**Ex-Vivo** heat shock protein 70-peptide-activated, autologous natural killer cells adoptive therapy: from the bench to the clinic

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Valeria Milani, MD, PhD
Munich
Agenda

1. NK ligands
2. HSP70-NK interaction
3. HSP70-activated NK cells adoptive transfer
4. Hyperthermia and NK cells transfer
1. NK ligands
NK ligands

**Missing self hypothesis**

NK ligands
NK ligands

NK cell receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
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<tr>
<td>KIR2DL1</td>
<td>HLA-C^w01</td>
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<td>KIR2DL2</td>
<td>HLA-C^w04</td>
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<td>HLA-C^w06</td>
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<td>HLA-B</td>
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<td>HLA-A</td>
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<td>NK92A</td>
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<td>NK92C</td>
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<td>NK92E</td>
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Nature Reviews Immunology
NK ligands
NK ligands

C-type lectin receptors

NK ligands
NK ligands

The NK cell response depends on the net effect of activating and inhibitory receptors with additional involvement of adaptor molecules.
2. HSP70-NK interaction
Hsp70-NK interaction

Tumor-specific HSP70 membrane expression

Tissue bank

- Solid tumors
  - Respiratory
  - Gastrointestinal
  - Urogenital
  - Head and neck
  - Sarcoma
  - Melanoma
- Hematological tumors
- Metastases

Hsp70 on tumor cells

Methods

- FACS
- Surface biotinylation
- Proteomic profiling

M. Ferrarini et al. (1992); Tamura et al. (1993); Chouchane et al. (1994); Muhlhoff et al. (1995), Altmeyer et al. (1996); Hantschel et al. (2000); Shin et al. JBC (2003)
Hsp70-NK interaction

Diagram showing Hsp70 localization in tumor and normal tissues, with higher expression in tumor cells (Cx+).
Hsp70-NK interaction

Receptor mediated HSP70-NK activation

NK cell proliferation $\uparrow$
Cytolytic activity $\uparrow$
INF-$\gamma$ production $\uparrow$

Hsp70-NK interaction
Hsp70-NK interaction

Mechanism of kill: Granzyme B (mi-APO) initiates apoptosis in Hsp70 positive tumors

Hsp70-NK interaction
Hsp70-peptide TKD (aa 450-463) (ENKASTIM)

Epitope of the therapeutic Hsp70 specific antibody (mi-TUMEX)

<table>
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<tr>
<th>Name</th>
<th>aa</th>
<th>Sequence</th>
<th>NK Cell Stimulation</th>
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<tr>
<td>NLL</td>
<td>454-461</td>
<td>N L L G R F E L</td>
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<tr>
<td>GIPP</td>
<td>454-466</td>
<td>N L L G R F E L S G I P P</td>
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<tr>
<td>TKD</td>
<td>450-463</td>
<td>T K D N N L L G R F E L S G</td>
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</table>

Hsp70-NK interaction

Hsp70 interaction with NK cells can lead to lysis or apoptosis depending on the presence of CD94 and the membrane bound Hsp70. Hsp70 can also be found in the cytoplasm of the cell.

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**Table:**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>NK Cell</th>
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<tr>
<td>NCL</td>
<td>454-461</td>
<td>0.15</td>
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<tr>
<td>GRP</td>
<td>454-461</td>
<td>0.15</td>
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<tr>
<td>TKO</td>
<td>486-483</td>
<td>0.00</td>
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</table>

**Note:**

- ATPase domain (conserved)
- Protease-binding domain (variable)
Hsp70-NK interaction

- Hsp70 membrane/cytoplasm
- Normal/Cx-
- Tumor/Cx+

From the bench to the bedside
3. Clinical trials
Phase I clinical trial

Leukapheresis → Reinfusion

NK phenotyping

1-10 x 10^8 NK cells

Washing steps

Hsp70 stimulation

Laboratory parameters

CD94 Density
Granzyme B ELISPOT

Phase I clinical trial

Treatment of colon and lung cancer patients with ex vivo HSP70-peptide-activated NK cells

Patients:
metastasised, therapy refractory colorectal cancer (n = 11) and non-small cell lung cancer (n = 1)

1) Feasibility and safety of Hsp70-reactive NK cells
   Excellent safety profile even at maximum dose

2) Escalation: cell dose, infusion cycles
   max 1-10 x 10⁸, 6 cycles every 4 weeks

3) In vitro efficacy
   In vitro efficacy in 10 of 12 patients (CD94, cytolytic activity)

