Innovations with NK Cells

Rizwan Romee, MD
Dana Farber Cancer Institute, Harvard Medical School
Disclosures

InnDura Therapeutics (co-founder & SAB)
Parker Institute for Cancer Immunotherapy (member/funding)
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xNK Therapeutics (SAB)
Skyline Therapeutics (sponsored research)
CRISPR Therapeutics (sponsored research)
NK Cells and their Key Functions

- Kill virus-infected cells
- Key contribution to human reproduction (dominant lymphocyte in pregnant decidua)
- Removal of the senescent cells

- Exhibit anti-tumor responses
  - Kill cancer target cells without prior sensitization
  - Key mediators of ADCC via Fc receptor (CD16a)

5-15% of PB lymphs
Regulation of NK Cell Responses

Killer immunoglobulin-like receptors (KIRs)

Romagnane et al, 2013

Demaria et al, Eur J Immunol 2021
KIR Ligand Mismatch and Decreased Risk of Relapse

Effectiveness of Donor Natural Killer Cell Alloreactivity in Mismatched Hematopoietic Transplants
Loredana Ruggeri et al.
Science 295, 2097 (2002);
DOI: 10.1126/science.1068440

<table>
<thead>
<tr>
<th>KIR ligand incompatibility in GVH direction</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of transplants</td>
<td>58</td>
<td>34</td>
</tr>
<tr>
<td>Donors displaying antirecipient NK clones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>AML</td>
<td>37</td>
<td>20</td>
</tr>
<tr>
<td>Transplantation outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rejection</td>
<td>15.5%</td>
<td>0%*</td>
</tr>
<tr>
<td>Acute GVHD, ≥ grade II</td>
<td>13.7%</td>
<td>0%*</td>
</tr>
<tr>
<td>Probability of relapse at 5 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>90%</td>
<td>85%</td>
</tr>
<tr>
<td>AML</td>
<td>75%</td>
<td>0%**</td>
</tr>
</tbody>
</table>

\(p \leq 0.01; \quad **p < 0.0008 \text{ (22).} \)
Donor KIR Haplotype and Relapse after Matched Stem Cell Transplantation

Cooley et al, Blood 2009 and 2010

Venstrom et al, NEJM 2012

Schetelig et al, Blood 2020
NK Cell Engagers (NKCEs) in Clinical Trials

Demaria et al, Eur J Immunol 2021

TriKE

<table>
<thead>
<tr>
<th>NK cell</th>
<th>Tumor cell</th>
<th>Killing</th>
<th>Cytokine production</th>
<th>Proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADCC</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Trispecific ANKET</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tetraspecific ANKET</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td></td>
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</tbody>
</table>

Innate Pharma/Vivier Group
Why Adoptive NK Cell Therapy?

Advantages

- ‘Ready to Kill
- No severe CRS or neurotoxicity
- No GvHD risk, no need to TCR KO
- Propensity to target class 1- cells

Major Challenges

- Short life spans of NK cells
- Limited in vivo persistence
- Low NK cells numbers

Miller et al, Blood 2005
Rubnitz et al, JCO 2010
Curti et al, Blood 2011
Bachanova et al, Blood 2014
Nahi et al, Cell Rep Med 2022
NK Cell CARs Demonstrate Safety and Promising Activity

Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors

8/11 (73%) ORR, 7/11 CR

No CRS, No neurotoxicity, No GvHD

Liu et al, NEJM 2020

Trogocytosis as a novel mechanism of relapse

Li et al, Nat Med 2022
Cytokine Activation Induces Human *Memory-Like* NK Cells

**Memory-Like NK Cell Properties:**
- Enhanced anti-tumor responses
- Prolonged survival in vivo
- Enhanced proliferation
- Enhanced ADCC
- May inhibit GVHD
- High CD25
- Low TGFβ receptor

Cooper et al, PNAS 2009
Romee et al, Blood 2012
Romee et al, Science TM 2016
Berrien-Elliot et al, Cancer Discov 2020
Terren I et al, Sci Rep, 2021
Berrien-Elliott et al, Science TM, 2022
Bednarski et al, Blood 2022
Shapiro, et, al, JCI, 2022
Phase 1 Study of CIML NK Cells in Rel / Ref AML

Day -1: Donor leukapheresis

NK cell purification

CD3-/CD56+

IL-12/15/18

12-16h

AML patient

Flu/Cy

Day 0

IL-2 1mIU/m² qOD x 2 weeks

Cell dose levels:
1. 0.5 x 10⁶/kg
2. 1.0 x 10⁶/kg
3. Max capped at 10x10⁶/kg

Endpoints:
Primary: Safety / MTD
Secondary: Leukemia Response In vivo biology
Safe and with Very Encouraging Response Rates

19 patients treated
No CRS, No GVHD
~50 CR rates

Romee et al, Science TM 2016
Dana Farber NK Cell Therapeutic Initiative

In Clinic

DFCI NK Cell Trials

1. Relapsed AML/MDS (*Enrolling*)
2. Head & Neck Ca (*Enrolling*)
3. MRD+ Myeloma (*Enrolling*)
4. Ovarian Cancer (*IND Approved*)
5. Kidney Cancer (*Regulatory*)
6. Pre-emptive Post HCT (*Regulatory*)
7. CD123 NK CAR (*Regulatory*)
8. NPM1c NK CAR (*Planning*)

In Lab

NK Cell Gene Manipulation Efforts

- CAR and TCR-mimetic CAR arming
- CRISPR Gene editing
- Non-CAR engineering
Can Memory-like NK Cells Target Relapse after Stem Cell Transplantation?

