NK Cells

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NATURAL KILLER CELLS IN THE MOUSE

ADADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen
vid Karolinska institutet officiellen
förvaras i Fysiologiska institutionens
föreläsningssal, Karolinska institutet,
tisdagen den 9 mars 1976 kl. 9

Av
ROLF KESSLING
Läk. ex.

STOCKHOLM 1976
Natural Killer Cells

- Activating receptor
- Inhibitory receptor
What really goes on
## NK cell abnormalities

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased cytotoxic activity of NK cells</td>
<td>NSC lung Ca HCC CRC H&amp;N Ca Breast Ca Squamous cell Ca Bronchogenic Ca Cervical Ca Ovarian Ca AML ALL B-CLL CML MM</td>
</tr>
<tr>
<td>Defective expression of activating receptors</td>
<td>HCC M. melanoma AML MM</td>
</tr>
<tr>
<td>Defective NK cell proliferation</td>
<td>Renal Ca Neuroblastoma Nasopharyngeal Ca CML</td>
</tr>
<tr>
<td>Increased number of CD56^{bright} NK cells</td>
<td>H&amp;N Ca Breast Ca</td>
</tr>
<tr>
<td>Defective expression of signalling molecules</td>
<td>Cervical Ca CRC Ovarian Ca Prostate Ca AML CML</td>
</tr>
<tr>
<td>Decreased NK cell counts</td>
<td>Nasopharyngeal Ca Neuroblastoma CML ALL ( Pediatric)</td>
</tr>
<tr>
<td>Defective cytokine production</td>
<td>AML ALL CML</td>
</tr>
</tbody>
</table>
NK cell therapy overview

Sutlu & Alici, JIM 2009
The expansion process

Day 0
CellGro SCGM
5% Human AB serum
IL-2 (500 U/ml)
OKT3 (10 ng/ml)

Every 2-3 days
Additional medium with IL-2, without OKT3

Detailed phenotypic characterization of NK cells
Degranulation and cytotoxicity assays

Day 0
Day 5
Day 10
Day 15
Day 20

CD3
CD56

13.6 15.6 14.7 39.6 72.4
1.48 2.91 14.7 2.91 6.58
18.2 17.4 2.36 2.36 1.37
66.7 64.1 43.3 43.3 19.6
1.48 2.91 14.7 2.91 6.58

cGMP certified expansion process
# A phase-I clinical trial with expanded NK cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Sex</th>
<th>Age (years)</th>
<th>PBL chimerism</th>
<th>Immuno-suppression</th>
<th>Location of metastases(^1)</th>
<th>Time since last DLI (months)</th>
<th>GvHD</th>
<th>Additional tumor debulking (months)</th>
<th>KIR allo-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>CRC</td>
<td>M</td>
<td>67</td>
<td>DC</td>
<td>No</td>
<td>1, 2, 3, 4</td>
<td>7</td>
<td>No</td>
<td>RFA liver met/(3,5)</td>
<td>No</td>
</tr>
<tr>
<td>P2</td>
<td>HCC</td>
<td>M</td>
<td>48</td>
<td>DC</td>
<td>Yes*</td>
<td>3, 4</td>
<td>2</td>
<td>No</td>
<td>RFA liver met/(16) IRD lung met/(16)</td>
<td>No</td>
</tr>
<tr>
<td>P3</td>
<td>RCC</td>
<td>M</td>
<td>50</td>
<td>DC</td>
<td>No</td>
<td>1, 2, 3, 4, 13</td>
<td>13</td>
<td>Limited cGvHD(^2)</td>
<td>No</td>
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<tr>
<td>P4</td>
<td>CLL</td>
<td>F</td>
<td>59</td>
<td>100% recipient CD19(^+) cells</td>
<td>Yes(^3)</td>
<td>5</td>
<td>11(^1)</td>
<td>No</td>
<td>surgery of lung met/(2)</td>
<td>Yes</td>
</tr>
<tr>
<td>P5</td>
<td>RCC</td>
<td>M</td>
<td>54</td>
<td>DC</td>
<td>No</td>
<td>1, 3</td>
<td>26</td>
<td>Limited cGvHD(^4)</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

*Tacrolimus 0.006–0.1 mg/kg BW; Prednisolon 0.8 mg/kg BW; \(^1\) LN = lymph node; \(^2\) liver; \(^3\) lung; \(^4\) pleura; \(^5\) LN abdomen; \(^6\) After booster stem cell infusion; \(^7\) No active chronic GvHD 11 months prior to the first NK/NK-like T-cell infusion.