4) Clinical outcome
   1 SD and 1 mixed response in 5 pts with > 4 cycles

Phase I clinical trial

Increased tumor kill by patient-derived, TKD-activated NK cells

TKD + IL2

IL2 alone

## Clinical studies on NK cell adoptive immunotherapy

<table>
<thead>
<tr>
<th>Setting</th>
<th>Source</th>
<th>Results</th>
<th>N</th>
<th>Tumor entity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous</td>
<td>Conclusion: feasible, increases NK-cell function <em>in vivo</em>, no improvement in outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Keilholz et al. <em>EJC</em> 1994</td>
<td>HD-IL2 +/- LAK iv or ia in liver MTS (objective responses 20%)</td>
<td>9</td>
<td>melanoma</td>
</tr>
<tr>
<td></td>
<td>Law T et al. Cancer 1995</td>
<td>cl IL-2 +/- LAK (objective responses 6%)</td>
<td>71*</td>
<td>renal cell carcinoma</td>
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<tr>
<td></td>
<td>Lister et al. CCR 1995</td>
<td>IL2 + NK after HD-PBSC (increase NK activity)</td>
<td>12</td>
<td>refractory lymphomas</td>
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<tr>
<td></td>
<td>Kruit et al. J Immunoth 1997</td>
<td>cl IL-2 +/- IFN + LAK (objective responses 24-37%)</td>
<td>72</td>
<td>renal cell carcinoma</td>
</tr>
<tr>
<td></td>
<td>Kimura et al. Cancer 1997</td>
<td>IL-2 + LAK adjuvant after chemo-radiotherapy (survival benefit?)</td>
<td>174*</td>
<td>lung carcinoma</td>
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<tr>
<td></td>
<td>Rosenberg et al, JEM 1997</td>
<td>HD-IL2 + LAK (objective responses 20%)</td>
<td>157</td>
<td>metastatic cancer</td>
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<tr>
<td></td>
<td>Burns et al. BMT 2003</td>
<td>IL-2 activated NK +/- IL2 (increase NK lysis + cytokines)</td>
<td>57</td>
<td>refractory lymphomas</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>Conclusion: feasible, eradication of leukemia, protection against GVHD</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Ruggeri et al. Science 2002</td>
<td>Haploidentical NK (KIR mismatch) in Allo-BMT setting</td>
<td>na</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>Miller et al. Blood 2005</td>
<td>Transfer and expansion of haplo-NK in non-BMT setting</td>
<td>43</td>
<td>metastatic cancer</td>
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</tbody>
</table>
4. Hyperthermia and NK transfer
Clinical hyperthermia

Clonogenic survival

No tumor destruction
Phenotypic changes

Indirect tumor destruction
Architectural/vasculature changes

Tumor destruction
Apoptosis, necrosis

37°C 38°C 39°C 40°C 41°C 42°C 43°C 44°C 45°C

<table>
<thead>
<tr>
<th>Immune response</th>
<th>Molecular response</th>
<th>Clinical setting</th>
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</thead>
<tbody>
<tr>
<td>Increased susceptbility to NK</td>
<td>Cell cycle; DNA repair</td>
<td>Thermosensitisation of chemotherapy and radiation</td>
</tr>
<tr>
<td>Increased lymphocytes</td>
<td>Apoptosis</td>
<td>Whole-body Hyperthermia</td>
</tr>
<tr>
<td>Release of HSPs and</td>
<td>Induction of intracellular heat shock proteins</td>
<td>Regional hyperthermia</td>
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<tr>
<td>Maturation/Activation od DC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>migration/infiltration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSP-PC</td>
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</table>

**Hyperthermia: vaccination *in situ*?**

**Tumor Necrosis and Release of HSP70 Activate the Immune System**

- **extracellular HSP**
- **Heat Shock**
- **Necrosis**
- **NK cells**
- **Cross-Presentation**
- **Maturation**
- **HSP70-Peptide Complexes**
  - CD91; TLR; CD40 etc.
  - APC
  - T Cells
  - Stimulation
  - Proliferation

**Antitumor Response**

Can we specifically activate NK cells against tumor?
Can we specifically activate NK cells against tumor?

- Heat upregulates **surface HSP70**
- Heat provides the **optimal immunological milieu** by release of HSPs
  - trigger the innate immune system
  - cross-talk DC-NK
  - cross-presentation of tumor antigens

*Multhoff et al. (1995); Asea et al. (2000); Srivastava (2000-5); Noessner et al. (2002); Parmiani et al. (2001-5); Milani et al. (2005); Massa et al. (2005); Pilla et al. (2005)*
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