37°C. For ADCC, target cells were preincubated with human monoclonal antibodies at various concentrations prior to the co-incubation with effectors at a fixed E:T ratio. The killing percentages were normalized to the corresponding negative controls (spontaneous death for cytototoxicity, killing in absence of mAb for ADCC).

Flow cytometry: CD16 density on the cells surface was determined by flow cytometry using a Qifikit® kit (DakoAgilent).

Conclusions
- ERL2 allows IL-2 independent growth of NK-92 through autocrine mechanism.
- Plasmid-based transduction allows for stable long-term expression of CD16.
- NK-92.CD16-ERIL2 (haNKTM) cells mediate potent ADCC, which is minimally affected by irradiation.
- NK-92 cells can be frozen with minimal loss of viability and no effect on cytotoxicity after a rest period of 24h.
- CD16 expression in plasmid-transfected cells is unchanged by freezing.
- ERL2 makes NK-92 easier and more cost effective to grow in large scale cultures, but avoids secretion of IL-2 in patients.
- A master cell bank is being established for use in a phase I/II trial with haNKTM cells in combination with a clinical grade mAb.

Disclosure Statement:
KSC: Scientific advisor for and recipient of research support from Conkwest, Inc.; holds a patent on CD16-bearing NK-92 cells

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