Abstract #2302

A GMP-Grade IL-2 Independent NK Cell Line Expressing The High-affinity Fc-receptor (haNK) to Augment Antibody Therapeutics: Combination of haNK™ With Anti-CD38 Monoclonal Antibody (Daratumumab) In Multiple Myeloma

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Background

- Natural killer (NK) cell antibody-dependent cell-mediated cytotoxicity (ADCC) is considered a major mechanism of action of IgG1 and IgG3 monoclonal antibodies (mAb) used in cancer therapy[1]
- Efficacy of these mAbs is limited by the fact that only about 12% of the normal human population is homozygous for expression of the high-affinity variant of FcγRIIIA (CD16-158V) that provides maximum ADCC[2,3]. Further, overall NK cell function may be compromised in many patients
- NK-92[4] (aNK) can be modified to express the high affinity CD16(158V) receptor (high affinity aNK or haNK), and the combination of mAbs with haNK has superior antitumor effects compared to mAb infusions alone
- Infusion of expanded aNK cells have been shown to be safe in phase I clinical trials with evidence of complete responses and durable remission
- Daratumumab (humanized anti-CD38 mAb) has demonstrated safety and efficacy as monotherapy in a clinical trial for patients with refractory multiple myeloma,[5] and its mechanism of action involves NK-mediated ADCC
- Here we review the generation and characterization of haNK with preliminary data in a human xenotransplant myeloma NSG mouse model and the CD38 mAb daratumumab

Methods

Cells and media:

K562, DoHH2, SKOV-3 Granta-519, NCI-H929, and SR-91 cells were cultured in RPMI 1640 medium + 10% FBS. NK-92 (aNK) cells were grown in X-Vivo™ 10 medium + 5% human serum, supplemented or not with human recombinant IL-2 at 500 IU/mL. aNK and haNK cells were subjected to a radiation dose of 1000 cGy.

Electroporation:

The pNeukv1 plasmid (CD16-158V_ERIL2) was synthesized *de novo*. CD19CAR mRNA was synthesized in vitro using mMessage mMachine T7 transcription kit. Electroporations were performed either using a Neon™ or a MaxCyte GT electroporator, according to manufacturer's instructions.

Cytotoxicity and ADCC assays:

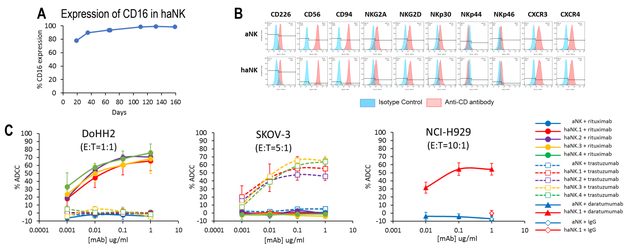
For cytotoxicity assays, cells were mixed in various effector-to-target (E:T) ratios and co-incubated for 4h at 37°C. For ADCC, target cells were pre-incubated with human monoclonal antibodies at various concentrations prior to the co-incubation with effectors at a fixed E:T ratio. The killing percentages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

In Vivo assays:

Eight-week-old NOD/SCID gamma null (NSG) mice were purchased from the Ontario Cancer Institute colony, and sublethally irradiated (200 cGy) before being inoculated with 10^7 CD38+ human multiple myeloma cell line NCI-H929 via tail vein. Six-week-old Nude mice were purchased from Charles River Laboratory and inoculated subcutaneously with 10^6 or 10^7 non-irradiated human cells. Mice were observed for tumor size, signs of illness and weight change until signs or symptoms of disease progression required humane sacrifice. Kaplan-Meier survival curves were generated, and log rank statistic determined to compare cohorts.

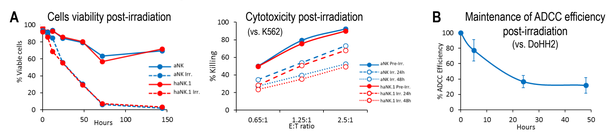
Results

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



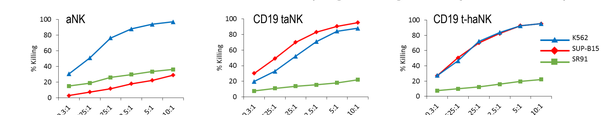
aNK cells were electroporated with a bicistronic plasmid vector (pNEUKv1 plasmid) expressing CD16-158V and intracellular retained IL-2. The resulting NK-92.CD16-ERIL2 cells (haNK.1, .2, and .3) grew in the absence of IL-2 in the culture medium, maintained a high expression of CD16(158V) over time (A), with no change in surface markers profile compared to the parental unmodified aNK cells (B). haNK cells mediated potent ADCC in combination with trastuzumab (anti-HER2/neu), rituximab (anti-CD20), or daratumumab (anti-CD38), against ovarian cancer (SKOV-3, Her2+), lymphoma (DoHH2, CD20+), and myeloma (NCI-H929, CD38+) cells lines in a flow-based assay (C).

