Natural Killer Cell Therapy of Cancer

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NK Cell Therapy of Cancer: Problems

- NK cells are difficult to obtain in large numbers
  - High effector : target ratios are likely to be required to achieve clinical effects
  - Ideal culture systems should not stimulate the expansion of T cells

- Some cancer subtypes are relatively resistant to NK cells
A Method to Specifically Expand NK Cells

- NK cells express 4-1BB
- 4-1BB ligation results in stimulatory signals

“K562-mb15-41BBL”

- IL-15 stimulates NK cells
- It is more powerful in its membrane-bound form

Imai et al. Blood 2005
NK Activation and Expansion System

Growth Potential of Human NK Cells

Pulses of K562-mb15-41BBL every 2-3 wks

senescence

Pulses of

Days of culture

Population doubling

Days of culture

Population doubling
Growth Potential of Human NK Cells

Pulses of K562-mb15-41BBL every 2-3 wks

Control  TERT

hTERT

β-actin

SKY-FISH  46, XY (Day 263)
NK Cell Expansion from:

- Peripheral blood from healthy donors
- Peripheral blood from patients with acute lymphoblastic leukemia in remission, multiple myeloma, and gastric cancer
- Cord blood
- Liver lymphocytes
Cytotoxic Capacity of Expanded NK Cells

- Hematologic malignancies
- Solid tumors
NK Cell Therapy of AML – Case Report

14-year old boy with AML secondary to osteosarcoma tx; relapse after MUD HSCT; no remission after salvage chemotherapy (30% blasts in BM)

NK cell therapy (Miller et al. Blood 2005)
- Fludarabine/cyclophosphamide lymphodepletion
- Apheresis from KIR-mismatched haplo donor
- T-cell depletion, IL-2 1000 IU/mL overnight
- $10^7$ NK/kg; 3 x $10^6$ IU/m² IL-2 3x/week for 2 weeks

BM MRD post-NK: day 14 = 0.3%; day 29 <0.01%; NK cells >50% in PB and BM

35 days post-NK infusion: T-cell depleted HSCT from NK donor (2Gy TBI)

6 mths post-HSCT: MRD-neg with complete donor chimera
Expanded NK Cells Are More Powerful Than Primary and IL-2-stimulated NK Cells

- U937
- HL60
- Primary AML

Expanded NK Cells Are More Powerful Than Primary and IL-2-stimulated NK Cells

Relative Sensitivity of Childhood Solid Tumors to NK Cell Cytotoxicity

EWS: Ewing sarcoma; RMS: Rhabdomyosarcoma; NB: Neuroblastoma; OS: Osteosarcoma

Cho et al. Clin Cancer Res 2010
Production of Clinical Grade Reagents

Large-scale cultures
Median NK cell expansion after 7 days of culture:
91.5 fold (range, 33-141; n = 12)
Eligibility: Relapsed/refractory AML, T-ALL, Ewing sarcoma and rhabdomyosarcoma

Procedure:
- Leukapheresis of haploidentical donor
- 7-day NK expansion with irradiated K562-mb15-41BBL cells
- T-cell depletion

Dose escalation: 1 x 10^6/kg, 1 x 10^7/kg and 5 x 10^7/kg; 3 patients in each group

Conditioning: Cyclophosphamide 60 mg/kg; Fludarabine 25 mg/m^2/day x 5; IL-2: 1 million IU/m^2 3x/wk x 2wks
Large-scale ex vivo expansion and characterization of natural killer cells for clinical applications

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CytOTHERAPY, 2012; 14: 1131–1143

(A) Day 0 Day 2 Day 4 Day 6 Day 8 Day 10
2x10⁶ NK feeding harvest
Bags

Day 0
2x10⁶ NK Day 10
no processing for up to 10 days
harvest

18x10⁹ cells in 40 197-mL bags

18x10⁹ cells in 20 G-Rex100s

(B) (C)
# NK cells (x10⁶) per vessel

Fold NK Expansion

Days

6 8 10
6 8 10

G-Rex Bags

*
Autologous NK Cell Infusion in Multiple Myeloma (NCT01313897)

Courtesy Dr. Frits van Rhee
NK Cytotoxicity Against Gastric Cancer Cell Lines
(K. Mimura, K. Kono)