All donors were siblings. KIR alloreactivity was determined by donor KIR ligand missing in the recipient.

BW: Body weight; CLL: Chronic lymphocytic leukemia; CRC: Colorectal carcinoma; DC: Total donor chimera of CD3\(^+\), CD19\(^+\), CD33\(^+\) cells; DLI: Donor lymphocyte infusion; F: Female; GvHD: Graft-versus-host disease; HCC: Hepatocellular carcinoma; IRD: Stereotactic irradiation; KIR: Killer immunoglobulin-like receptor; M: Male; met: Metastases; PBL: Peripheral blood lymphocytes; RCC: Renal cell carcinoma; RFA: Radiofrequency ablation.
Patient follow-up

<table>
<thead>
<tr>
<th>Patient</th>
<th>3 months</th>
<th>6 months</th>
<th>Inf1</th>
<th>Inf2</th>
<th>Inf3</th>
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</thead>
<tbody>
<tr>
<td>P1</td>
<td>P (lung, liver)</td>
<td>SD (lung, liver)</td>
<td>DLI1</td>
<td>DLI2</td>
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</tr>
<tr>
<td>P2</td>
<td>SD (lung, liver)</td>
<td>SD (lung, liver)</td>
<td></td>
<td></td>
<td>INF3</td>
</tr>
<tr>
<td>P3</td>
<td>P (pleura, lung)</td>
<td>SD (pleura, lung)</td>
<td></td>
<td></td>
<td>INF3</td>
</tr>
<tr>
<td>P4</td>
<td>P (LN meta)</td>
<td>SD (LN meta)</td>
<td></td>
<td></td>
<td>INF3</td>
</tr>
<tr>
<td>P5</td>
<td>P (pleura, liver)</td>
<td>SD (pleura, liver)</td>
<td></td>
<td></td>
<td>INF3</td>
</tr>
</tbody>
</table>

*RECIPT: Response Evaluation Criteria in Solid Tumors
*Fatal outcome as a result of disease progression
LN: Lymph node, met: Metastases

α-FP levels (ng/ml)

Time (months after SCT)
The lifetime risk of getting MM is 1 in 159 (0.63%).

In 2015:
- 20,000 new cases
  - 11,000 men and 9,000 women
- 10,650 deaths
- 5-year survival rate: 35%
- 10-year survival rate: <2%
Various immune dysfunctions are observed in MM patients.

Tumor-induced immune dysfunctions regarding NK cells in MM:
- Increased level of soluble IL-2 receptors
- High levels of M-component
- Defective expression of activating receptors
- Impaired NK cell cytotoxicity and abnormal NK cell counts

Adoptive transfer of IL-2 activated NK cells prolongs survival in animal models of MM.
Expanded cells have cytotoxicity against autologous MM cells

Against autologous MM cells

Against autologous non-MM cells

% Lysis

0 20 40 60 80
E:T

0.3:1 1:1 3:1 10:1

Day 0  Day 5  Day 20
Phenotypic changes

Adhesion / Activation

Inhibition

Chemokine receptors

N=7
Different strategies for scaling up
NK cells from bioreactor expansions degranulate more efficiently.
Phenotypic differences

- % Degranulation
  - MFI
  - $r^2$: 0.1160 $p$: 0.409
  - $r^2$: 0.2292 $p$: 0.230
  - $r^2$: 0.6046 $p$: 0.012

Adhesion / Activation

- Inhibition
- Chemokine / cytokine receptors

N=4
- First-in-man, Phase I/II
- Open, single arm study
- Primary objective:
  - Safety and tolerability
- Secondary objective:
  - Effect on serum Ig levels
- Inclusion:
  - 20 MM patients eligible for ASCT
  - 3 escalating infusions/patient (Weekly)
    - $10^6$, $5 \times 10^7$ and $10^8$ cells/kg
- Evaluation:
  - 4 weeks after infusion,
  - 6 months follow up.
Daratumumab treatment
Improving lentiviral and retroviral gene delivery to NK cells
How could we help the viral gene delivery process?
Time dynamics of lentiviral transduction on NK cells