haNK cells maintain activity after irradiation



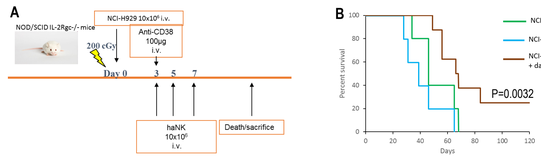
(A) Irradiation of haNK cells with a dose of 1000 cGy affects their viability and cytotoxic properties in the same manner as parental aNK cells. (B) Irradiated haNK cells have significant ADCC activity early after radiation (clinical scenario) and are still able to perform ADCC for up to 48 h.

haNK cells CAR-modified to become t-haNK (targeted-high affinity natural killer)



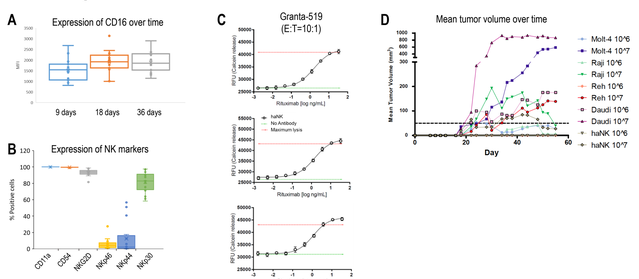
haNK and aNK cells were electroporated with mRNA coding for a first-generation CD19-CAR. (A) CAR-modified haNK (t-haNK) showed CAR-mediated killing of the CD19-positive, resistant SUP-B15 cell line as efficiently as CAR-modified aNK cells (taNK), and in a CD19-specific manner as shown by the absence of killing of the CD19-negative, NK-resistant SR-91 cell line.

haNK + daratumumab combination significantly improves survival in a multiple myeloma NSG mouse model



(A) Irradiated NSG mice were inoculated i.v. with $10x10^7$ CD38-positive multiple myeloma NCI-H929 cells (MM) on day 0. Anti-CD38 antibody (daratumumab) was delivered by i.v. injection on day 3 (100ug per injection). Irradiated haNK cells were delivered by i.v. injection on days 3, 5 and 7 (doses of $10x10^6$). (B) Survival curves for NCI-H929 myeloma mice treated with haNK and daratumumab. There was a significant increase in survival in mice treated with haNK cells in combination with daratumumab compared to those given haNK only (* P=0.0032).

haNK GMP-grade master cell bank for clinical trials



Single clones derived from haNK cells by limiting dilution in 96-well plates were established and characterized. (A) Expression of CD16(158V) in the established clones was stable for at least 36 days, and (B) all haNK clones displayed similar expression of a panel of characteristic aNK surface markers. All established haNK clones were tested for in vitro ADCC activity against B-cell lymphoma (CD20-positive Granta-519 cell line) in combination with rituximab. (C) ADCC cytotoxicity assay data for three representative haNK clones (Calcein assay). Subcutaneous injection of non-irradiated haNK cells into Nude mice did not cause any tumor formation, in contrast to other human leukemia/lymphoma cell lines (D).
Several haNK clones were selected for master cell banking based on phenotype and ADCC potency, and three Master Cell Banks have been prepared and cryopreserved under GMP conditions and are being validated to be released for use in clinical applications.

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
- haNK cells mediate potent ADCC in vitro 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 t-haNK
- haNK and aNK cells perform similarly after irradiation, and haNK cells continue to mediate detectable ADCC for up to 48 h
- haNK in combination with daratumumab significantly (P=0.0032) increases survival in a murine model of multiple myeloma
- Single haNK clones for clinical application have been generated by limiting dilution, and show excellent ADCC activity and no change in surface markers profile
- GMP-grade haNK Master Cell Banks have been established for use in phase I/II trials in combination with clinical grade mAbs, such as daratumumab

References

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