PI: Dr. Roman Shapiro
NCT04024761

Safe, 4/6 CR/CRi

Shapiro & Birch et al, J Clin Invest 2022
Mem-like NK Cells Traffic to the Tumor Site

Shapiro & Birch et al, J Clin Invest 2022
Mem-like NK Cells Traffic & ‘Evolve’ in vivo

Shapiro & Birch et al, J Clin Invest 2022
Allogeneic Memory-like NK Cells Plus CTLA-4 Blockade in Head & Neck Cancer

Anti-CTLA-4 antibody mediated Treg depletion to facilitate NK cell activity
NK cell infiltration associated with favorable outcomes in H&N ca patients

PI: Dr. Glenn Hanna
NCT04290546

Romee et al, Blood 2018

Recurrent, metastatic head and neck cancers
- Prior platinum exposure (optional)
- Prior PD-1/L1 exposure (optional)
- Any smoking history
- Any HPV status or tumor site
- ECOG PS 0-2

Lymphodepleting chemotherapy
- Fluorouracil + Cyclophosphamide

Ipilimumab*

N-803 IL-15sa SC (q21d)

12-16 hours

wash

CIML NK cell infusion

-7 -6 -5 -4 -3 -2 -1 0 1 22 43 64...

outpatient ambulatory care setting

* PART 1: without lead-in, PART 2: with CTLA-4I lead-in
Allogeneic Memory-like NK Cells Plus CTLA-4 Blockade in Head & Neck Cancer

Patients screened: 13
- Ineligible: 2
- Expired: 1

Started Flu-Cy conditioning: 11

Received CIML NK cells: 10
- Evaluable: 10

Gr 1-2 CRS in 6 patients

Median PFS of 3.43 months
Disease Response and *in vivo* NK Cell Expansion

**Responder**

**Progressive disease**

Prelim results presented at AACR 2022
CTLA-4 Blockade Impacts NK Cell Phenotype?

Skewing towards CD16^+CD57^+ NK cells
Can we Arm Memory-Like NK Cells?
BaLV allows High Transduction Efficiency of Mem-like NK Cells

Modified from Dong et al, PNAS 2022
Generation of CAR-NK Cells Specific for AIQ-HLA-A2 Complex

CAR targeting a neoepitope derived from NPM1c protein

Xie et al, Nature Biomed Eng 2021
Dong et al, PNAS 2022
Gene Expression and Specificity of NPM1c ML-CAR

Dong et al, PNAS 2022
Are CAR NK & CAR T Cells Similar in Efficacy?

Dong et al, PNAS 2022
CIML NK CAR Targeting Membrane Proximal Domain

Region I (MUC16, MORAb-009, SS1P, HN1)
Region II (YP187, YP223)
Region III (YP218)
Conformation (YP3)

Day 5 OV8

Day 5 SKOV3

Cell viability (% control)

E:T ratio

Cell viability (% control)

E:T ratio

IFN $\gamma$ + cells (%)

OV8
SKOV3
OVCAR3

✱✱✱✱ ns ❄️

Copy of D53 OV8 day 5

Copy of SKOV3 Day 5

SS1PYP218

0 10 20 30 40 50

IFN $\gamma$ + cells (%)

OV8
SKOV3
OVCAR3

✱✱✱✱ ns ❄️
MSLN CIML NK CARs Demonstrate activity against PDOTs & TTFs

Meso+ PDOTs

Tumor tissue

NK cells

IFNγ pg/mL

0 10 20 30 40

Control CIML MSLN-CAR CIML

1x10^11

5 x 10^10

Days
Do NK Cell CARs Traffic Out of Peritoneum?

NK Cell Trafficking

Tumor Spread
Phase 1 Trial of Autologous CIML NK Cells with PD1 Blockade & IL-15

Pre-activation, NK cell enrichment and culture

Autologous NK cell apheresis

Intraperitoneal CIML NK cell infusion

Lymphodepleting chemo
(Intravenous Fludarabine + Cyclophosphamide)

NIZ985 subcutaneously weekly
3wks on/1 wk off x 16 weeks

Disease progression

Days -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 21-day cycles

Tislelizumab, 200 mg intravenously every 3 weeks, until progression

Eligibility: recurrent (3+ lines) high grade ovarian cancer
• Cohort 1: without tislelizumab
• Cohort 2: with tislelizumab

IND Approved
Summary

- NK cells play an important role in mediating graft versus tumor effect after stem cell transplantation.

- Lack of severe CRS, neurotoxicity, GvHD, and intrinsic tumor targeting mechanisms are some of the major advantages of NK cells.

- Memory-like NK cells with enhanced proliferation, expansion and in vivo persistence make them an attractive platform for NK cell-based therapies.

- Novel CAR and non-CAR gene editing approaches are leading the ‘revolution’ in the NK cell immunotherapy field.
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CMCF Staff

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