Resting NK

IL-2 NK

Expanded NK

E:T 4:1
N = 6
Acute Lymphoblastic Leukemia
Relative Sensitivity to NK Cell Cytotoxicity

E:T 4:1

E:T 1:1

% cell killing

AML B-lin. ALL T-ALL

0 20 40 60 80 100

% cell killing

0 20 40 60 80 100

AML B-lin. ALL T-ALL
Redirecting the Specificity of NK Cells

Single chain variable domain - scFv
(binds to molecule on the surface of target cells)

Imai et al. Leukemia 2004; Blood 2005
NK Cell Transduction with Anti-CD19 Chimeric Antigen Receptors

- High expression (median transduction efficiency, ~70%)

- Significantly higher IFN$\gamma$ and GM-CSF production upon contact with CD19+ cells

- Marked increase in specific cytotoxicity against CD19+ cells in vitro and in vivo

Imai et al. Blood 2005
NKCD19 (NCT00995137)

- **Eligibility:** Relapsed/refractory B-lineage ALL

- **Procedure:**
  - Leukapheresis from haploidentical donor
  - 7-day NK expansion with irradiated K562-mb15-41BBL cells
  - T-cell depletion
  - Transduction with anti-CD19-BB-ζ

- **Dose escalation:** 1 x 10^6/kg, 1 x 10^7/kg and 5 x 10^7/kg; 3 patients in each group

- **Conditioning:** Cyclophosphamide 60 mg/kg; Fludarabine 25 mg/m^2/day x 5; IL-2: 1 million units/m^2 3x/wk x 2wks
Recent Technical Developments

- Cell engineering by electroporation
- Enhanced cell killing
Expression of Anti-CD19-BB-ζ mRNA in Immune Cells by Electroporation

- primary NK cells: 45%
- activated T cells: 74%
- expanded NK cells: 69%
- CIK cells: 72%

Shimasaki et al. Cytotherapy, 2012
Transient Expression of Anti-CD19-BB-ζ mRNA after Electroporation

Shimasaki et al. Cytotherapy 2012
NK Cells Electroporated with Anti-CD19-BB-ζ mRNA in a Mouse Model of ALL

<table>
<thead>
<tr>
<th>Day</th>
<th>2</th>
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Shimasaki et al. Cytotherapy, 2012
NK Cell Receptors and Their Ligands

NKG2D-DAP10 Signaling

Lanier, Nature Immunol 2008
Genetic Modification of NK Cells with NKG2D

Mock

NKG2D

DAP10

CD3ζ

IRES

FLAG

Ampicillin resist.

5'-LTR

3'-LTR

pMSCV-CD3ζ-NKG2D-IRES-DAP10ζ

NKG2D expression (MFI)

P<0.0001

Mock

NKG2D-DAP10-CD3ζ

DAP10

NKG2D

Mock

NKG2D-DAP10-CD3ζ

kD

82

38

30

15

reducing

non-reducing

NKG2D-CD3ζ

CD3ζ

CD3ζ
NKG2D-DAP10-CD3ζ Improves NK Cytotoxicity

Chang et al. Cancer Res. 2013
Specificity of NKG2D-DAP10-CD3ζ Signaling

Suppression of cytotoxicity with an anti-NKG2D inhibitory Ab

CD107a assay after stimulation with anti-NKG2D agonistic Ab

Cytokine production after stimulation with anti-NKG2D agonistic Ab
NKG2D-DAP10-CD3ζ Improves Cytotoxicity Against Osteosarcoma
NKG2D-DAP10-CD3ζ Expression by Electroporation
Donor

Cell expansion

NK

Patient

AML, T-ALL
Ewing, rhabdo
Myeloma

Cell expansion

and genetic modification

B-lineage ALL/B-NHL

Osteo, prostate
Neuroblastoma

Patient

Neuroblastoma
Breast, GI

Patient

Autologous

Cell expansion

plus antibody

AML, T-ALL
Ewing, rhabdo
Myeloma

NK

Patient

B-lineage ALL/B-NHL

Osteo, prostate
Neuroblastoma
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Ko Kudo
Paolo Lorenzini
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