N=4

% GFP+ NK cells

With BX795
Without BX795

Day of transduction

0 1 2 3 4

n.s. ** *** ***
Transduction process does not effect NK cell function

With BX795

FSC
SSC

52.1
42.4

eGFP

CD56
CD107a

79.8
78.4

Without BX795

FSC
SSC

86.8
10.5

eGFP

CD56
CD107a

81.4
80.8
Intracellular anti-viral defense mechanisms can limit viral gene delivery in NK cells and this can be reduced by using small molecule inhibitors.
A more targeted molecule: VyOxo
Enhancing lentiviral gene delivery to NK cells: Vy-Oxo
NK-Tumor interactions

Genetic modification of NK cells from patients with hematological malignancies

- Immunotherapy with genetically modified NK cells
- Increase detection of patient-specific target ligands
LeGo-Vectors

- Self- inactivating-vector of the 3rd generation
- replication incompetent
- can infect a broad group of cell types
- Backbone, packaging, envelope and regulation plasmid
- Pseudotyped with VSV-G
- HEK293FT as producer cell line
Genetically modified NK cells

<table>
<thead>
<tr>
<th>Genes</th>
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</tr>
</thead>
<tbody>
<tr>
<td>CD94</td>
<td>2B4</td>
<td>NKp46</td>
<td>NKG2E</td>
<td>KIR2DL4</td>
</tr>
<tr>
<td>CD16a</td>
<td>2B4T</td>
<td>NKp44</td>
<td>NTBA</td>
<td>KIR2DL5</td>
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<tr>
<td>CD160</td>
<td>CD27</td>
<td>NKp30</td>
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<td>KIR3DL1</td>
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<tr>
<td>CD161</td>
<td>CRACC</td>
<td>NKG2A</td>
<td>KIR2DL1</td>
<td>KIR3DL2</td>
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<tr>
<td>CD2</td>
<td>Siglec7</td>
<td>NKG2C</td>
<td>KIR2DL2</td>
<td>KIR3DL3</td>
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<tr>
<td>DNAM1</td>
<td>Siglec9</td>
<td>NKG2D</td>
<td>KIR2DL3</td>
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</tbody>
</table>
Protocol overview

Transduction with different vectors

Activating receptors
- CD16
- Nkp44
- DNAM1
- CD160
- CD27
- Nkp30
- CD161
- CRACC
- NKG2A
- CD2
- NKG2C
- Nkp46
- 2B4
- Nkp80
- NKG2D

Inhibitory receptors
- KIR2DL1
- KIR2DL4
- KIR3DL2
- KIR2DL2
- KIR2DL5
- KIR3DL3
- KIR2DL3
- KIR3DL1
- Siglec7
- KIR2DL1
- KIR2DL4
- Siglec9
- KIR2DL3
- KIR3DL1
- Siglec9
- NKG2A

Degranulation assays
- CD16
- NKp44
- DNAM1
- CD160
- CD27
- Nkp30
- CD161
- CRACC
- NKG2A
- CD2
- NKG2C
- Nkp46
- 2B4
- Nkp80
- NKG2D

Viral vector
- Gene of interest
- IRES
- GFP

Unmodified NK cell

Transduced NK cell

Phenotyping
- PBMCs
- magnetic separation

25 November 2016
Goal: Patient-specific data

Transduction with different vectors

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
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<td>19</td>
<td>20</td>
<td>21</td>
<td>22</td>
<td>23</td>
<td>24</td>
</tr>
</tbody>
</table>

Degranulation assays

- No target
- Autologous PBMCs
- Autologous tumor

% Degranulation

![Chart showing % Degranulation for different samples labeled 1 to 24.](image-url)
A new discovery: VyX
Vy-X effects *in vitro*

**Perforin**
- Medium
- VyX
- Control A
- Control B
- IL-2 + VyX
- IL-2 + Control A
- IL-2+ Control B

**Granzyme B**
- Medium
- NKx
- Control
- Control B
- IL-2 + NKx
- IL-2 + Control A
- IL-2+ Control B

*Note:* The graphs show the mean fluorescence intensity (MFI) for Perforin and Granzyme B across different conditions. The asterisks indicate statistical significance: 
- ** represents p < 0.05
- *** represents p < 0.01
- **** represents p < 0.0001
16h Cytotoxicity assay

![Graph showing cytotoxicity percentages for different E:T ratios and conditions.]

- IL-2+VYX
- IL-2+Control A
- IL-2+Control B

% Cytotoxicity vs E:T Ratio
Direct VyX in vivo delivery

![Graph showing percent survival over days for different conditions]

- **5T33**
- **5T33VyX**
- **PBS**
Identification of genetic predispositions to treatment resistance to identify optimum personalized therapy for MM patient.
Acknowledgments