Background

- Natural killer (NK) cell antibody-dependent cell-mediated cytotoxicity (ADCC) is considered a major mechanism of action of IgG and IgG monoclonal antibodies (mAbs) used in cancer therapy.
- Efficacy of these mAbs is limited by the fact that only about 12% of the normal population is responsive for the expression of the high-affinity variant of Fc receptor for IgG (FcyRIIIa-158V+) that mediates ADCC.
- Further, NK cell function may be compromised in many patients.
- NK-2 (aNK) cells can be readily modified to express CARs to broaden the specificity of GMP-grade IL-2 independent NK cell lines expressing Chimeric Antigen Receptor (CAR) for clinical applications.

Methods

Cells and media:

- aNK cells from Cynomolgus macaque were used. aNK cells were cultured in RPMI 1640 medium supplemented with 10% FBS and human recombinant IL-2, 5-15 units/mL, and IL-15, 15 units/mL.

Electroporation:

- The protocol’s electroporator, according to Message Machine T7 transcription kit. Electroporations were performed using a Neon electroporator, according to the manufacturer’s instructions.

Cytophotometry and ADCC assays:

- For cytotoxicity assays, cells were seeded in various effector to target (E:T) ratios and incubated for 4 h in a 37°C, 5% CO2 incubator. For ADCC assays, target cells were incubated with aNK cells and monoclonal antibodies for 30 min before the addition of 5% Alamar Blue for 2 h.

Results

hANK cells grow independently of exogenous IL-2 and mediate potent ADCC in vitro

hANK cells maintain activity after irradiation

hANK cells CAR-modified to become f-hANK (targeted-high affinity natural killer cells)

Conclusions

- Plasmid-based transduction successfully produced IL-2 independent aNK cells with stable long-term expression of CD16 (hANK).
- Expression of transgenes did not modify the expression of aNK surface markers.
- aNK cells mediate potent ADCC in vivo in combination with the FDA-approved mAbs rituximab, trastuzumab, and daratumumab.
- hANK cells can be readily modified to express CARs to broaden the specificity of target recognition producing f-hANK.
- hANK and aNK cells similarily perform after irradiation, and hNK cells continue to mediate detectable ADCC for up to 48 h.
- hNK in combination with daratumumab significantly (P<0.0032) increases survival in a murine model of multiple myeloma.

References


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